EFFECT OF ASPIRIN INTERVENTION ON PLATELET
AGGREGATION IN NON-KAVA DRINKING AND KAVA
DRINKING FIJIAN AND INDO-FIJIAN HEALTHY
VOLUNTEERS AND DETERMINATION OF ASPIRIN
RESISTANCE

by
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Degree of Doctor of Philosophy in Biology

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DECLARATION

I, Vaishali Khatri, declare that this thesis is my own work and that, to the best of my knowledge, it contains no material previously published or substantially overlapping with material submitted for the award of any other degree at any institution, except where due acknowledgement is made in the text.

Signature ........................................ Date: ................................

The research in this thesis was performed under our supervision and to our knowledge is the sole work of Vaishali Khatri (student ID# S11046711).

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Abstract

Fiji is a Pacific island country, located in the southwest Pacific. It is made up of 350 islands out of which 100 are inhabited. 95% of the population lives on the two main islands viz, Viti Levu and Vanua Levu. Fiji has two main ethnic groups—the original Melanesian/Polynesian inhabitants of the islands, and the descendants of the Indian laborers, who were brought in the late 19th century to work on the sugar plantation.

Changing disease patterns due to urbanization and modern life style in Fiji is reflected clearly in mortality rates and risk factors in indigenous Fijians and Indo-Fijians. Mortality and morbidity due to cardiovascular disease and ischaemic heart disease are on the rise.

Platelet reactions contribute to thrombus formation and these can be inhibited by various platelet inhibiting agents. Therefore, pharmacological inhibition of platelets is considered to be the cornerstone in acute and prophylactic treatment of ischaemic heart disease, peripheral vascular disease and stroke. Management of cardiovascular disease in Fiji includes anti-platelet drugs, especially aspirin which is prescribed routinely. Several laboratory studies have reported variations in response to aspirin treatment and also platelet function has been demonstrated to be normal in a considerable proportion of patients despite taking aspirin due to decreased sensitivity to aspirin, a phenomenon which is also referred to as aspirin resistance. At present no tests are being done to check if aspirin treatment of individual patients has been beneficial in terms of decreasing platelet aggregation.

Numerous interactions between herbal medicines and conventional drugs have been documented. Non-steroidal anti-inflammatory drugs (NSAIDs), particularly aspirin, have the potential to interact with herbal supplements that are known to possess antiplatelet activity.

Kava (Piper methysticum) is an ancient crop of the western Pacific. Kava use is so widespread throughout Oceania that it is considered an integral part of their culture that binds together most of the people of Oceania. Kava preparation and extracts are very popular in the Pacific as well as western society and the potential remains for herbal supplements like kava to interact negatively with other drugs like aspirin in vivo which needs to be thoroughly explored.
This thesis describes studies undertaken to determine the effect of aspirin intervention (100 mg and 300 mg) on platelet aggregation (PA) in non-kava drinkers (NKD) and kava drinkers (KD) (subdivided into occasional kava drinkers (OKD) and regular kava drinkers (RKD), Fijian and Indo-Fijian healthy adult volunteers and thereby to determine aspirin sensitivity or aspirin resistance amongst these groups. The Fijian volunteers were divided into three groups, NKD (n=58), OKD (n=60) and RKD (n=58). Similarly the Indo-Fijian volunteers were also divided into three main groups, NKD (n=58), OKD (n=58) and RKD (n=52).

PA aggregation was measured using a whole blood platelet aggregometer (Chronology Corp) and collagen was used as the aggregating agent. Required ethical approval was obtained from the Fiji National Research Ethics Review Committee (FNRERC) and from the National Health Research Committee (NHRC).

Data were analysed by appropriate statistical tests such as repeated measures ANOVA, Mauchly’s test of sphericity, one way ANOVA with post hoc Scheffe test and independent t test. Comparisons were made within and between the Fijian and the Indo-fijian population to see if there was a difference in PA before giving aspirin and after 100 mg and 300 mg aspirin intervention and the prevalence of aspirin resistance was determined. Differences in PA were also evaluated taking gender, BMI and smoking status into consideration.

Overall PA remained uniform and within the normal range (15-27 Ω) in the NKD Fijians (21.4±3.9) and Indo-Fijians (20.9±3.2) before aspirin intervention. Similarly the kava drinkers (OKD and RKD) in the Fijian and the Indo-Fijian group exhibited PA within the normal range. PA also did not significantly differ among NKD, OKD and RKD Fijians (p>0.05) and Indo-Fijians (p>0.05).

After a single dose of 100 mg of aspirin, PA was inhibited (<15 Ω) in most of the NKD and OKD in both Fijians and Indo-Fijians, whereas a large number of participants in both ethnic groups of RKD were found to have decreased aspirin sensitivity or aspirin resistance as referred to in this thesis. In the Fijian RKD group, 58.9% (33/56) participants were found to be aspirin resistant and therefore showed no inhibition of platelet aggregation after 100mg of aspirin, whereas 94.6% (55/58) in the NKD and 93.3% (56/60) in the OKD group showed
significant (p<0.01) inhibition. Similarly in the Indo-Fijians, after a single dose of 100mg of aspirin, 93.1% (54/58) participants in the NKD group showed a decrease in PA and 6.9% (4/58) participants were found to be aspirin resistant, whereas in the OKD group, 93.1% (54/58) participants had reduced PA and 6.9% (4/58) were aspirin resistant. In the RKD group 67.3% (35/52) participants were found to be aspirin resistant. The aspirin resistant participants had their PA within the normal range (15-27 Ω) even after administration of aspirin. There was a significant difference (p<0.05) in PA among the three kava drinking groups.

PA after 300 mg aspirin was compared and analysed between the NKD, OKD and RKD Fijians. All the participants in the NKD group showed a decrease, 98.3% (59/60) in the OKD group showed a decrease and 96.4% (54/56) in the RKD group showed a decrease in PA (mean 6.95 Ω). Similarly in the Indo-Fijians all the three groups, NKD, OKD and RKD showed a reduction in PA (<15 Ω) after the administration of 300 mg of aspirin. However, the difference was statistically non- significant (p>0.05).

Comparing the PA between two ethnic groups, the Fijian NKD (21.4±3.9 Ω) and the Indo-Fijian NKD (20.8±3.2 Ω) before aspirin intervention showed no significant difference (p>0.05) and after 100 mg (9.1±3.9 and 10.3±3.9 Ω) and 300 mg (5.4±3.6 and 7.1±4.1 Ω) aspirin dosages. No significant difference in PA was seen on comparing the Fijian OKD with the Indo-Fijian OKD (p=0.345), but after 100mg aspirin (8.4±4.8 and 10.3±3.1), a significant difference (p=0.013) was recorded, aggregation values being higher in the Indo-Fijian group as compared to the Fijians. No significant difference between these two ethnic groups (OKD) was observed after 300 mg of aspirin (p>0.080).

Ethnic comparisons did not reveal significant difference before aspirin intervention in RKD individuals. After 100 mg aspirin (15.3±6.0 and 15.7±4.4), though the PA was found to be slightly higher in the Indo-Fijians, the difference was not statistically significant (p=0.654) in the Fijians. No difference in PA was seen after 300mg (6.9±4.7 and 8.5±4.6; p>0.081).

Gender effect on PA was studied among all groups and both aspirin dosages. Results showed that when PA in the females was compared with the males in the NKD Fijian group before aspirin, the females had a higher mean PA (23.5±3.4 Ω) as compared to the males (19.6±3.4 Ω) and the difference was statistically highly significant (p<0.01). No significant difference
in PA was seen after aspirin intervention. Similarly, comparing the PA in males (18.6±3.1 Ω) and females (22.5±2.7 Ω) of the OKD Fijian group showed that there was a highly significant difference ($p<0.01$) before aspirin, whereas, no significant difference in PA was seen between the males and the females after aspirin intervention. In the RKD Fijian group, gender difference for PA was statistically non significant before and after aspirin intervention.

A highly significant difference ($p<0.01$) was recorded for PA in NKD Indo-Fijian participants before (22.2±2.8 in females; 19.8±3.1 Ω in males) and after aspirin interventions (100mg: 11.5±3.6 in females and 9.3±3.0 Ω in males; 300mg: 9.0±3.9 in females and 5.6±3.5 Ω in males). In the OKD Indo-Fijian group, the gender difference for PA was significant ($p<0.01$) before aspirin intervention only. The difference was non-significant after aspirin intervention. There was no significant difference ($p>0.05$) in PA between males and females in the RKD Indo-Fijian group before and after aspirin intervention.

The PA in all the three groups of BMI was within the normal range (before aspirin intervention). The differences were non significant among the three kava drinking groups. A significant difference in PA was seen between the smokers and non-smokers of the NKD and RKD Indo-Fijian groups. Mean PA in the smokers was found to be slightly higher than the non-smokers. The results of both these groups were statistically significant.

No correlation between age and platelet aggregation was found before and after aspirin intervention in Fijian non-kava drinking, occasional kava drinking and regular kava drinking participants. A highly significant correlation was seen between age and platelet aggregation in the Indo-Fijian participants (non-kava, occasional and regular kava drinkers). A significant negative correlation was seen between age and platelet aggregation in the Indo-Fijian occasional kava drinkers after 100mg aspirin.

The results of this study definitely raise concern about the dose of aspirin required to be administered in kava drinking populations. Non-steroidal anti-inflammatory drugs, particularly aspirin have the potential to interact with herbal supplements like kava, which possesses antiplatelet activity. Health-care professionals should be aware of the potential adverse interactions between herbal supplements and analgesic drugs. Further research is suggested to understand the mechanisms of the effect of kava on platelets and to assess the clinical significance of these potential interactions.
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DEDICATION

This thesis is dedicated to my parents, Jeevanlal Khatri and Veena Khatri for being there always. I would also like to dedicate this to my sisters, Varsha and Priya for the love they have always showered on me. Finally to my son Abhijeet for his love, patience and understanding throughout this research project.
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<td>AA</td>
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<tr>
<td>ADP</td>
<td>Adenosine-diphosphate</td>
</tr>
<tr>
<td>AHA</td>
<td>American Heart Association</td>
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<tr>
<td>ANOVA</td>
<td>Analysis of Variance</td>
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<tr>
<td>ASA</td>
<td>Acetylsalicylic Acid</td>
</tr>
<tr>
<td>ASPECT</td>
<td>Aspirin Induced Platelet Effect Study</td>
</tr>
<tr>
<td>BMI</td>
<td>Body Mass Index</td>
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<tr>
<td>Ca2+</td>
<td>Calcium</td>
</tr>
<tr>
<td>CAD</td>
<td>Coronary Artery Disease</td>
</tr>
<tr>
<td>COL</td>
<td>Collagen</td>
</tr>
<tr>
<td>COX</td>
<td>Cyclooxygenase</td>
</tr>
<tr>
<td>CNS</td>
<td>Central Nervous System</td>
</tr>
<tr>
<td>CWM</td>
<td>Colonial War Memorial</td>
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<tr>
<td>DNA</td>
<td>Deoxyribonucleic Acid</td>
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<tr>
<td>EPI</td>
<td>Epinephrine</td>
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<tr>
<td>F</td>
<td>Fijians</td>
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<tr>
<td>F(ANOVA)</td>
<td>F (ANOVA) test</td>
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<tr>
<td>GABA</td>
<td>Gamma Amino Butyric Acid</td>
</tr>
<tr>
<td>IF</td>
<td>Indo-Fijians</td>
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<tr>
<td>IgG</td>
<td>Immunoglobulin</td>
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<td>IHD</td>
<td>Ischemic Heart Disease</td>
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<td>IL-6</td>
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<td>JPAD</td>
<td>Japanese Primary Prevention of Atherosclerosis with Aspirin for Diabetes</td>
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<td>KD</td>
<td>Kava Drinkers</td>
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<tr>
<td>MAO-B</td>
<td>Monoamineoxidase B</td>
</tr>
<tr>
<td>MI</td>
<td>Myocardial Infarction</td>
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<tr>
<td>mRNA</td>
<td>Messenger Ribonucleic Acid</td>
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<tr>
<td>NKD</td>
<td>Non-kava Drinkers</td>
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<tr>
<td>NO</td>
<td>Nitric Oxide</td>
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<tr>
<td>NSAID</td>
<td>Non Steroidal Anti-Inflammatory Drug</td>
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<td>Occasional Kava Drinkers</td>
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<td>PA</td>
<td>Platelet Aggregation</td>
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<td>PCV</td>
<td>Packed Cell Volume</td>
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<td>Platelet Count</td>
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<td>PDGF</td>
<td>Prostaglandin Derived Growth Factor</td>
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<td>TXA2</td>
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<td>TXS</td>
<td>Thromboxane Synthase</td>
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<tr>
<td>vWF</td>
<td>Von Willebrand Factor</td>
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<td>WBA</td>
<td>Whole Blood Aggregometer</td>
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* t test
CHAPTER 1: INTRODUCTION

1.1 General Introduction

Ischaemic heart disease (IHD) is largely considered to be a disease of western civilization but from the past decade or two, this view has been changing as high prevalence of IHD is being reported from developing countries. Epidemiological studies show that heart disease of all types are extremely common in Fiji and the incidence of chronic non-communicable disease such as IHD has lead to the highest mortality rate in Fiji amongst the Pacific Island nations (Ram and Cornelius, 1991). Fiji is a Pacific island country, located in the southwest Pacific. It is made up of 350 islands out of which 100 are inhabited. 95% of the population lives on the two main islands viz, Viti Levu and Vanua Levu. Fiji has two main ethnic groups—the original Melanesian/Polynesian inhabitants of the islands, and the descendants of the Indian laborers, who were brought in the late 19th century to work on the sugar plantation. Fiji is a country where rapid changes have occurred in the social and economic aspects since its independence in 1970, and cardiovascular diseases have emerged as the major public health problem (Toumilehto et al., 1984).

There is an acute awareness of race and especially of the entitlements, advantages and disadvantages of the two main groups Fijian and Indian (Indo-Fijians). Each group has strong stereotypes about the other. Most of the research studies done in Fiji present extensive tables under the racial headings Fijian, Indian, and Others, even though these terms are not clearly defined in the medical literature (Sorokin et al., 1973). Further the authors have indicated that hospital admissions and mortality due to cardiovascular disease has been increasing steadily over the past 20 years, shortening the life span significantly but also adding to the health burden in terms of requiring secondary level care (Ram et al., 1983). They were seen more frequently in the Indian population but now an increase is the prevalence rate has been noticed among the Melanesian population. IHD has been known to be the main cause of mortality and morbidity among the Indian population.

Management of cardiovascular disease in Fiji includes anti-platelet drug, especially aspirin which is prescribed routinely. No tests are being done to check if aspirin has been beneficial in terms of decreasing platelet aggregation or reducing the clinical outcome.
1.2 Platelets and Aspirin

Platelets, the third formed element of the blood (after red blood cells and white blood cells) are anuclear, disc shaped particles produced in the bone marrow. They measure about 1.5-3.0 micrometers in diameter and play an important role in hemostasis. Physiologically platelets are inactive in the blood circulation. The role of platelets in blood coagulation is extensively reviewed (White, 1979; Spencer et al., 1997; Guyton and Hall, 2005). Platelets initiate the formation of blood clots by sticking together - a process called platelet aggregation. When the vessel wall is damaged, platelets are activated when they come in contact with collagen (which is exposed when the endothelium lining the blood vessel is damaged). The platelets release the contents of their storage granules such as adenosine diphosphate (ADP), which then causes the platelets to swell to a more spherical shape, extend pseudopods and adhere to each other. These clumps of platelets together with fibrin form the blood clot. However if blood clots (thrombus) are formed inside the artery, they can block the flow of blood to the tissue that the artery supplies, leading to ischemia (Guyton and Hall, 2005).

Platelet reactions contribute to thrombus formation and these can be inhibited by various platelet inhibiting agents. Therefore, pharmacological inhibition of platelets is considered to be the cornerstone in acute and prophylactic treatment of ischaemic heart disease, peripheral vascular disease and stroke. (Poulsen and Mickley, 2004). A number of drugs have been shown to be capable of inhibiting platelet function at various stages, both in vitro and in vivo. Drugs which inhibit platelet function include the non-steroidal anti-inflammatory drugs (NSAID). Aspirin inhibits platelet aggregation and daily low dose aspirin improves the outcome in patients who are at an increased risk of occlusive vascular events (Mustard, 1972).
Aspirin is a drug in the family of salicylates often used as an analgesic, antipyretic and anti-inflammatory agent. Aspirin or acetylsalicylic acid (ASA) is a potent and inexpensive drug prescribed to prevent first and recurrent myocardial infarcts and thrombotic cerebrovascular events including transient ischaemic attacks. It is also prescribed after thrombolytic therapy and in acute myocardial infarction (Bochner and Lloyd, 1993).

Thromboxanes are eicosanoids produced from damaged platelets cell membrane that are responsible for the aggregation of platelets and which help stabilize blood clots. Aspirin prevents platelet plug formation by blocking the production of thromboxane A-2 by platelets. Long term, low doses of aspirin irreversibly block the formation of thromboxane A2 by inhibiting the enzyme cyclooxygenase (COX-1) in platelets, producing an inhibitory effect on platelet aggregation. Since platelets have no nucleus or DNA, they are unable to synthesize...
new COX once aspirin has inhibited it. This irreversible inhibition of platelet aggregation by aspirin makes it useful for reducing the incidence of heart attacks.

**Figure 1:** Site of action of aspirin- Aspirin inhibits COX-1 and decreases the production of Thromboxane A2 (source: Gasparyan, A Y. et al. J Am Coll Cardiol 2008; 51: 1829-1843)

Regular doses as low as 75mg /day have been shown to be effective. Aspirin produced for this purpose often comes in 75mg or 100mg tablets and is sometimes called Junior Aspirin. (Eikelboom *et al.*, 2002).

Reviews from the USA food and drug administration have suggested that the physicians should need to look at aspirin in the context of complete care, as part of the whole treatment plan for people at risk of heart attack or stroke (Awtry and Loscalzo, 2000).
While other NSAIDs such as ibuprofen also inhibit the COX-1 enzyme, aspirin is the preferred NSAID for use as an antiplatelet agent because its inhibition of the COX-1 enzyme lasts much longer than the other NSAIDs (aspirin's antiplatelet effect lasts days while the other NSAIDs' antiplatelet effects last only hours). Aspirin is widely used either alone or in combination with other antiplatelet agents to prevent blood clots from forming in arteries. When aspirin is given in low doses (75 mg/day), the complete inhibition of the COX-1 enzyme and hence maximal antiplatelet effect occurs. It is prescribed in low doses on a long-term basis to patients with prior heart attacks or strokes and to patients with TIAs (transient ischemic attacks or mini-strokes) and exertional angina to prevent heart attacks and ischemic strokes and for prevention of heart attack or stroke in patients with risk factors of these conditions including longstanding diabetes, vascular disease (previous heart attack or stroke, or poor circulation to the legs), or angina (Gasparyan et al., 2008).

Thus, aspirin at low doses (75-150 mg/day) is used for the long term prevention of heart attacks and strokes, whereas moderate doses (160-325 mg/day) of aspirin are given in situations where an immediate anti-clotting effects are needed (such as in the treatment of acute heart attacks and unstable angina). At a dose of 160-325 mg/day, the maximal antiplatelet effect of aspirin occurs within 30 minutes. It is prescribed in moderate doses for patients who are having heart attacks to limit the extent of damage to the heart's muscle by preventing blood clot formation in the blood vessels of the heart and therefore to prevent additional heart attacks and improve survival. At moderate doses it produces an antiplatelet effect rapidly (within 30 minutes). The current recommendation is to give aspirin immediately to almost all patients as soon as a heart attack is recognized at a dose of 160-325 mg/d and to continue it for one month. Aspirin is easy to use, safe at the low doses used for its antiplatelet action, and fast acting. The only reason for not using aspirin is a history of intolerance or allergy to aspirin or evidence of obvious active bleeding (such as actively bleeding stomach ulcers) (Antiplatelet Trialist collaboration, 2002).

The Antiplatelet Trialist’s collaboration study thoroughly evaluated aspirin as an antiplatelet drug and found that it prevented non vascular events by about 30% and vascular deaths by about 15% in a meta-analysis of more than fifty secondary prevention trials in various groups of patients (Antiplatelet Trialist collaboration, 2002).
The interaction of platelets with damaged vessel walls leads to the formation of platelet-fibrin thrombi and this may also contribute to the development of atherosclerotic lesions because platelets adherent to exposed collagen and release a mitogen that stimulates smooth muscle cell proliferation. Therefore most physicians recommend aspirin in healthy subjects who have one or more risk factors for developing atherosclerosis (Kawasaki et al., 2003).

1.3 Aspirin resistance (Aspirin sensitivity)

The benefits of aspirin have been studied extensively and its place in therapy has been established through many landmark clinical trials. Despite the proven efficacy in clinical studies, there is a growing concern regarding patients who continue to experience vascular thrombotic events despite receiving aspirin therapy. Several laboratory studies have reported variations in response to aspirin treatment and also the platelet function has been demonstrated to be normal in a considerable proportion of patients taking aspirin daily (Poulsen, 2004 and Hankey, 2004). According to available data and ongoing research, it has been shown that about 20% of patients are resistant or rather less sensitive to the thrombus-reducing effects of aspirin, or the doses they are taking are too low to inhibit platelet aggregation.

Aspirin resistance can be defined as the lack or decreased antiplatelet effect despite therapeutic doses of aspirin or the incidence of recurrent vascular events in patients taking aspirin. This term has been used interchangeably in the literature to describe biochemical as well as clinical phenomenon (Sanderson et al., 2005).

This lack of anti-platelet response to aspirin increases the risk of developing heart attacks, strokes, and related deaths. Aspirin resistance is different from other causes of aspirin failure such as patient non-compliance or drug interference from concomitant use of ibuprofen.

In these aspirin-resistant individuals, aspirin does not inhibit the formation of thromboxane-A2. Therefore failure of aspirin to suppress thromboxane production and therefore to inhibit platelet aggregation in vitro has been linked to inadequate protection against atherothrombotic events (Blinc, 2007). Mechanisms to determine aspirin resistance are being studied, but aspirin resistance is still considered to be an enigma and the data available on aspirin resistance is scarce. Millions of people with heart disease who take aspirin need to
know whether it is effective for them. Not finding out whether the patient is aspirin resistant would be comparable to diagnosing a patient with high blood pressure, giving him/her medication for blood pressure and then not monitoring the blood pressure. However aspirin resistance, also called ‘Aspirin non-responsiveness’ or simply treatment failure, is a heterogeneous phenomenon still without a generally accepted definition and with unclear clinical implications.

Aspirin resistance may be classified as (Patrono, 2003).

i. laboratory resistance (the failure of aspirin to inhibit platelet thromboxane-A2 production or inhibit platelet aggregation or persistent platelet activation, demonstrated by platelet function tests (biochemical aspirin resistance)

ii. clinical resistance (the failure of aspirin to prevent clinical thromboembolic ischemic events in patients prescribed aspirin) or the recurrence of vascular events in patients prescribed usual therapeutic doses of aspirin (clinical aspirin resistance). The clinical concept is nonspecific and might be preferably labeled as clinical treatment failure.

One of the ways in which resistant individuals can be identified in research settings is by finding high levels of 11-dehydrothromboxane B2 (a metabolic breakdown product of thromboxane-A2) in the urine while taking aspirin. These individuals have a higher risk of heart attack and strokes than subjects with lower urine levels of 11-dehydrothromboxane B2 (Eikelboom, 2002).

Another way of identifying aspirin resistance in research settings is by using the whole blood platelet aggregometer. Aspirin non-responders identified by this method were found to have higher rates of heart attacks, strokes, and death than aspirin responders (Anderson, 2003).

The potential benefit of aspirin therapy may be significantly reduced in patients with aspirin resistance, creating a clinical and economic burden on the healthcare system. It is estimated that 29 billion aspirin tablets are consumed each year by Americans, with the most popular indication for the use of aspirin being the prevention of cardiovascular disease (Chow and Cheung, 2006). However studies have estimated that 5% to 45% of patients taking aspirin are experiencing suboptimal antiplatelet effects (Helgason et al., 1993; Gum, 2001), hence the potential impact of aspirin resistance is highly significant.
Therapeutic failure due to aspirin resistance can have a major impact on the cost of treating patients with coronary heart disease and stroke. The American Heart Association (AHA) estimates that $112 billion per year is spent on direct costs, including hospital, nursing home, physician and drug cost on these diseases. The cost on society adds another $88 billion per year in lost productivity and loss of future earnings. AHA also estimates that 2.4 million procedures, including angioplasty, percutaneous intervention and cardiac revascularization are performed yearly. Since aspirin resistance prevalence is estimated to be between 5% and 45%, the cost of treatment and the number of interventions can be significantly reduced if patients can be identified with validated laboratory tests and treated appropriately according to their resistance to aspirin (Awtry and Loscalzo, 2000).

It is clear that clinically defined aspirin resistance is a major concern, and there is growing evidence that patients with laboratory evidence of aspirin resistance are at a greater risk of thromboembolic events than aspirin responders (Campbell, 2005). However, further studies are needed to clarify the relation between platelet aggregation, aspirin resistance testing, pharmacological management, and clinical outcomes (Grotemeyer et al., 1993), and to study the prevalence of aspirin resistance in different populations of subjects and patients.

1.4 Kava and platelet function
Kava (Piper methysticum), also called the pepper plant, is an ancient crop of the western Pacific. It is found in Polynesia, Melanesia, and Micronesia. Kava is the term used for both the plant and the beverage made from its roots. Kava has a peppery taste and has long been a part of religious, political and cultural life throughout the Pacific. Differences in the kava plant are seen throughout the pacific, and these differences are due to the appearance of the plant (plant morphology) such as the color of its stem, stem thickness, and shape of the leaves. The differences are also due to the content of kava-lactones in different kava plants.
The kava root is ground to a powder and the brownish powder is then mixed with water and drunk, without being fermented or any other processing. Extracts from the kava root are placed in capsules and sold as kava or kava kava. More recently, kava kava has also gained popularity with the native people of Hawaii, Australia and New Guinea where it is used medicinally as well as recreationally. Kava also is effective as a pain reliever, and can be used instead of aspirin, paracetamol and ibuprofen. According to recent studies, kava kava may interact with anticoagulants or antiplatelet drugs to increase risk of bleeding. One \textit{in vitro} study showed that the kava-lactone, kawain, appears to decrease thromboxane-2 production and inhibit cyclo-oxygenase, indicating that kava may have significant inhibitory effect on platelet aggregation (Gleitz \textit{et al.}, 1997). All of the above kava compounds tested demonstrated better or similar COX-1 inhibition activities as compared to ibuprofen, aspirin and naproxen (Wu \textit{et al.}, 2002).

Kava drinkers may be arbitrarily classified as light, moderate or heavy depending on the quantity of kava they drink or as regular and occasional drinkers based on their frequency of
drinking. In the study carried out by Ruci (2001), the degree of kava consumption was classified according to the classification used in the Australian Aborigines studied by Mathews as non-users, occasional users (100gram/week), heavy users (300grams/week) and very heavy users (440grams/week) (Ruci, 2001; Mathews et al., 1988). The absorption of kava in the gastrointestinal tract is rapid so the effects are felt almost immediately. The peak plasma levels occur about 1.8 hrs after an oral dose and elimination half life of kava lactones is 9 hours. Elimination occurs primarily by the kidneys and the faeces (Malani, 2002). According to the available literature it is very difficult to quantify the amount of kava that people consume. This is because the different varieties of kava contain kava-lactones in different concentrations and also the preparation of kava and volume drunk may vary.

When kava is drunk, the active chemicals are absorbed through the stomach into the bloodstream and pass quickly to the brain. Kava acts as sedative and soporific (sleep inducing). It also induces generalized muscle relaxation (Alexander et al., 1987). Depending on the strength of the kava mixture, it can have a psychoactive effect. While kava does not contain alcohol, people talk about getting 'drunk' on kava. A person is thought to be drunk on kava if he or she cannot walk or talk properly, is very friendly, dizzy, sleepy or is acting 'funny' (Watson, 1988).

Increased packed cell volume (PCV), decreased platelet count (PC), decreased lymphocyte count, reduced serum albumin; tachycardia and electrographic abnormalities have been reported in heavy users of kava (Ulbricht, 2005). Though it is unclear whether these observations represent true adverse effects or are due to other factors.

There have been a number of sudden deaths of young, adult men during heavy exercise after they had drunk large amounts of kava. These young men had diseased hearts (Young et al., 1999). It is now well known that smoking, alcohol, high blood pressure, diabetes, lack of exercise, excessive weight and poor nutrition all contribute to cardiovascular disease. It is also known that unaccustomed exercise puts a strain on a diseased heart, increasing the risk of sudden death. Dehydration from heavy sweating and not drinking water can also be dangerous. Therefore, circumstantial evidence suggests that kava consumption may be associated with IHD and sudden cardiac arrest particularly among young aboriginal sportsmen. Some young Aboriginal people have cardiovascular disease without knowing it. Some of these young people drink alcohol heavily and smoke and so are already at risk of
further damaging their hearts (Young et al., 1999). Drinking kava may add to the list of risks in the South Pacific region.

It is important to note that apart from the documented effects like drowsiness, liver damage, abnormal muscle spasms or involuntary muscle movements, kava may interact with several drugs taken concomitantly (Schelosky et al., 1995).

1.5 Importance of this study
Evidence which shows that aspirin as an important drug used to prevent heart attacks and strokes. Aspirin does this by inhibiting the enzyme COX1 in platelets and therefore preventing platelet aggregation. Aspirin has been prescribed for decades by cardiologists to patients who have suffered heart attacks, strokes or other thromboembolic event to prevent recurrence (Sanderson et al., 2005). However, after it has been prescribed, no tests are done to check whether it is working for the patients. Research shows that some patients may not respond to aspirin or maybe less sensitive to the antiplatelet effect of aspirin and this makes them susceptible to recurrence of the heart attack

So far no report is available in the literature on platelet aggregation in the kava drinkers or the effect of aspirin on platelet aggregation in the kava drinkers in Fiji.

To explore this issue, a series of experiments have been conducted to study platelet aggregation in response to collagen (as an agonist) in healthy Fijians (F) and Indo-Fijians (IF), kava drinking (KD) and non-kava drinking (NKD) adult volunteers treated with a single dose of 100 mg and 300 mg of aspirin.

This study is important as it looks into the effect of aspirin on platelet aggregation in the kava drinkers and compares it with non-kava drinkers in the two major populations in Fiji, the Fijians and the Indo-Fijians. By doing interventional studies in the volunteers, it would be possible to determine the subjects’ response to aspirin and to record the prevalence rate of aspirin resistant. Maybe then, alternative antiplatelet medicines could be advised to the aspirin resistance individual.
The questions that need to be answered by this research are

1. Is there a difference in platelet aggregation between the F and IF population?
2. Is there any gender variation in platelet aggregation in the F and IF population?
3. What is the effect of aspirin on platelet aggregation in the NKD and KD?
4. Is there an interaction between kava and aspirin and what is their effect on platelet aggregation?
5. What is the effect of 2 different doses of aspirin (100mg and 300mg on platelet on aggregation in the NKD and KD?

To get insight of these questions, this study puts forward a broad aim with specific objectives as given below.

1.6 Aims

To examine the effect of two different doses of aspirin on platelet aggregation in non-kava drinkers and kava drinkers in the Fijian and Indo-Fijian population and to determine the prevalence of aspirin resistance in these groups.

1.7 Objectives

1. To determine platelet aggregation before a dose of aspirin
2. To determine platelet aggregation after a single dose of 100mg of aspirin
3. To determine platelet aggregation after a single dose of 300mg of aspirin
4. To analyse and compare if there is any difference in the platelet aggregation before and after 100mg and 300mg of aspirin
5. To determine aspirin resistance in Fijian and Indo-Fijian individuals which are further subdivided into categories of:
   a) non-kava drinkers (control)
   b) occasional kava drinkers
   c) regular kava drinkers
6. To determine the effects of gender, smoking, BMI and age on platelet aggregation.
CHAPTER 2: REVIEW OF LITERATURE

2.1 Introduction
In this chapter, the structure, function and the mechanism of platelet aggregation have been reviewed. The various antiplatelet agents, their mode of action and the methods for platelet function testing have been listed down. The mode of action of aspirin and its role as an antiplatelet agent has been elaborated. Various clinical studies have been mentioned regarding the effect of aspirin on platelets and the mechanism of aspirin resistance. Finally the different effects of kava and specifically its effect on the platelets have been described.

2.2 Discovery of Platelets
The presence of particles in blood which appeared to be smaller than the white blood cells and red blood cells is dated way back to the end of the eighteenth century. It was between 1865 and 1877 that these particles were clearly described, although their origin, significance and function still remained an enigma.

In the years 1881 -1882, Giulio Bizzozzer, an Italian Pathologist was the first to establish the key role of platelets in physiological haemostasis, and thrombosis. The discovery of blood platelets and their function took place long before the knowledge about the mechanisms of blood clotting was discovered completely and described. Probably, Hewson in 1780 was the first person to fully describe platelets as very small undefined particles in blood. In 1842, Alfred Donne (1801 -1878), a French histologist, named platelets as “globulin du chyle” which meant small globules extracted from plasma. They appeared as small globular, pale, opaline cells which were not visible in blood. Beale in 1850 described these same particles as “germinal matter” (Bioplasma kornchen) and Zimmermann in 1860 referred to them as “small corpuscles” (Körperchen) (Gazzaniga and Ottini, 2001).

Although red blood cells had been known since van Leeuwenhoek, it was the German anatomist Max Schultze (1825-1874) who first offered a description of the platelet in his newly-founded journal Archiv für mikroskopische Anatomie. Max Schultze (1825-1874) was the first to give a detailed description of these blood components, in 1865, and expressed them as “clumps of irregular shape and different size, up to 80 u, composed by small globules or colorless granules, having a diameter of 1-2µ. He described these particles as having
sharp-corners and sometimes showing granular appearance. They seemed to lack spontaneous motility and irregular radial extensions were seen starting from the periphery of these clumps whenever the process of blood clotting was initiated being related to the fibrin filaments. Based on this morphological and structural observation, especially the granular appearance, Schultze, concluded that they probably were degenerated or disintegrated leukocytes (Gazzaniga and Ottini, 2001).

George Hayem (1878 and 1879) who was a renowned haematologist and an expert on haemolytic anaemias published a clear description of the platelets. He described them as small corpuscles having a diameter between 1.5-4.5µ which were present along with the red and white blood cells. They appeared to be very pale and delicate. On coming in contact with surfaces like glass, they rapidly changed shape, having pseudopodia like extensions and becoming stick. They could stick to each other and form aggregates. During the same period another researcher, Reiss, debated that the origin of the platelets was from leucocytes (Gazzaniga and Ottini, 2001).

In 1880, Neumann, proposed that when venipuncture was done in an incorrect manner to draw blood, the RBC’s broke up and the remnants were termed as the platelets. A long line of debates followed over the years and finally in 1882 Giulio Bizzozzero (1846-1901) was the first to clearly establish the significance of the particles. By doing a number of in vivo and in vitro investigations, he was able to demonstrate that they were visible not only in blood extracted from veins, but also in circulating blood which he observed using a Hartnack microscope. He firmly stated that these corpuscles were the third morphological element of blood, totally unrelated to white and red cells (Gazzaniga and Ottini, 2001).

Bizzozzero also confirmed their adhesive properties, not only to glass but also to the damaged vasculature. Moreover, he clearly stated they did not contain haemoglobin nor nuclear material, and therefore they could not be considered as precursors of erythrocytes. He is especially noted for discovering that platelets clump at the site of blood vessel injury, a process that precedes the formation of a blood clot. This observation confirmed the role of platelets in coagulation. Wright, between 1906 and 1910 was the first to identify bone marrow megakaryocytes as the precursor of blood platelets (Dianzani, 1994).

During 1961, research on thrombotic mechanisms was mainly concerned with blood coagulation, but then, a shift of emphasis toward the study of platelets dramatically evolved.
The recent history of research on platelets and its applications in medicine started with the introduction of ex vivo methods for studying platelet behavior. The Witness Seminar held on 25 November 2003, chaired by Professor Tom Meade, considered the detailed study of platelets starting with the recognition of their role not only in haemostasis, but both in thrombotic and bleeding disorders (Tansey, 2005).

2.3 Structure and functions of platelets

Over the years great progress has been made in our knowledge regarding the discovery and the basic cellular mechanisms of blood platelets which have helped to clarify the role of platelets. Platelets play a very critical role in response to injury that involves the process of haemostasis, thrombus formation, vascular and connective tissue healing (Savage et al., 1998).

Platelets are non nucleated, disc shaped particles produced in the bone marrow. They measure about 1.5-3.0 micrometres in diameter and are produced from fragmentation of the megakaryocyte. The megakaryocyte is derived from pluripotential haemopoietic stem cells in the bone marrow. Platelets lack DNA but contain mRNA and the translational machinery needed for protein synthesis. Cytokines like IL-6 and IL-11 are thought to increase platelet production from megakaryocytes. The average lifespan of a platelet is between 8 and 12 days. After leaving the bone marrow, platelets circulate freely in the blood. Their primary function is to stop hemorrhage after tissue trauma and vascular injury (White, 1979).

Two possible mechanisms have been postulated regarding the fragmentation of megakaryocytes to platelets, i.e. transition from cells in the bone marrow to circulation cells in the bloodstream. One theory is that megakaryocytes themselves are released from the bone marrow and are carried to the pulmonary capillaries, where they fragment into individual platelet. Another is that the bone marrow endothelium has special properties that encourage formation of pseudopods extending from mature megakaryocytes to bone marrow sinuses which then fragment and directly release platelets into the blood (Cramer, 2001).

Thrombopoietin is the hormone which regulates the production of megakaryocyte and platelets. It is produced in the liver and kidneys. Each megakaryocyte produces between 5,000 and 10,000 platelets. The physiological range for platelets is 150-400 x 10^9 per liter. An average healthy adult produces around 1 x 10^{11} platelets each day. Senile or old platelets
are destroyed in the spleen and the liver (Kupffer cells) by the process of phagocytosis. Spleen also stores platelets which are released when needed by sympathetically-induced splenic contraction. Platelets contain numerous intracytoplasmic granules. These store numerous proinflammatory mediators. Platelets are therefore known to be the greatest single source of vasoactive amines in the body and are also a rich source of thromboxane A2 (Guyton and Hall, 2006).

The primary components of platelets are the membrane structures, microtubules, and granules. Platelet membrane, overlying glycolecty, and the submembrane structures mediate responses to platelet stimulation and express specific antigenic characteristics. The surface glycoproteins are known to act as receptors and therefore play an important role in the process of platelet adhesion and contraction. Contractile proteins in the platelets are myosin and actin filaments which are anchored to the surface of the platelet by the Trans membrane glycoprotein a-actinin. They mediate the initial change in the shape of the platelets and therefore help in retraction of the formed clot. The platelet membrane invaginates to form the canalicular system which provides a direct connection between the interior and the exterior surface of the platelet and hence provide a direct route through which plasma constituents can move into the platelet as well as platelet components can exit in connection with the release reaction. The dense tubular system is entirely enclosed and is the major site for storage of Ca2+. On stimulation of the platelet, the cytoplasmic concentration of Ca2+ rises and calmodulin is activated which then combines with myosin light-chain kinase. This enzyme phosphorylates myosin, leading to the combination of myosin with actin, which brings about the initial changes in the shape of the platelet and, ultimately, retraction of the formed clot (Comfurius et al., 1985). The dense tubular system is also the location of cyclooxygenase, the critical enzyme which helps in the conversion of membrane-derived arachidonic acid to unstable endoperoxide precursors of prostaglandins and thromboxanes (Gerrard et al., 1976).

Various granules are present in the platelets such as the alpha-granules, dense granules and lysosomal granules. These vary from each other in size and content. These granules contain substances like the platelet factor 4, β-thromboglobulin, fibrinogen, vWf, PDGF, fibronectin, thrombospondin, IgG, clotting factors V, VIII and XIII. Most of the proteins found in the platelets are absorbed from the plasma and not synthesized by the megakaryocyte (Harrison and Cramer, 1993).
PGDF (prostaglandin derived growth factor) is stored in the granules and is released during platelet aggregation. It induces proliferation of fibroblasts, microglia, and smooth muscle. PDGF may also serve as a chemotactic agent for inflammatory cells. Activated platelets may also release chemokines that can trigger the recruitment of monocytes during the process of inflammation or promote their differentiation into macrophages (McNicol and Israels, 1999).

Platelets are also known to play a central role in angiogenesis as they are a rich source of stimulators and inhibitors.

### 2.4 Platelet activation and aggregation

Platelets are important components of hemostasis and play a key role in atherothrombosis due to their capacity to adhere to injured blood vessels and to accumulate at the site of injury. These are the first cells to be recruited at a wound site, where they form a mechanical plug to seal the injury. Platelet aggregation is the process in which the platelets stick together and thereby initiate the formation of blood clots. Platelet adhesion and activation are physiologic repair responses to the sudden rupture or fissuring of an atherosclerotic plaque. But uncontrolled progression of this process can lead to the development of a thrombus, vascular occlusion and transient ischaemia or infarction. Various mechanisms have been postulated to explain the process of platelet aggregation (Kulkarni et al., 2002).

Normally, platelets circulate freely and because of their negative surface potential and also due to the smoothness of the vessel wall, they are not attracted to the endothelium. After injury of a blood vessel, the collagen gets exposed along with other subendothelial fibers and platelets then get attracted and immediately adhere to the exposed collagen. Due to the adhesion, the platelets swell up and become irregular in shape. Collagen exposure leads to the secretion of a number of factors from granules within the platelet, including adenosine diphosphate (induces aggregation), serotonin (a vasoconstrictor) (Goldsmith and Turitto, 1986). Factors released from the platelet membrane include arachadonic acid (AA) which leads to thromboxane A2 production which induces platelet aggregation. Other substances released during this activation process include:

1) fibrinogen - which is used to stabilise the clot,
2) platelet factor 4 (acts as anti heparin),
3) inositol trisphosphate (controls Ca\(^{++}\) release). Ca\(^{++}\) is also used in the clotting mechanism to activate some of the clotting factors of the cascade system (Ginsberg et al., 1980).

The initial binding of platelets at sites of vascular injury is mediated by glycoprotein Ib/V/IX, which is a structurally unique receptor complex expressed in megakaryocytes and platelets. vWF is the major ligand for one component of this complex, glycoprotein-I\(b\). Besides glycoprotein-I\(b\), several collagen receptors with a tethering function are found on the platelet surface, notably glycoprotein-VI and glycoprotein-I\(a\) which are members of the immunoglobulin superfamily (Tschopp et al., 1973).

vWF is released by endothelial cells and is involved in platelet adhesion and later in platelet aggregation. Some hormones such as adrenaline control the release of vWF. Therefore a situation in which adrenaline is released such as stress and exercise indirectly increase vWF levels (Coller, 1985).

After the initial adhesion of platelets to the extracellular matrix, the repair process requires a rapid response to autocrine and paracrine mediators, including ADP, thrombin, epinephrine, and thromboxane A\(_2\). These mediators amplify and sustain the initial platelet response and they recruit circulating platelets from the flowing blood to form a growing haemostatic plug. Most agonists that activate platelets operate through G-protein–coupled receptors. The final pathway for all agonists is the activation of the platelet integrin glycoprotein IIb/IIIa. Several adhesive substrates bind to glycoprotein IIb/IIIa. Fibrinogen plays an important role in maintaining the stability of a thrombus, by bridging glycoprotein IIb/IIIa integrins between platelets. vWF is necessary to facilitate interplatelet bridges at low shear rates \textit{in vitro}. Quiescent platelets contain the pre-mRNA of the molecule termed tissue factor, the primary initiator of the coagulation cascade that leads to the conversion of prothrombin to thrombin and fibrinogen to fibrin. Signal-dependent splicing of tissue-factor pre-mRNA allows for the synthesis of bioactive tissue-factor protein and therefore provides platelet-derived tissue factor for propagating and stabilizing the thrombus (Phillips et al., 1988; Ruggeri, 2003).

The activation of platelets is induced by the interaction of several agonists with receptors expressed on the platelet membrane. TXA\(_2\) is synthesized by activated platelets from arachidonic acid (AA) through the cyclooxygenase pathway. Once formed, TXA\(_2\) can diffuse
across the membrane and activate other platelets. In platelets, there are two splice variants of the TXA2 receptor: TPα and TPβ, which differ in their cytoplasmic tail. TPα and TPβ couple to the proteins Gq and G12 or G13, all of which activate phospholipase C (PLC). This enzyme degrades the membrane phosphoinositides (such as phosphatidylinositol 4,5-bisphosphate [PI(4,5)P2]), releasing the second messengers inositol triphosphate (IP3) and diacylglycerol (DAG). DAG activates intracellular protein kinase C (PKC), which causes protein phosphorylation. The release of IP3 increases cytosolic levels of Ca2+, which is released from the endoplasmic reticulum. ADP is released from platelets and red cells. Platelets express at least two ADP receptors, P2Y1 and P2Y2, which couple to Gq and Gi, respectively. The activation of P2Y2 inhibits adenylate cyclase, causing a decrease in the cyclic AMP (cAMP) level, and the activation of P2Y1 causes an increase in the intracellular Ca2+ level. The P2Y2 receptor is the major receptor able to amplify and sustain platelet activation in response to ADP. Thrombin is rapidly generated at sites of vascular injury from circulating prothrombin and, besides mediating fibrin generation, represents the most potent platelet activator (Roth et al., 1975).

2.5 Antiplatelet agents

The multiple pathways of platelet activation limit the effect of specific receptor/pathway inhibitors, resulting in limited clinical efficacy. Various agents inhibit platelet activation and aggregation by different ways (Table 1) (Coccheri, 2010)

**Table 1:** Antiplatelet agents

<table>
<thead>
<tr>
<th>Drug</th>
<th>Mechanism of action</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspirin</td>
<td>Inhibition of cyclooxygenase enzyme and therefore inhibits thromboxane production.</td>
</tr>
<tr>
<td>ADP Receptor blockers</td>
<td>Block the ADP dependant pathway of platelet activation.</td>
</tr>
<tr>
<td>Phosphodiesterase Inhibitors</td>
<td>Inhibits the platelet cyclic AMP breakdown by phosphodiesterase inhibitors.</td>
</tr>
<tr>
<td>GPIIb/IIIa inhibitors</td>
<td>Antagonist of GPIIb/IIIa fibrinogen receptors. Block the GPIIb/IIIa receptor and prevent the binding of vWF, fibrinogen and adhesive molecules to GPIIb/IIIa receptors.</td>
</tr>
</tbody>
</table>
2.6 Platelet Function Testing

Measurement of platelet aggregation can be done by various methods. They include various platelet function tests and direct determination of the ability of aspirin to inhibit TXA2 synthesis. Basically they are done to measure the platelets ability to aggregate. These tests have been challenged in terms of reliability, accuracy, being cumbersome and time consuming. Ability to assess platelet function efficiently is of critical importance in the haemostatic management of patients especially for those who have a history of clinically significant bleeding.

Table 2: Different methods for analysis of platelet aggregation (Harrison, 2005).

<table>
<thead>
<tr>
<th>Bleeding Time methods: Duke, Ivy, Mielke</th>
<th>Assess in vivo platelet function</th>
<th>On puncture measures the time taken for the bleeding to stop. (platelet plug formation)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Platelet rich plasma (light transmission aggregometry)</td>
<td>in vitro</td>
<td>Measurement of light transmission through the platelet rich plasma is recorded before and after addition of the aggregating agent</td>
</tr>
<tr>
<td>Whole Blood Method: Whole Blood aggregometer</td>
<td>in vitro</td>
<td>Measurement of passage of current through the electrodes before and after the aggregation of platelets.</td>
</tr>
<tr>
<td>Lumiaggregation</td>
<td>Firefly luciferin is added to the sample. When platelets are stimulated, the ATP released acts upon the luciferan to form luciferase and the amount of luminescence is measured.</td>
<td></td>
</tr>
<tr>
<td>Platelet function analyser: PFA 100</td>
<td>Monitors platelet adhesion and aggregation on the membrane as whole blood is aspirated under controlled flow conditions through a microscopic aperture cut in the membrane and measurement of the time required for the platelet plug to occlude the aperture.</td>
<td></td>
</tr>
<tr>
<td>The clot signature analyser (CSA)(XYLUM)</td>
<td>Downstream pressure is measured dynamically as Whole blood flows through a tubing system containing two holes. As platelets aggregate and plug the holes, down stream pressure increases. The time required for the pressure to increase to a predetermined level is measured and is a direct measure of platelet function. It is a turbidimetric based optical detection system that measures platelet induced aggregation as an increase in light transmittance.</td>
<td></td>
</tr>
</tbody>
</table>

<p>| The Verify Now Ultegra System (Accumetrics) |                                                                                      |                                                                                      |</p>
<table>
<thead>
<tr>
<th><strong>Plateletworks (Helena Laboratories)</strong></th>
<th><strong>Calculates the percentage of platelet aggregation by measuring a change in the baseline platelet count compared to the platelet count of blood that is added to an agonist tube.</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Serum Thromboxane B2 production</strong></td>
<td><strong>Determined by radioimmunoassay</strong></td>
</tr>
<tr>
<td><strong>Urinary 11-dehydro-thromboxane B2</strong></td>
<td><strong>Determined by ELISA</strong></td>
</tr>
</tbody>
</table>

### 2.7 Discovery of Aspirin

The father of modern medicine was Hippocrates, who lived sometime between 460 B.C and 377 B.C. Hippocrates recommended a tea made from yellow leaves for pain relief. He left historical records of pain relief treatments, which included the use of a powder made from the bark and leaves of the willow tree to help heal headaches, pains and fevers but it wasn't until the 1800's that scientists discovered what was in the willow tree that relieved pain and reduced fever. The substance was named salicylic acid (Anonymous, 2007).

The ancient Romans recorded the use of the willow bark as a fever fighter. The leaves and bark of the willow tree contain a substance called salicin, which was the name given to a naturally occurring compound similar to acetylsalicylic acid, the chemical name for aspirin.

From antiquity to the 1800’s, salicylates from plant sources (white willow, salix alba) have been known to provide a folk remedy for pain and fever. In the mid 1800’s salicylic acid was synthesized in Europe and this was soon followed by the synthesis of acetylsalicylic acid (ASA). The Bayer Company developed ASA as a commercial pharmaceutical product and trademarked the compound as aspirin.

In 1826, two Italian scientists, Brugnatelli and Fontana, were successful in obtaining salicin in a highly impure form and during the same period, Johann Buchner, Professor of Pharmacy at the University of Munich, was able to isolate a small amount of a bitter tasting yellow, crystalline substance, which he named salicin.
By 1829, Henri Leroux, a French chemist, had worked on improving the extraction process and was able to obtain about 30g from 1.5kg of bark. In 1838, an Italian chemist Raffaele Piria, working at the Sorbonne in Paris, succeeded in splitting salicin into a sugar and an aromatic component (salicylaldehyde) and applying the process of hydrolysis and oxidation, converted it to an acid of colorless crystalline nature. This product was named as salicylic acid. Therefore, it was Henri Leroux who was able to extract salicin, in its crystalline form for the first time, whereas Raffaele Piria had succeeded in obtaining the salicylic acid in its pure state.

Felix Hoffmann, a German chemist, produced a stable form of acetylsalicylic acid, more commonly known as aspirin, in 1897. Hoffmann was searching for something to relieve his father's arthritis. He studied French chemist Charles Gergardt's experiments and rediscovered acetylsalicylic acid—or aspirin, as we now know it.

Since 1899, acetylsalicylic acid has attained a leading position world-wide in the prescription-free therapy of painful, feverish and inflammatory states.

Dr. Lawrence Craven, a California general practitioner, in 1948, noticed that the 400 men he had prescribed aspirin to, hadn't suffered any heart attacks. He regularly recommended to all patients and colleagues that an aspirin a day could dramatically reduce the risk of heart attack.

Since then, a lot of research was being done on aspirin and several investigators in the 1940’s reported that aspirin seemed to be a more potent antihaemostatic than salicylic acid. Almost a decade passed before it was reported that aspirin can induce clinically significant bleeding if taken for a long period of time. Craven suggested that the drug might be useful as an antithrombotic agent and this was confirmed by several investigators in the 1960s, who then went on to describe the ability of aspirin to suppress platelet function.

**2.8 Actions of aspirin**

1. It acts as an antiplatelet agent by irreversibly inhibiting and acetyltating cyclooxygenase in circulating platelets and possibly in megakaryocytes.
2. It buffers and transports the protons. It uncouples oxidative phosphorylation in cartilaginous and hepatic mitochondria, by diffusing from the inner membrane space as a proton carrier into the mitochondrial matrix, where it ionizes once again to release protons.

3. It induces the formation of NO-radicals in the body. Experiments with mice have shown this to be an independent mechanism of reducing inflammation.

4. Recent data suggests that salicylic acid and its derivatives modulate signaling through NF-kB which is a transcription factor complex that plays a central role in many biological processes including inflammation (Vane and Botting, 2003).

2.9 Aspirin as an antiplatelet agent

In his research for which he was awarded both a Nobel Prize in Physiology or Medicine in 1982 and a knighthood, John Robert Vane who was then employed by the Royal College of Surgeons in London, showed, that aspirin suppress the production of prostaglandins and thromboxanes. He went on to describe that this happens because cyclooxgenase enzyme that participates in the production of prostaglandins and thromboxanes is irreversibly inhibited when aspirin acetylates it. This makes aspirin different from other NSAIDS which are reversible inhibitors.

After Vane published his observation that aspirin inhibits PG synthesis, the work brought together two separate areas of inquiry (PGs and NSAIDs) and stimulated intense study of the agonist (PG)-inhibitor (NSAID) relationship. PGs mediate responses, such as inflammation, that are blocked by NSAIDs. NSAIDs act by blocking PG synthesis. When used in high doses, such as greater than 4 g/d for rheumatoid arthritis, aspirin may affect pathways of inflammation that do not involve PGs but in most clinical settings, the drug works through its effect on PGS. Even though aspirin was in use for over 70 years its mode of action remained unknown. In 1960 H.O Collier and colleagues determined that aspirin worked, in part, through modulation of the activation of the pathways involved in the synthesis of the prostaglandins. In the 1970s it was determined that aspirin and other non-steroidal anti-inflammatory drugs (NSAIDs) all exerted their effects through the inhibition of PG synthesis via the inhibition of the bifunctional enzyme called cyclooxygenase (Tansey, 2005).
2.10 Clinical Studies - Aspirin and platelet aggregation

Inhibitory effect of aspirin on platelet aggregation has been researched extensively and the role of aspirin as an antiplatelet agent has been established through various trials.

A collaborative meta-analysis trial (Anonymous, 2002), comprising of 287 studies and involved 135000 patients was undertaken to compare and assess the antiplatelet therapy versus the control. The conclusion of this study was that low dose aspirin is an effective antiplatelet especially for long term use and that it is protective in most patients who are at an increased risk of occlusive vascular events but in an acute setting an initial loading dose of 150mg may be given.

Epidemiological studies have indicated that irrespective of the dose of aspirin, very low or very high doses (30–1500 mg), they seem to have effective anti-platelet action, suggesting that inhibition of platelet COX-1 is the key role of aspirin. This supports the view that inhibition of platelet COX-1 by low doses of aspirin is sufficient to provide the cardio-protective effects observed in clinical trials. Aspirin is also known to reduce the risk of secondary events by about 25% in cardiovascular diseases (Catella-Lawson et al., 2001).

The analyses done by the Antiplatelet trialist’s collaboration (1994), showed conclusively that aspirin definitely reduces the risk of nonfatal stroke, non fatal myocardial infarction and vascular death by 25-32% in patients who are at high risk of arterial thrombosis.

In a large multi-center study which was the Second International Study of Infarct Survival of the ISIS-2 trial (1988), conducted on patients having acute heart attacks, treatment within 24 hours of the onset of symptoms with aspirin (160 mg/d) was found to reduce mortality from the heart attacks by 23%. The improved survival has been attributed aspirin's ability to quickly prevent further blood clots and hence the expansion of existing clots is limited and therefore decreases the damage to the heart muscle.

Aspirin in low doses is used in the prophylaxis of platelet aggregation. Jalal et al. (2000), observed the inhibitory effect of aspirin on platelet aggregation using adenosine diphosphate as the stimulating agent. 36 male and 35 female were recruited for this study. The method of Born and Mustard was used to analyze platelet aggregation by using platelet rich plasma. It
was concluded that aspirin reduced platelet aggregation by 5.96% which proves that aspirin has a significant inhibitory effect on platelet aggregation.

There seems to be considerable debates and discussions regarding the right dose of aspirin to prevent strokes and cardiovascular events and though the available data does not confirm that low doses of aspirin (<100mg) are more effective than the higher doses, the safety profile of low dose definitely outweighs the high doses (Dabaghi et al., 1994). Some of the studies mentioned below highlight the efficacy of single low dose aspirin.

Dabaghi, et al. (1994) documented that platelet aggregation was fully inhibited by a single dose of aspirin when arachidonic acid was used as the aggregating agent regardless of the dose given (low or high dose). They also documented that low doses of aspirin effectively inhibit thromboxane B2 metabolism. These results are similar with the results of Kuster and Frolich (1986), who found that platelet aggregation, was entirely blocked by 50mg of aspirin using arachidonic acid as the stimulating agent. They also showed that increasing the dose of aspirin did not result in increased inhibition of platelet aggregation. Both these studies reported the inhibition of thromboxane B2 with low levels of aspirin. Thus a review of these studies provides the information that thromboxane metabolism is inhibited by a single low dose of aspirin and that if arachidonic acid as the aggregating agent, treatment with low dose aspirin (80-100 mg) completely inhibits the platelet aggregation.

In the same study by Kuster and Frolich (1986), collagen was used as the stimulating agent for platelet aggregation. Results showed that a greater degree of inhibition of platelet aggregation was achieved with a single, high dose of aspirin (500mg) compared with a single low dose (100 mg).

Mehta and Aquila (1984) compared the effects of different doses of aspirin on platelet aggregation and showed that a dose of aspirin as low as 40mg was capable of decreasing platelet aggregation when epinephrine was used as the stimulating agent and that the magnitude of inhibition of platelet aggregation did not increase with higher doses.

Whether small, single doses of aspirin are effective in adequately inhibiting platelet aggregation is an important clinical consideration. The therapeutic goal of the cardiologist
caring for a patient in the setting of an acute ischemic attacks, myocardial infarcts or strokes is to achieve the fullest platelet inhibition possible without unacceptable adverse effects.

Certain clinical studies have also demonstrated the effect of gender and ethnicity on platelet aggregation.

Mohammad et al. (2010) evaluated the gender variability in platelet aggregation in response to the agonists like ADP, epinephrine, arachidonic acid and collagen. Platelet aggregation was measured in 36 healthy men and women in the age group of 22–36 years, of Caucasian, Hispanic, and African-American origin, who had not taken any antiplatelet medication. Platelet aggregation was measured using a platelet ionized calcium aggregometer (Chrono-Log Co.). The results showed that platelet aggregation response to all tested agonists was higher in females than in males regardless of ethnicity. The most significant differences were observed with collagen ($p < 0.01$). Among the ethnic groups, Caucasian women were most prone to platelet aggregation. This study concludes that gender is an important determinant of agonist effects on platelet aggregability in healthy subjects.

Ethnic variations have been observed in platelet aggregation. Gader et al. (1991) analysed platelet aggregation in various ethnic groups residing in Riyadh, the capital city of Saudi Arabia. The aggregating agents used were ADP, adrenaline, collagen, arachidonic acid and ristocetin. The subjects selected for this study were healthy and were divided according to their ethnic origin into Saudi Arabs, Westerners (Europeans and Americans), South East Asians (Koreans and Filipinos) and West Africans. Significant differences in the platelet aggregation responses were found between these four ethnic groups. Saudi Arabs and Westerners showed better aggregation responses to ADP than Asians and Africans. Aggregability in response to collagen was greater in Saudis and Africans than in Westerners and Asians. There was remarkable inhibition of adrenaline induced-aggregation in Asians while other populations produced comparable results. Inhibited responses to arachidonic acid were most prevalent among Westerners and to a lesser extent in Asians than in Saudis and Africans. Ristocetin-induced aggregation was significantly inhibited in Africans and less so in Asians and Arabs and most pronounced in Westerners. These ethnic differences should be taken into account when assessing aggregation responses in patients. Gan et al. (2002) assessed the effect of increasing dose of aspirin on the degree of platelet aggregation induced by collagen among stroke patients in which 16 stoke patients were prescribed aspirin at daily
doses of 40, 80, 160, 325, 650 and 1300mg, each dose taken for 14 days. This study concluded that the inhibition of collagen induced platelet aggregation by aspirin appeared to be optimal at 80-160mg /day in the post stroke patients.

The benefits of aspirin have been studied extensively and its place in therapy has been established through many landmark clinical trials, but despite the proven efficacy in clinical studies, there is a growing concern regarding patients who continue to experience vascular thrombotic events despite receiving aspirin therapy.

Recently several studies have demonstrated an association between aspirin resistance and outcomes, shifting this problem to the forefront of current clinical practice.

2.11 Aspirin resistance

In recent years aspirin resistance has been in focus and the mechanisms to determine aspirin resistance are being studied, but aspirin resistance is still considered to be an enigma and the data available on aspirin resistance are scarce. Several laboratory studies have reported variations in response to aspirin treatment and also the platelet function has been demonstrated to be normal in a considerable proportion of patients taking aspirin daily. However according to the available data and ongoing research, it has been shown that about 20% of the patients are resistant to the antiplatelet effects of aspirin, or the doses they are taking are too low to affect platelet aggregation (Bhatt, 2004).

Failure of aspirin to suppress thromboxane production and therefore to inhibit platelet aggregation in vitro has been linked to inadequate protection against atherothrombotic events. Millions of people with heart disease who take aspirin need to know whether it is effective for them. Not finding out whether the patient is aspirin resistant would be comparable to diagnosing someone with high blood pressure, giving him medication for blood pressure and then not monitoring his blood pressure (Hankey and Eikelboom, 2004).

However, aspirin resistance also called ‘aspirin non-responsiveness’ or simply treatment failure, is a heterogeneous phenomenon still without a generally accepted definition and with unclear clinical implications.
Aspirin resistance may be classified as (Gum et al., 2001):

1) Laboratory resistance which is defined as the failure of aspirin to inhibit platelet thromboxane A2 production or inhibit platelet aggregation.
2) Clinical resistance is the failure of aspirin to prevent clinical thromboembolic ischaemic events in patients prescribed aspirin.

### 2.12 Table 3: Possible postulated mechanisms for aspirin resistance as reviewed by Cambria-Kiely and Gandhi (2002).

<table>
<thead>
<tr>
<th>Possible postulated mechanisms for aspirin resistance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concurrent intake of certain NSAIDs</td>
</tr>
<tr>
<td>Inadequate dose of aspirin</td>
</tr>
<tr>
<td>Poor compliance</td>
</tr>
<tr>
<td>Reduced bioavailability</td>
</tr>
<tr>
<td>Enhanced platelet function</td>
</tr>
<tr>
<td>Biosynthesis of TXA2 by pathways that are not blocked by aspirin, e.g. by COX-2 in monocytes and macrophages</td>
</tr>
<tr>
<td>Increased platelet sensitivity to ADP and collagen</td>
</tr>
<tr>
<td>Hypercholesterolemia, usually accompanied by increased thrombin generation</td>
</tr>
<tr>
<td>Hypercoagulable states following MI and unstable angina</td>
</tr>
<tr>
<td>Increased release of platelets from bone marrow in response to stress, i.e. after coronary artery bypass surgery, introducing to blood newly formed platelets unexposed to aspirin</td>
</tr>
<tr>
<td>Genetic polymorphisms</td>
</tr>
<tr>
<td>Transcellular arachidonate metabolism between aspirinated platelets and endothelial cells</td>
</tr>
<tr>
<td>Inadequate blockade of red cell-induced platelet activation</td>
</tr>
<tr>
<td>Polymorphism of platelet glycoprotein IIb/IIIa; carriers of PIA2 allele are less sensitive to antithrombotic action of aspirin in vivo</td>
</tr>
<tr>
<td>Polymorphisms of platelet collagen receptors</td>
</tr>
<tr>
<td>COX-2 variants in patients after coronary artery bypass surgery</td>
</tr>
<tr>
<td>FXIII Val34Leu polymorphism leading to variable inhibition of FXIII activation by low-dose aspirin</td>
</tr>
<tr>
<td><strong>Other probable factors</strong></td>
</tr>
<tr>
<td>Increased levels of norepinephrine (excessive physical exercise, mental stress)</td>
</tr>
<tr>
<td>Smoking</td>
</tr>
<tr>
<td>Oxidant stress and biosynthesis of 8-iso-PGF2a</td>
</tr>
</tbody>
</table>

The potential benefit of aspirin therapy may be significantly reduced in patients with aspirin resistance, creating a clinical and economic burden on the healthcare system. It is estimated that 29 billion aspirin tablets are consumed each year by Americans, with the most popular use of aspirin being the prevention of cardiovascular disease. However studies have estimated that 5% to 45% of patients taking aspirin are experiencing suboptimal antiplatelet effects (Gum et al., 2001).
The potential impact of aspirin resistance is highly significant since large populations of patients are taking aspirin and therapeutic failure due to aspirin resistance can have a major impact on the cost of treating patients with coronary heart disease and stroke.

The American Heart Association (AHA) estimates that $112 billion per year is spent on direct costs, including hospital, nursing home, physician and drug cost on these diseases. The cost on society adds another $88 billion per year in lost productivity and loss of future earnings. AHA also estimates that 2.4 million procedures, including angioplasty, PCI (percutaneous intervention) and cardiac revascularization are performed yearly. Since aspirin resistance prevalence is estimated to be between 5% and 45%, the cost of treatment and the number of interventions can be significantly reduced if patients can be identified with validated laboratory tests and treated appropriately according to their resistance to aspirin (Gum et al., 2001)

There is growing evidence that patients with laboratory evidence of aspirin resistance are at a greater risk of thromboembolic events than aspirin sensitive or aspirin responders.

2.13 Clinical Studies- Aspirin Resistance

A number of clinical studies have been done in an attempt to explain the phenomenon of aspirin resistance, but they seem to be insufficient in explaining the phenomena of aspirin resistance. Same research may yield different results and, the characteristics in different population exhibiting aspirin resistance may add to the complexity. Aspirin resistance cannot be explained by only one pathway. More studies are required to investigate the mechanisms in different population (Zhang and Zhang, 2007).

The first study to demonstrate variability in response to aspirin was published nearly 50 years ago. Since then numerous trials evaluating responsiveness to aspirin in a variety of different settings, have been undertaken. No trial till date has ever found a uniform response to aspirin despite using a wide range of techniques.

Various studies have reported a prevalence of aspirin resistance in healthy volunteers and in patients with various manifestations of atherosclerosis in frequencies ranging from 5.5% to 60%. So far only a few published papers have provided knowledge on the clinical relevance of aspirin resistance. Overall these trials support the hypothesis of an association between aspirin resistance and an increased risk of suffering future thrombotic complications.
Table 4: Prevalence of aspirin resistance reported in selected studies (Modified from Poulsen et al., 2005).

<table>
<thead>
<tr>
<th>Author</th>
<th>Sample size</th>
<th>Aspirin dose mg/day</th>
<th>Platelet analysis method used</th>
<th>% aspirin resistant</th>
<th>Aggregating agent used</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grotemeyer et al. (1993)</td>
<td>180</td>
<td>1500</td>
<td>Platelet reactivity index</td>
<td>33</td>
<td>Aggregation induced by blood collection</td>
</tr>
<tr>
<td>Helgason et al. (1994)</td>
<td>306</td>
<td>325</td>
<td>Optical aggr</td>
<td>25</td>
<td>AA, ADP, Collagen, Epinephrine</td>
</tr>
<tr>
<td>Mueller et al. (1997)</td>
<td>100</td>
<td>100</td>
<td>Whole blood aggregometer</td>
<td>60</td>
<td>ADP, collagen</td>
</tr>
<tr>
<td>Buchanan et al. (2000)</td>
<td>289</td>
<td>325</td>
<td>Bleeding time</td>
<td>55</td>
<td></td>
</tr>
<tr>
<td>Peters et al. (2001)</td>
<td>19</td>
<td>100</td>
<td>PFA-100</td>
<td>63</td>
<td>Collagen, ADP, Epinephrine</td>
</tr>
<tr>
<td>Macchi et al. (2002)</td>
<td>72</td>
<td>160</td>
<td>PFA-100</td>
<td>29</td>
<td>Collagen/Epinephrine</td>
</tr>
<tr>
<td>Andersen et al. (2002)</td>
<td>129</td>
<td>75-160</td>
<td>PFA-100</td>
<td>37</td>
<td>Epinephrine, ADP</td>
</tr>
<tr>
<td>Christiaens et al. (2002)</td>
<td>50</td>
<td>&gt;75</td>
<td>PFA-100</td>
<td>20</td>
<td>Collagen/Epinephrine</td>
</tr>
<tr>
<td>Hezard et al. (2002)</td>
<td>50</td>
<td>75-300</td>
<td>PFA-100, Optical aggr.</td>
<td>52</td>
<td>ADP</td>
</tr>
<tr>
<td>Ziegler et al. (2002)</td>
<td>52</td>
<td>100</td>
<td>PFA-100</td>
<td>10</td>
<td>Collagen/Epinephrine</td>
</tr>
<tr>
<td>Sane et al. (2002)</td>
<td>88</td>
<td>325</td>
<td>Flow cytometry, aggr.</td>
<td>57</td>
<td>Collagen, ADP</td>
</tr>
<tr>
<td>Macchi et al. (2003)</td>
<td>72</td>
<td>160</td>
<td>PFA-100</td>
<td>29</td>
<td>Collagen/Epinephrine</td>
</tr>
<tr>
<td>Gum, et al. (2003)</td>
<td>326</td>
<td>325</td>
<td>PFA-100, optical aggr.</td>
<td>9.5</td>
<td>AA, ADP</td>
</tr>
<tr>
<td>Wang et al. (2003)</td>
<td>422</td>
<td>81-325</td>
<td>Ultegra RPFA</td>
<td>23</td>
<td>ADP</td>
</tr>
<tr>
<td>Grundmann et al. (2003)</td>
<td>53</td>
<td>100</td>
<td>PFA-100,</td>
<td>34</td>
<td>Collagen/Epinephrine</td>
</tr>
<tr>
<td>Chen et al. (2004)</td>
<td>151</td>
<td>80-325</td>
<td>Ultegra RPFA</td>
<td>19</td>
<td>Incidence of myonecrosis measured</td>
</tr>
<tr>
<td>Cotter et al. (2004)</td>
<td>82</td>
<td>100</td>
<td>TxB2</td>
<td>12</td>
<td>Measured health outcome</td>
</tr>
<tr>
<td>Alberts et al. (2004)</td>
<td>129</td>
<td>&lt;162 162-325</td>
<td>PFA-100</td>
<td>56</td>
<td>Analysed mean closure time</td>
</tr>
</tbody>
</table>

aggr.=platelet aggregation, Ultegra RPFA®=Ultegra Rapid Platelet Function Assay®, PFA-100®=Platelet Function Analyser-100

In 2004, the New York Times reported that up to 40% of aspirin users are resistant to aspirin. Patients prescribed aspirin to prevent atherothrombotic vascular disease need to know if they are resistant to aspirin and if so, what the implications are.
In the study conducted by Eikelboom et al. (2002) a comparison was made between 488 patients who suffered myocardial infarction or stroke or died from cardiovascular causes with 488 control subjects who had no cardiovascular event. All the participants from both the groups took aspirin. Urinary 11-dehydrothromboxane B2 excretion levels were measured as a reflection of platelet aggregation. The patients in the highest quartile of urinary 11-dehydrothromboxane B2 excretion levels (i.e. those who were least affected by aspirin intake) had a 3.5-fold higher risk of cardiovascular death compared with those in the lowest quartile.

Sane et al. (2002) conducted a study on 88 outpatients who had suffered from congestive cardiac failure and had been treated with aspirin 325 mg/day for more than one month. The stimulating agents for platelet aggregation were 5 μM of adenosine diphosphate (ADP), 1 μg/ml of collagen or 5 μM of epinephrine, and aggregation was assessed using a Chronolog Lumi-Agregometer. Whole blood aggregation was determined using the Chronolog device and the sample was stimulated with 4 μg/ml of collagen. The study considered patients to be aspirin non-responsive when 4 of the following 5 parameters were observed: collagen-induced aggregation >70%, adenosine diphosphate induced aggregation >60%, whole blood aggregation >18 ohms. Persistent platelet activation despite aspirin therapy was detected in 50 of the 88 patients (56.8%).

De Gaetano and Cerletti (1985) outlined the possible mechanisms that might be responsible for the phenomenon of aspirin resistance and these were further summarized by Cambria-Kiely and Gandhi. They include: 1. bioavailability of aspirin; 2. platelet function; 3. polymorphisms; 4. platelet interactions with other blood cells and cell-derived products; 5. several other factors i.e. stimulation of platelet aggregation by cigarette smoking. Aspirin resistant platelet aggregability by increased levels of norepinephrine, as seen during excessive exercise or periods of mental stress biosynthesis of F-isoprostane 8-iso-prostaglandin (PGF2 alpha), a bioactive product of arachidonic acid peroxidation and increased platelet sensitivity to collagen.

Friend et al. (2003) defined aspirin resistance as poor platelet responsiveness to aspirin and therefore aggregation of ≥ 50% of platelets. Gum et al. (2001) defined aspirin resistance based on the aggregating agents used. The percentage aggregation by using different stimulating agents was measured. Aggregation of ≥ 70% with 10 μM ADP, and of ≥ 20% with 0.5 mg/ml arachidonic acid constituted aspirin resistance. This study shows that the
aggregating or the stimulating agents also need to be considered while defining aspirin resistance.

Weber et al. (1999, 2002) classified aspirin resistance into three main categories. Type-1 (pharmacokinetic type) entails the inhibition of platelet thromboxane formation \textit{in vitro} but not \textit{in vivo}. Type-2 (pharmacodynamic type) is characterized by the inability of aspirin to inhibit platelet thromboxane formation both \textit{in vivo} and \textit{in vitro}. Type-3 (pseudoresistance) involves thromboxane-independent platelet activation. The results also suggested that the inducible isoform of cyclooxygenase in platelets, COX-2, confers aspirin resistance, although this opinion was challenged by Patrignani et al. (1999).

According to Koksch et al. (2001) aspirin resistance involves, besides thromboxane formation, an impaired inhibition of platelet aggregation and an increased expression of \(\alpha\)-selectin, a marker of \(\alpha\)-granule secretion associated with the progression of atherosclerosis.

As stated by Halushka and Halushka (2002), that despite the demonstrated benefit of aspirin in secondary prevention and its possible beneficial effects in selected individuals for primary prevention, there remains a large segment of the population at risk that does not benefit from aspirin.

It is clear that further large prospective studies are needed to further clarify the clinical significance of aspirin resistance

Hence aspirin resistance and aspirin non-responsiveness are the terms used for describing both the failure of aspirin to protect individuals from severe vascular events and to cause inhibition of platelet function. Several studies utilizing a broad range of platelet function tests have shown that some subgroups of individuals exhibit a reduced or completely missing antiplatelet response to aspirin. The clinical significance of aspirin non-responsiveness for the prediction of clinical endpoints remains, however, to be determined. Thus far, only a few prospective clinical trials have demonstrated a possible relationship between aspirin non-responsiveness and subsequent vascular events. Most platelet function tests used in respective clinical studies cannot be reliably performed in clinical routine and are not interchangeable for monitoring antiplatelet treatment. There is a need for a simple and reliable assay for predicting the clinical efficacy of antiplatelet therapy (Patrono, 2003; Sanderson et al., 2005).
Several studies have demonstrated, using various platelet function tests (PFTs) that subgroups of patients taking acetylsalicylic acid (ASA) failed to produce the desired antiplatelet effect. This phenomenon as well as the clinical failure of ASA to protect patients from thromboembolic events has been termed as ASA resistance (AR) or ASA non-esponsiveness. Several different methods like optical aggregometry, platelet aggregation in whole blood, platelet function analyzer (PFA-100), platelet reactivity test or platelet aggregate ratio, flow cytometry and thromboxane B2 generation have been used to determine platelet function and hence aspirin resistance. The widespread clinical use of these platelet function tests is substantially limited due to complex preanalytic factors, reduced specificity and poor reproducibility and hence, there is a need for a simple and reliable assay for predicting the clinical efficacy of antiplatelet therapy (Haubelt et al., 2005).

Studies have shown that the antithrombotic effects of aspirin are variable among individuals and this might explain, in part, why the absolute risk of recurrent vascular events in patients receiving aspirin therapy remains relatively high (8% - 18% after 2 years). Although formal diagnostic criteria are lacking, aspirin resistance generally describes the failure of aspirin to produce an expected biological response or the failure of aspirin to prevent atherothrombotic events. Aspirin resistance has been reported to occur in 5% to 60% of the general population, therefore, its detection is potentially of clinical importance (Benedek et al., 1995; Buchanan et al., 1995).

Estimates suggest that the prevalence of aspirin resistance is between 5.5% and 60% depending upon the definition used and parameters measured and also the methods used to measure platelet aggregation. Only a limited number of clinical studies which are of a sufficient scale, well designed, and prospective, with aspirin used at standard doses have convincingly investigated the importance of aspirin resistance. Also, most studies do not sufficiently address the issue of non-compliance to aspirin as a frequent, yet easily preventable cause of resistance to this antiplatelet drug. The clinical implications of aspirin resistance needs to be explored in various cardiovascular disease states, including diabetes mellitus, hypertension, heart failure, and other similar disorders where platelet reactivity is enhanced.
2.14 Kava as a recreational drink in South Pacific

It is difficult to have definite theories about the origin of kava usage in Oceania because there has been no documented evidence about kava before European contact. Since the Pacific islands did not seem to have any oral tradition or a written one. Certain theories have been proposed by some researchers based on study regarding the origin of kava.

Newell (1947) put forth a theory that it was the Polynesians who were responsible for spreading the kava throughout Oceania and that kava originated from New Guinea-Indonesia area. It is believed that kava might have originated from the Asian subcontinent. Handy (1972) linked the kava ceremony with the Chinese tea ceremony. Williamson (1939) said that kava came from the southern part of India. Migrants who were unable to find betel nuts to chew turned to kava for their needs and therefore it has been proposed that kava drinking is related to betel chewing (Handy and Handy, 1972; Pollock 1995).

Vincent Lebot’s theory is the most recent and well-argued theory. It has used botanical evidence to prove that kava originated somewhere in Melanesia, either on Vanuatu, the Solomon Islands, or New Guinea.

Kava (*Piper methysticum*) is an ancient crop of the western Pacific. Kava is the term used for both the plant and the beverage made from it. The beverage is prepared from the root of this shrub also called the pepper plant. It is found in Polynesia, Melanesia, and Micronesia. The kava root is ground to a powder and the brownish powder is then mixed with water and drunk as a beverage, without being fermented. Extracts from the kava root are placed in capsules and sold as kava or kava kava. Kava has a pungent taste and has long been a part of religious, political, and cultural life throughout the Pacific (Moulds and Malani, 2003; Davis and Brown, 1999). Kava kava has also gained popularity with the native people of Hawaii, Australia and New Guinea where it is used medicinally as well as recreationally. Kava continues to occupy a central place in the everyday life of the people in the islands concerned.

Kava has acquired for itself important symbolic meanings and has associated with it large number of rules and procedures for its preparation, distribution and consumption. The kava use is so widespread throughout Oceania that it is considered an integral part of their culture that binds together most of the people of Oceania. Besides being the social beverage for
chiefs and noblemen, it was also used to welcome distinguished guests at formal gatherings, at initiation and completion of work, in preparing for a journey or ocean voyage, installation in office, validation of titles, ratification of agreements, celebration of birth, marriage and death (Pollock, 1995; Lebot et al., 1997).

It has been documented that the Swedish botanist Daniel Scholander and Sydney Parkinson who accompanied James Cook on his first voyage in the Endeavour (1768-1771), were probably the first white men who saw and recorded the kava plant. However the credit for the first detailed description of the kava plant is given to Johann Georg Forster (Forster, 1771). Forster named the kava plant *Piper methysticum* or intoxicating *Pepper methysticum*, being the Latin transcription of the Greek methustikos and derived from Methu which according to Steinmetz (1960) means intoxicating drink (Lebot et al., 1997).

### 2.15 Traditional preparation

Kava is consumed by preparing it in various ways throughout the Pacific Ocean cultures of Polynesia, Vanuatu, Melanesia and also in some parts of Micronesia and Australia. Traditionally it is prepared by chewing, grinding or pounding the roots of the kava plant. Dead coral is used for the grinding purpose and then the ground root or the bark is mixed with little water, as the fresh root also releases moisture during grinding. Pounding is done using a large stone and a small log. The product is then mixed with water and consumed. The beverage is slightly pungent in taste, while the aroma which is so distinctive depends on whether it was prepared from dry or fresh plant, and also on the variety of kava used for the preparation. The color is grey to tan to opaque greenish. Traditionally, kava is drunk from the shorn half-shell of a coconut which is usually referred to as the Bilo (Kevin, 2005).

### 2.16 Modern preparation

In modernized countries kava root powder is used to make the kava beverage. The root powder is prepared by grinding the dried root. The powder is then mixed in water and allowed to stand for about for 30 minutes to allow powdered fibers to soak the water and the mixture is strained through the straining cloth. The powdered pulp enclosed within the straining cloth, is massaged for five to thirty minutes in water, then the liquid is squeezed out which causes the kavalactones to be released from it (Pollock, 1995).
2.17 Pharmacology

Kava lactones (kava pyrones), are the biologically active compounds present in the kava drink, made traditionally by mixing powdered kava roots with water. The anti-anxiety, sedative, anti-insomnia, anti-convulsing, anti-spasmodic, diuretic, and diaphoretic properties of kava are due to the kavalactones (Leung and Forster, 1986).

Lebot and Levesque have stated that sixteen different kavalactone are present, whereas in another study, Singh, has documented presence of 18 kavalactones. But both these studies show that out of the kavalactones isolated from kava, there are six major ones which are: kawain, methysticin, demethoxyyagonin, dihydrokawain, dihydromethisin and yagonin and these accounts for about 96% of the organic extract (Lebot and Levesque, 1989). It has been reported that individual administration of the lactones does not induce the same degree of pharmacological activity as does whole kava extract (Lebot, 1992, 1997). Total kavalactone content ranges from 3% to 20% on a dry weight basis.

Fresh kava rootstock contains on an average 80 percent water, whereas dried, rootstock consists of approximately 43 percent starch, 20 percent fibers, 12 percent water, 3.2 percent sugars, 3.6 percent proteins, 3.2 percent minerals and 15 percent kavalactones, In another study, Lebot et al., (1992) have reported that the dried root contains almost upto 20% kava lactones, however the lactone composition can vary between 3-20% depending on the age, variety and geographical location of the plant.

2.18 Effects of Kava

It was drunk to unclog urinary tract and there are reports of kava being prescribed by the British herbalists for urinary problems at the beginning of the twentieth century. It was also consumed to lose weight and get relief from asthma and rheumatism. Drinking kava is thought to be good for headaches, cramps, and to cure syphilis and gonorrhea. Many Pacific islanders believe that kava restores strength; it soothes stomach pains and helps to cure ailments as boils.

The key ingredients of kava, the kavalactones are believed to cause mild sedation, and a slight numbing of the gums, tongue and mouth. The numbing of the mouth is caused by the two kavalactones, kavain and dihydrokavain which cause the constriction of the blood vessels
in these areas acting as a local topical anesthetic. This anesthetic effect can also cause the stomach to feel numb and therefore cause a sensation of nausea (Mathews et al., 2005).

Kava has also been reported to improve cognitive performance and promote a cheerful mood. Kavalactones are thought to directly interact with the voltage gated ion channels and therefore act as Muscle relaxant, anaesthetic, anticonvulsive and anxiolytic. Current studies suggest that kavalactones potentiate GABA activity but do not alter levels of dopamine and serotonin in the CNS. In heavy, long-term kava drinkers, there seems to be no reduction of ability in saccade and cognitive tests but it is known to be associated with elevated liver enzymes (Cairney et al., 2003).

Desmethoxyyangonin, which is one of the six major kavalactones, probably, is a reversible MAO-B inhibitor (Ki 280 nM) and is therefore able to increase dopamine levels in the nucleus accumbens. This finding might relate to the slightly euphoric action of kava (Uebelhack et al., 1998; Baum et al., 1998).

Kavain, another of the kavalactones, inhibits the reuptake of noradrenalin which is an important neurotransmitter in the brain but not of serotonin (SERT) and this elevated levels of extracellular NA in the brain may account for the reported enhancement of attention and focus (Thompson et al., 2004).

2.19 Kava and Platelets

According to recent studies, kava may interact with anticoagulants or antiplatelet drugs to increase risk of bleeding. One in vitro study showed that the specific kavalactone, kawain, appears to decrease thromboxane 2 production and inhibit cyclo-oxygenase, indicating that kava may have significant inhibitory effect on platelet aggregation. The anti-inflammatory activities of natural products are determined mainly by evaluating their ability to inhibit cyclooxygenase (COX) enzymes. Kavain, one of the six major kavalactones present in the kava roots, was determined to have antithrombotic action on human platelets. It was also shown to prevent the formation of prostaglandin E2 and Thromboxane A2 by inhibiting the action of COX and thromboxane synthase enzyme (Gleitz et al., 1997). Though a few in vitro studies have been done to study the effect of kava on platelets especially the COX enzyme, Except for the study by Gleitz, the in vivo effect of kava on the platelet aggregation has not
been studied. Hence this study has been undertaken to see the effect of kava on platelet aggregation.

2.20 Adverse Effects

In Fiji, in an interview, the wives of heavy kava drinkers expressed that their husbands spent the money on kava due to which they were deprived of the basic needs.

The hepatotoxic effects of kava have been researched and though the direct toxic effects of kava might be less, the potential for drug interactions and toxic interactions with other compounds is large and cannot be ruled out, though the mechanism is not well understood. It has also been known to potentiate other medications such as barbiturates and Xanax.

Mathew et al. (1988) studied the effects of heavy use of kava in an Aboriginal community in Arnhem Land. They observed that heavy kava use in the community was associated with overall poor health, a puffy face, scaly rash which is usually referred to as dermopathy and a slight increase in patellar reflexes. Health status was assessed in 39 kava users and 34 non-users in a coastal Aboriginal community. Twenty (27%) respondents were very heavy (mean consumption, 440 g/week) users of kava; 15 (21%) respondents were heavy (310 g/week) users of kava and four (5%) respondents were occasional (100 g/week) users of kava. Kava users usually exhibited poor health, puffy face, and were more likely to have a typical scaly rash, and slightly-increased patellar reflexes. Very heavy users of kava were found to be 20% underweight and their levels of gamma-glutamyl transferase (GGT) were also greatly increased. Albumin, plasma protein, urea and bilirubin levels were decreased in kava users and high-density lipoprotein cholesterol levels were increased. Kava users were more likely to show haematuria and to have urine which was poorly acidified and of low specific gravity. The use of kava was also associated with an increased red-cell volume, with a decreased platelet volume and with a decreased lymphocyte count. Shortness of breath in kava users was associated with tall P waves on a resting electrocardiogram, which provided suggestive evidence of pulmonary hypertension. In common with other Aboriginal communities, there was evidence of decreased lung volumes and a high carriage rate of hepatitis B surface antigen.

Animal studies show that kava lactones alter neuronal excitation through direct interactions with voltage-dependent ion channels, giving rise to kava's muscle relaxant, anaesthetic,
anxiolytic and anticonvulsive properties. Several isolated cases of psychotic and severe dystonic reactions following kava use suggest that kava also has psychoactive properties, yet there is no conclusive evidence that kava interferes with normal cognitive processes. There may be risk-factors for severe motor and psychiatric responses to kava use, although these are not well-understood. Given the increasingly widespread use of kava, further investigation is necessary to gain an understanding of its immediate neuropsychiatric effects and long-term cognitive effects (Cairney et al., 2002).

In the past few years, about 35 cases of severe liver toxicity associated with kava intake have been reported in Europe and the US, with the occurrence of severe hepatic toxicity possibly associated with the consumption of products containing kava (i.e., kava kava or *Piper Methysticum*). A total of 11 patients who used kava products had liver failure and underwent subsequent liver transplantation. FDA (food and drug administration) continues to advise consumers and health-care providers about the potential risk associated with the use of kava containing products (Moulds and Malani, 2003; Escher et al, 2001).

It is important to investigate the effects recreational drinks might have on different haematological parameters and different organ systems especially when taken concomitantly with NSAID’s. In this study analysis of PA has been done in non-kava drinkers and kava drinkers.
CHAPTER 3: METHODOLOGY

3.1 Introduction
This chapter describes the aspects of this clinical study, study site, and experimental design for the determination of platelet aggregation in individuals that were classified as non-kava drinkers (NKD) and kava drinkers (KD), belonging to Fijian (F) and Indo-Fijian (IF) groups, subjected to aspirin ingestion. The methods and protocols that were used for determining whole blood platelet aggregation (base line study and aspirin intervention), platelet count (PC), packed cell volume (PCV), and statistical analysis of the data are outlined. This was a clinical study; therefore, the guidelines regarding clinical studies and their application in this study have also been described.

3.2 Study location
This study was carried out in Suva city, Fiji Islands (latitude 18° 00' S and longitude 175 ° 00' E). The analytical part of the research work was conducted at the Fiji School of Medicine (FSM), Pacifica Campus Research Laboratory located on Extension Street, Suva. The lab is well equipped both, for teaching and research. The platelet aggregometer was installed in this research lab.

3.3 Clinical Study
The undertaken research work was a clinical study. The guidelines regarding clinical studies have been followed based on the following definitions (Anonymous, 2009a).

Clinical studies are biomedical or health related research in human beings that follow a certain protocol. They usually include interventional as well as observational types of studies. Studies in which the research subjects are assigned to a particular treatment or other intervention, and their outcomes are measured, are considered as interventional studies, whereas observational studies are those in which individuals are observed by the investigator and their outcomes are measured.
Based on this definition this research study was classified as interventional clinical study since the participants were required to ingest two doses of aspirin (100mg and 300mg). Platelet aggregation was determined before and after this intervention.

3.4 Ethical consideration and approval

Required human ethics approval was sought from the Fiji National Research Ethics Review Committee (FNRERC) and from the National Health Research Committee (NHRC) before the commencement of the experiment. The approval was granted, FNRERC Reference Number: 2008-001 dated 14th July, 2008.

3.5 Instrumentation

Reagent preparation, instrument & technique(s) standardization with reference values, possible chances of error, precautions to be taken and quality control are described in this section. The instrument was purchased from Chronolog whole blood aggregometer distributor from Australia. This is the only instrument available in Fiji for measurement of whole blood platelet aggregation.

3.5.1 Installation of the instrument

The Chronolog whole blood aggregometer (WBA) was set in a clean dry area. The voltage indicator switch located on the rear panel of the instrument was set to the proper voltage (115 or 220). The aggregometer was plugged into a 115 or 220 volt AC, grounded power receptacle. The power switch was set at the ON position. The instrument was allowed to warm up for 15 minutes to allow the heat block to come up to temperature before the testing began (the temperature control indicator should read 37°C before testing begins).

3.5.2 Calibration of the instrument

The instrument was electronically calibrated when it was assembled and therefore required no adjustment. At the beginning of each test the baseline button on the instrument had to be pressed which initiated a baseline setting.

3.5.3 Cleaning the instrument

The exterior surface of the instrument was cleaned by wiping the surface with a mild detergent. Care was taken to keep the liquids from entering the internal areas of the
instruments. A cotton swab moistened with a mild detergent was used to clean the incubation and assay wells. No cotton remained behind in the well after cleaning.

### 3.5.4 Quality Control

Whole blood specimen was collected using the 21G needle (1 ½ inch), in sodium citrate tubes/blue top tubes (3.2% sodium citrate) (purchased from Pasifica Medica, Suva). A stir bar was inserted into a cuvette with disposable probe for each reagent to be run. To each cuvette, 0.5ml of saline was added and prewarmed.

### 3.5.5 Reagent

Sterile physiological saline was used for irrigation (0.85-0.9%w/v) for chrono-par reagent preparation and dilution of the whole blood specimen. Blood bank saline was avoided as the osmolarity is not constant. Cell counter diluents could not be used because they contain EDTA, which inhibits platelet aggregation. Infusion salines were also found to be unsuitable because they contain benzyl alcohol or other preservatives which can inhibit platelet function. Collagen was used directly as supplied by the distributor (chronology corporation, Victoria, Australia). As the collagen fibrils are in suspension, the vial was inverted or swirled before use. Collagen was stored at about 4°C and not frozen. Collagen does not contain any preservative and because of its very low pH, organisms do not grow in it. If aseptic techniques are used (sterile syringes and needles) remaining reagent if stored at 2-8°C is stable until expiration date.

### 3.5.6 Operation of the Chronolog WBA

The Chronolog WBA (Plate-1) uses electrical impedance to measure platelet aggregation in whole blood. A diluted specimen of whole blood is placed in the sample cuvette which is then placed in the instrument reaction well (Plate-2). The sample cuvette includes a disposable electrode (Plate-2). The platelets in the blood sample adhere to the two fine precious metal pins on the electrode, forming a uniform monolayer of platelets on the pins. A small voltage difference is applied across the two pins and the impedance caused by the platelets coating the pins is measured. In the absence of an aggregating agent or agonist, the interaction between the platelets and the electrodes stabilize and the impedance between the two electrodes becomes constant. When an agonist such as collagen, ADP, or ristocetin is added platelets in the specimen are activated and begin to aggregate. The coating of platelets
thickens on the pins over the next several minutes with a corresponding increase in electrical impedance between the electrode wires. The change in impedance is directly proportional to the extent of platelet aggregation and is indicated on the digital display, in ohms (Ω) after six minutes.

In the whole blood impedance method, an AC voltage in milivolt range is applied to the circuit. A stable resistance value is created during the phase of equilibration when the monolayer of platelets covers the wire. After the addition of agonists (collagen was used in this study), platelet start aggregating and their accumulation increases the resistance to the flow of electric current in the circuit, which is measured in ohms.

**Picture 1:** Whole Blood Platelet Aggregometer- 2 channel (Chronolog corporation)
3.6 Measurement of platelet aggregation

3.6.1 Preparation and handling of whole blood sample
Extreme care was taken in the collection, handling and preparation of the participant’s whole blood specimen. As the study was conducted in the research lab, protocols regarding the handling of blood samples were strictly followed. Samples were handled as if they contained infectious material in accordance with national guidelines for biosafety /hazard (in accordance as mentioned in the quality manual at the Colonial War Memorial Hospital laboratory, Suva)

3.6.2 Precautions while handling blood samples
Blood samples were not pipetted by mouth. Disposable gloves were worn during all specimen and assay manipulation. No smoking, eating or drinking was allowed in the laboratory area. Hands were washed thoroughly on completion. The remaining blood samples were discarded according to the lab procedures. It was collected in the yellow plastic bags and taken to the incinerator to be destroyed along with the used syringes, needles, gloves, cotton swabs and the cuvettes.

3.6.3 Sample analysis
The chronolog model 591 whole blood aggregometer was used for platelet function testing of whole blood specimens using impedance aggregometry. The principle associated with this
method is the capability of the platelets to aggregate in response to a physiological agonist, when added to platelet rich plasma or whole blood. The Chronolog whole blood aggregometer (WBA) is a very simple and compact instrument designed to be used by medical professionals in a laboratory setup for routine determination of platelet function in whole blood.

3.6.4 Determination of platelet aggregation
Platelet aggregation was studied using the whole blood aggregometer manufactured by Chronolog Corporation. It is a reliable, qualitative and quantitative tool for measuring platelet aggregation, and thus detecting aspirin use and for measuring the therapeutic effect of aspirin therapy. The whole blood analysis requires only 1 ml of whole blood for a complete aspirin screening and results can be reported within 12 to 15 minutes of receiving and analysing the blood sample.

3.6.5 Handling Conditions
Testing was performed within 4 hrs after venipuncture. Cuvettes containing disposable electrodes, stir bars and 500µl saline were prewarmed. Blue top tubes were gently inverted to mix sample. When the testing began, 500µl of blood was pipetted into a prewarmed cuvette with disposable electrodes, stir bar and saline. The cuvette was warmed for 5 minutes in the incubation well. Lintless wipes such as kimwipes were used. Syringe was used to extract collagen from vial. A normal blood sample obtained from any of the laboratory personnel who was not on any medication was run whenever reagents were reconstituted or thawed to check the test results which should fall within normal ranges established in this laboratory.

3.6.6 Specimen preparation and testing
(The experiments were performed as mentioned in the Chronolog Corporation manual for Whole Blood Platelet Aggregometer, 2006).

Aggregometry was performed at 37°C with a constant stir bar speed of 1000 rpm. The Aggregometer was switched on and warmed to 37°C.

- the test cuvettes (polycarbonate cuvettes) containing 1ml prewarmed diluted blood (0.5ml blood mixed with 0.5ml saline) and stir bar were placed in each reaction well.
- the impedance electrodes were inserted into the sample.
• the amber status indicator on the instrument lit up to indicate that the baseline was being monitored for stability (The amber status indicator lit up after pressing the baseline button). When the baseline (automatically calibrated by the instrument) was stable, a green status indicator flashed, signaling that the test was ready to start.

• if the baseline did not stabilize after one minute, the amber status indicator flashed. Then the ‘set baseline’ button had to be pressed once to allow WBA monitor baseline to set for another minute.

• once all baselines were stabilized the green status indicators on the aggregometer started flashing.

• the ‘set baseline’ button has to be pressed once again. The green status indicator changed to a steady green, indicating that the test was starting. The amber status indicator button also light briefly while the baseline was being set.

• Immediately after the amber indicator went off and the baseline returned to ‘0’, appropriate aggregating agent-collagen (chrono-par reagent no. 385) was added. The aggregometer began analysing and displaying the incremental increase values in ohms.

• 5µl collagen reagent equals 5µg/ml final concentration.

• After 6 minutes the aggregometers digital display stopped blinking to display the final calculation of aggregation in ohms. This reading was recorded (digital values were obtained and these were recorded).

• Impedance electrodes were removed from the samples using a whisking motion and the probe was rinsed in distilled water. Electrodes were wiped dry using lintless wipes such as kimwipes being careful not to bend electrode wires. They were rinsed again in saline and wiped with a lintless wipe. A visual check of the wires was performed to be certain of cleanliness. The samples were removed from the reaction well and discarded.

Electrodes were not unplugged from the instrument when cleaning between tests. A beaker of water or saline was held in front of the instrument and the electrode were brought to the beaker for washing as leaving electrode unplugged in during washing prevented a large shift in baseline for next test.

At the end of the day, the electrodes were whisked in water and wiped with a clean kimwipe. Then they were whisked in 1:10 bleach dilution. Electrode were unplugged from instrument and rinsed under fresh running deionized or distilled water to thoroughly remove bleach from
wires. They were dried with kimwipes and stored in a dry cuvette. The final rinse before storage was in water to avoid salt deposits.

The results of the whole blood aggregation test were measured in ohms (Ω) by the instrument with its digital readout.

**Plate 3**: Temperature readout and amber and green indicators on the aggregometer.

![Plate 3](image)

**Plate 4**: Incubation and sample wells.

![Plate 4](image)
3.7 Relevant Haematological Analysis

3.7.1 Whole blood Platelet count (PC)
Out of the sample of blood collected a small volume was used for PC. It was performed in the research lab by following a standard method of Rees-Ecker (Dacie and Lewis 1995; Sloan, 1951). The principle associated for this manual method is to dilute the whole blood with a solution of brilliant cresyl blue. The diluents prevented blood coagulation. PC was determined by haemocytometry using Neubauer’s counting chamber and values were expressed as lakhs/mm³.

3.7.1.1 Reagent for PC
The diluents used for platelet count met the following requirements:
It provided fixation to reduce the adhesiveness of the platelets, prevented coagulation and haemolysis and also provided a low specific gravity so that the platelets could settle in one plane.

3.7.1.2. Composition of Rees-Ecker Solution
The composition of Rees-Ecker diluting fluid was as follows (Dacie and Lewis 1995).

- Sodium citrate .. .. .. ..3.8 g.
- Formaldehyde (neutral 40% solution).. .. 0.2 ml.
- Brilliant cresyl blue .. .. .. .. .. 0.05 g.
- Distilled water .... 100.0 ml.

3.7.1.3 Counting and calculation
It was basically an isotonic solution of sodium citrate, to which is added a fixative and stain. The stock solution was kept in a glass-stoppered bottle in a refrigerator until required. The stock solution can be stored for at least six months. Before every count the samples had to be filtered and examined to exclude the presence of particles which could be mistaken for platelets.

Care was taken that pipettes and counting chambers were absolutely clean to avoid contact haemolysis. Red blood cell (RBC) diluting pipette was used for diluting the blood. Blood was drawn into the pipette until the column of blood reached the mark 0.5. Diluting fluid was then drawn in to the mark 101, and the pipette was shaken for three minutes. About one-third of
the contents of the pipette were discarded and then the chamber was charged with the fluid. To allow the platelets to settle to the same optical plane, the slide was allowed to stand from 10 to 40 minutes between two pledges of moist cotton-wool under a Petri dish to reduce evaporation.

The cells were counted in 25 medium squares (the squares in which RBCs are counted) and each of these squares has 16 smaller squares. The area covered by 25 medium squares is 1mm².

Platelet count (µl or mm³) = number of platelets counted x dilution volume of fluid.

Where, dilution is 200; volume of fluid for 1mm³ = 1 x 1/10 = 1 x 0.1 = 0.1µl

Therefore, PC per µl of blood = number of PC x 200/0.1

i.e. number of platelets counted x 2000

3.7.1.4 Precautions for PC
- The glassware was absolutely clean (the debris and dust are the main sources of error as they can be easily mistaken for platelets).
- The diluting fluid was filtered just before use.
- As venous blood was used, the count was done within two hours (delay can cause disintegration and clumping of platelets).
- Blood was rapidly diluted as the platelets might form clumps.
- The charged chamber was kept for 15 minutes under Petri dish to prevent evaporation and for the cells to settle down.

3.7.2 Packed cell volume
Haematocrit was checked to rule out volunteers with anaemia. It was done by the microcentrifuge method.

The microcentrifuge was obtained from the CWM hospital. The capillaries were filled with blood and sealed at one end. They were carefully placed in the centrifuge. The centrifuge was run for 5 minutes at a constant speed (3400 rpm). The capillaries were then removed from the centrifuge and the PCV reading was done using the microcentrifuge reader. Samples which
showed a low packed cell volume or haematocrit were discarded (Quality Manual, CWM Hospital, Suva).

3.8 Participation of Individual

3.8.1 Subject selection

The guidelines for selection of participants in this clinical study included the consideration of both inclusion and exclusion criteria which helped to produce reliable results (Anonymous, 2009b).

Inclusion criteria are factors that allow a person to be selected as a participant and exclusion criteria are factors that don’t allow someone to participate in the research. These criteria are based on such factors as age, gender, BMI, the type and stage of a disease, previous medical history, other medical conditions and social history. The criteria were used to select appropriate participants and therefore ensure that this research would be able to answer the questions in the study.

The volunteers for this research work were selected randomly i.e. convenience sampling was done. A request for participation in this study was sent out to the academic and support staff at the Fiji School of Medicine, The University of the South Pacific and Fiji Institute of Technology via e-mail and information about this study was attached (Appendix-A). Participation in this study was voluntary. Friends and families of many of the staff members agreed to participate in this study. To maintain consistency and to prevent bias both males and females were included as volunteers in the study. The age group of the participants was between 21 - 50yrs and comprised of Indo-Fijian and Fijian non-kava and kava drinking volunteers. The legal age in Fiji Islands is 21 years; thereafter parental consent is not required. It was therefore convenient to select participants 21 years and above and if they agreed to participate in this study, they could sign the consent form themselves.

Majority of the staff working in these institutions where the e-mail requesting participation in this study was sent, were between the age group of 21-50 years which made it easier to select the participants in these age groups.
Most of the staff members who were above 50 years of age did agree to participate in this study but since most of them were suffering from heart diseases or other lifestyle diseases like diabetes or blood pressure or were already on certain medications, it was decided to select participants between the age group of 21-50 years. Out of those who agreed to participate, apparently healthy adult participants were selected as volunteers.

3.8.2 Consenting Procedure

Informed consent was obtained from the participants. It is the process of educating the participants about the clinical study before they decide whether or not to participate. The researchers explained orally about the study and intervention to the participant based on which the selected individual had a choice to decide whether to participate or not. Information details (Appendix- A) included the aims and objectives of this study and the names of the principal researchers were given to the participant. Translation assistance was also provided for the participant whose native language was not English. The informed consent form (Appendix- B) included details about the study, including its purpose, duration, required procedures, and key contacts. It also included the risks and potential benefits of the study. The participant then decided whether or not to sign the document. The participant was under no binding and was free to withdraw from the study whenever he/she wanted.

After signing the consent form, the volunteers also completed a structured questionnaire (Appendix- C) giving details about their lifestyle, social and medical history and other anthropometric information relevant to the study. The names or identity of the volunteers was not registered and therefore confidentiality has been maintained. The volunteers were given identity numbers on the basis of which they were identified. Participation in this study involved an in-person interview which elicited information on demographic and medical history, family and social history. The participants were requested to report at different times for the interview to maintain privacy.

To participate in the study the participants were requested not to take any drugs that included NSAID’s (such as aspirin, ibuprofen or any herbal medication containing garlic or *Gingko biloba* which could potentially impair platelet function) for a period of 2 weeks before the initial sampling. Women taking oral contraceptives or estrogen based therapy or other
hormonal based medication, were required to discontinue with the medications for a period of one cycle.

The health of the participant was checked, (basically height, weight, blood pressure and pulse) by the principal researcher at the beginning of the study and the participants were given specific instructions regarding the participation in the study. Since the participants were asked to ingest a single dose of 100mg and then 300mg aspirin (single dose) they were monitored constantly during the study to check for any side effects.

3.8.3 Classification of kava drinkers
Since there seems to be no standard classification of kava drinkers, various studies have classified them according to convenience. In this study the the participants were grouped as non-kava drinkers (NKD) and kava drinkers (KD). The non-kava drinking (NKD) volunteers were those who abstained from drinking kava completely. The kava drinking (KD) volunteers were divided into two groups. One group comprised of individuals who drank kava occasionally i.e. only once a week. These were classified as occasional kava drinkers (OKD). The other group included individuals who drank kava regularly i.e. every day and more than or equal to 20 bowls per day and were classified as regular kava drinkers (RKD). Regular drinkers acquire a reversible ichthyosiform eruption known as Kani kani in Fijian (Gounder, 2006). Many of the regular kava drinking volunteers exhibited Kani kani. Both the occasional and the regular kava drinkers had been drinking kava for more than 2 years. Verification regarding the kava drinking habits was obtained from the spouse, friends or colleague (whosoever could be contacted). Different varieties of kava are available in the market and the method of preparation may also vary. This study does not take those factors into account but in general analyses the effect of kava on platelet aggregation.

Aspirin 100mg (Cartia coated) and 300mg (generic, uncoated), were purchased from the local pharmacy. The volunteers were paid remuneration to cover the cost for their transport and meals. None of the participants withdrew from the study. Low PCV was observed in two participants and therefore they were excluded from the study.
3.8.4 Sample size

When human subjects are involved, opportunities for experimentation are limited due to ethical constraints and priority is always given to the safety of the participants especially in interventional studies where the participants have to be monitored closely. Taking into consideration the budget constrains, experimental design (blood sample to be withdrawn three times from each participant-before aspirin, after 100mg and 300mg aspirin), and constant monitoring of the participants for any side effects of aspirin, the ethics and health research committee, in consultation with the biostatistician agreed to a number of 50 participants in each group (±10, in case certain participants withdrew from the study) to maintain the scientific validity of this study. The sample size (Table-5) was determined based on these considerations so as to maintain the scientific validity of this study and to obtain significant results.

Table 5: Sample size used in the research work: two main ethnic groups: Fijians and Indo-Fijians

<table>
<thead>
<tr>
<th>NKD</th>
<th>Occasional</th>
<th>Regular</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Fijian volunteers</strong></td>
<td>n=58</td>
<td>n=60</td>
</tr>
<tr>
<td><strong>Indo-Fijians volunteers</strong></td>
<td>n=58</td>
<td>n=52</td>
</tr>
</tbody>
</table>

3.8.5 Confidentiality

All the data pertaining to the participants was entered in excel spreadsheets. It was password protected and could be accessed only by the principal researcher. The informed consent forms and the participant information sheets were filed properly date wise and kept securely locked in the filling cabinet. Neither names nor any identification of the participants will be reported in any reports or publications.

3.9 Establishment of collagen and aspirin doses

3.9.1 Baseline Studies in Control volunteers

Before the start of actual research, development of methodology was done to ascertain the procedure for sampling. To develop a standard method of quantification of aspirin sensitivity,
A pilot study was undertaken on a small number (n=5) of normal volunteers. The volunteers were the researcher and the personnel working in the laboratory at FSM. The protocols mentioned above (sample selection and consenting procedures) were strictly adhered to while selecting the participants for the baseline studies. The participants from both ethnic groups (Fijians and Indo-Fijians) were included.

This lab experiment involved the repeated performance of collagen dose response curves on the platelets to determine the reproducibility of the curves testing various amounts of collagen and after different doses of aspirin as mentioned below in study 1 and study 2. These studies were done to determine the normal range of platelet aggregation on addition of different doses of collagen. It would be easy to determine the inhibitory effect of aspirin on platelet aggregation if the normal reference range is determined. Inhibitory effect of aspirin was determined when the values obtained after aspirin ingestion were below the normal values.

In this study the subjects having platelet aggregation within the normal range after aspirin ingestion have been considered as aspirin resistant or aspirin insensitive, though the degree of aspirin sensitivity (semi-responders) has not been analysed in this study.

3.9.1.1 Study 1: Sensitivity to the amount of collagen (n=5)

Flowchart-2: Effect of different doses of collagen on platelet aggregation in healthy volunteers
A test was run to determine the most appropriate dose of collagen for analysis of PA. It was done according to the standard method outlined in the principles of operation, whole blood platelet aggregometer manual, model 591/592 Chronolog Corporation (as mentioned in 3.6)

1) From the cubital vein 5 ml of blood was drawn with minimum stasis. (Only 2ml of blood was required but 5ml of blood was drawn from the subjects in case of spillage or any other subjective errors). The remaining blood was discarded according to the FSM laboratory protocols. The blood samples were collected in blue top tubes containing 3.2% sodium citrate.

2) Four aliquots (cuvettes) were prepared each containing 0.5ml blood sample mixed with 0.5ml saline.

3) The test cuvettes (polycarbonate cuvettes) containing 1ml diluted blood and stir bar were prewarmed (37°C) by placing them in the reaction well of the platelet aggregometer.

(Chromo- lume reaction is both time and temperature dependant. It is therefore important to incubate each sample for about two minutes before starting the test)

4) The impedance electrodes were inserted into the cuvettes containing the sample.

5) The prewarmed cuvettes containing the sample were then placed into the test wells.

6) Different concentrations of collagen (0.5, 1, 3, 5µg) were added in four prewarmed cuvettes (flowchart-1). These are the commonly used concentrations of collagen which are selected in platelet aggregation studies. Results obtained after experiments with different doses helps to determine the dose of collagen to be used in the study. The doses which give results showing large deviation of the curves or skewed curves are not considered

7) PA was measured using the whole blood platelet aggregometer to determine the effective collagen concentration with aggregation value within the range of standard values as specified in the chronology corporation manual, 2006.

This procedure was repeated 3 times (test 1, test2, test3) with each collagen dose, in the same participants with a gap of about 1 week between each sampling to determine reproducibility of the results (table 6 and Fig 1) that could be used to compare with other unknown samples (inter- and intra-subject variability).
Table 6: Study using four different doses of collagen on platelet aggregation (ohms)

<table>
<thead>
<tr>
<th>n</th>
<th>0.5µg collagen</th>
<th>1µg collagen</th>
<th>3µg collagen</th>
<th>5µg collagen</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>test1 test2 test3</td>
<td>test1 test2 test3</td>
<td>test1 test2 test3</td>
<td>test1 test2 test3</td>
</tr>
<tr>
<td>1</td>
<td>0 7 0</td>
<td>17 10 10</td>
<td>21 19 22</td>
<td>20 22 21</td>
</tr>
<tr>
<td>2</td>
<td>1 2 0</td>
<td>9 8 14</td>
<td>18 10 18</td>
<td>15 17 15</td>
</tr>
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<td>3</td>
<td>3 0 2</td>
<td>11 15 13</td>
<td>24 24 23</td>
<td>17 20 21</td>
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<td>4</td>
<td>0 1 1</td>
<td>18 20 19</td>
<td>15 15 14</td>
<td>19 19 19</td>
</tr>
<tr>
<td>5</td>
<td>0 5 0</td>
<td>17 17 17</td>
<td>2 11 10</td>
<td>23 22 22</td>
</tr>
</tbody>
</table>

Mean ±SD | 14.4 ±4.1 | 14.0 ±4.9 | 14.6 ±3.5 | 16.0 ±8.5 | 15.8 ±5.8 | 17.4 ±5.5 | 18.8 ±3.0 | 20 ±2.1 | 19.6 ±2.8 |

Test with each dose of collagen was repeated 3 times to check for reproducibility. The test study conducted with 0.5 µg of collagen did not yield any results. After addition of 0.5 µg collagen, the aggregometer frequently displayed a reading of ‘zero’. The values obtained were compared with the reference values mentioned in the Chronolog Corporation, whole blood platelet aggregometer manual.

Figure 3: Bar graphs showing mean platelet aggregation with 1µg, 3µg and 5µg collagen.
The results obtained with each dose of collagen were reproducible and exhibited uniformity. Among the three concentrations of collagen, 5µg was considered to be the optimum concentration as an aggregating agent as the results were comparable with the reference values mentioned in the instrument manual (table 7)

**Table 7:** Normal range in whole blood as given in Chronolog corporation instrument manual

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Concentration</th>
<th>Aggregation (ohms)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Collagen</td>
<td>5µg/ml</td>
<td>15-31</td>
</tr>
</tbody>
</table>

Hence in this study 5µg collagen was used as the aggregating agent.

The derived PA values in response to the aggregating agent collagen were in the range of 15-27 ohms and these were used as reference values for this study.

**Table 8:** Platelet aggregation range in ohms after addition of 5µg of collagen

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Concentration</th>
<th>Aggregation (ohms)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Collagen</td>
<td>5µg/ml</td>
<td>15-27</td>
</tr>
</tbody>
</table>
3.9.1.2 Study 2: Dose Response curve for aspirin (n=5)

Flowchart-3: Effect of aspirin intervention on platelet aggregation in healthy volunteers

The acute effect of a single dose of aspirin (100mg and 300mg) on the PA was measured in 5 healthy volunteers

1) From the cubital vein 5 ml of blood was drawn with minimum stasis and collected in blue top tubes containing 3.2% sodium citrate

2) The volunteers were asked to ingest a single dose of 100mg aspirin tablet

3) After about 4-6 hours a sample of 3ml of blood was again drawn from these volunteers

4) PA was determined from these blood samples collected before and after ingestion of a single dose of 100mg aspirin

5) There was a gap of about one week before the subject ingested 300mg of aspirin.

Blood sample was collected and analysed for PA

6) Three sets of tests were carried out on each participant to check for reproducibility of the results on three different occasions. There was a gap of one week between test 1 and test 2 and between test 2 and test 3

Table 9: Study of aspirin intervention on platelet aggregation (ohms, Ω) with 5µg collagen as the aggregating agent

<table>
<thead>
<tr>
<th>n</th>
<th>Control</th>
<th>100mg aspirin</th>
<th>300mg aspirin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Test 1</td>
<td>Test 2</td>
<td>Test 3</td>
</tr>
<tr>
<td>1</td>
<td>24</td>
<td>23</td>
<td>24</td>
</tr>
<tr>
<td>2</td>
<td>20</td>
<td>18</td>
<td>19</td>
</tr>
<tr>
<td>3</td>
<td>21</td>
<td>23</td>
<td>23</td>
</tr>
<tr>
<td>4</td>
<td>17</td>
<td>17</td>
<td>16</td>
</tr>
<tr>
<td>5</td>
<td>27</td>
<td>24</td>
<td>25</td>
</tr>
<tr>
<td>Mean ±SD</td>
<td>21.8 ±3.83</td>
<td>21.0 ±3.24</td>
<td>21.4 ±3.78</td>
</tr>
</tbody>
</table>
PA was reduced (less than 15 ohms) after the ingestion of 100 mg and 300 mg of aspirin. Since the normal range of PA considered in this study was 15-27 ohms (Ω), results below 15 ohms were considered to be due to the effect of aspirin. If PA was found to be within normal range after ingestion of aspirin, the subjects were classified as aspirin resistant or aspirin non-responders.

**Figure 4:** Study of platelet aggregation with aspirin interventions (test1, test2, test3) using 5µg collagen as the aggregating agent

Inhibition of platelet aggregation was seen after administration of aspirin (100mg and 300 mg). Values obtained below 15Ω were considered as platelet inhibition.
3.9.1.3 Study 3: Aspirin resistance/aspirin sensitivity

Once the PA baseline reference values with collagen (5µg) were established, aspirin resistance participants were identified if their platelet aggregation values were found to be within the normal range (15-27Ω) after the ingestion of aspirin.

Flowchart 4: Determination of aspirin resistance with aspirin intervention

3.10 Studies in non-kava drinkers and kava drinkers

After determining the collagen dose to be used and the baseline reference value for platelet aggregation, proper experiment was conducted with non-kava drinking, occasional kava drinking and regular kava drinking healthy Fijian and Indo-Fijian volunteers.

Flowchart 5: Effect of a single dose of aspirin and determination of aspirin resistance in NKD, OKD and RKD volunteers
Aspirin resistance was measured based on the results of PA after a dose of 100 mg and 300 mg of aspirin in all volunteers. If the PA was found to be within the normal range (15-27 ohms) after the dose of aspirin, those subjects were classified as aspirin resistant or aspirin non-responders.

3.10.1 Procedure

The volunteers reported at the teaching laboratory located at FSM. Each participant was given a specific identity number. The participants were interviewed and the research questionnaire form was completed based on the information given by the participants. Blood was collected from them on three different successive visits at weekly interval thus the sample collection spanned for over three weeks for each volunteer.

WEEK 1: Before aspirin: This sample acted as a control so that each subject had his own control before aspirin intervention.
WEEK 2: 100mg aspirin: The subjects were given a single dose of 100 mg of aspirin. After a period of about 4-6 hrs, a sample of blood was collected and analysed for PA.
WEEK 3: 300mg aspirin: The subjects were given a single dose of 300 mg of aspirin and the blood was collected after a period of 4-6 hrs and analysed for PA.

During the first visit, 10 ml blood was withdrawn from the subjects taking all aseptic precautions in blue top tubes containing 3.2% sodium citrate. This sample was then analysed for research related haematological profiles such as PC and PCV (as mentioned in 3.7). PA was measured following the method outlined mentioned in 3.6.

Volunteers with a normal PC and PCV were selected for study with aspirin intervention. Adverse effects from a single dose of low dose aspirin are highly unlikely; however, participants were warned and monitored for any side effects. They were asked to report immediately in case of any side effects like nausea, vomiting, gastro intestinal bleeding etc.

3.7 Statistical Analysis of data

The collected data were classified and transferred in to Microsoft Excel. Data on spreadsheets were used for an array of statistical tests using SPSS v18 to derive the results. The data were subjected to the following statistical tests as described by Tabachnick and Fidell (2007).
1) Tests for normality assumption- Kolmogorov-Smirnov and Shapiro-Wilk
2) Homogeneity of variance test was supported by $F_{\text{max}}$
3) Repeated measures ANOVA test and Mauchly’s test of sphericity
4) One-way ANOVA and post hoc Scheffé test for inter and intravariability between the groups and within the groups
5) Two sample independent $t$-test for comparing two groups.
6) Determined Pearson correlation coefficient between age and PA
CHAPTER 4: RESULTS

4.1 Introduction

In this clinical study the platelet aggregation was studied in the non-kava drinking and the kava drinking Fijian and Indo-Fijian population before and after administration of a single dose of 100mg and 300mg of aspirin. A comparison was done within the groups to see if there is a difference in platelet aggregation before giving aspirin and after 100mg and 300mg aspirin. A comparison was also done between the groups to see if there is any difference in platelet aggregation between the Fijian and the Indo-Fijian population before aspirin and after a single dose of 100mg and 300mg of aspirin.

The prevalence of aspirin resistance was determined after a dose of 100mg and 300mg aspirin. Difference in platelet aggregation was also evaluated taking gender into consideration and results were analysed before and after aspirin intervention to see if there is any difference in platelet aggregation between the males and females in the Fijian and Indo-Fijian population (NKD, OKD and RKD).

Ethnic differences in PA were also analysed. Comparison of PA between the Fijian and Indo-Fijian volunteers was done in all the groups (NKD, OKD, RKD), before and after aspirin intervention (100mg and 300mg).

The different groups (NKD, OKD and RKD) in the Fijian and Indo-Fijian participants were also categorized according to their smoking/ non-smoking status and BMI. PA in all the participants before aspirin intervention was measured and comparison of PA between smokers/non-smokers and also between different BMI groups was analysed. Thus the effect of confounding variables like BMI and smoking/non-smoking on PA before aspirin intervention in all the groups was determined.
4.2 Fijian volunteers: Comparison within the group

4.2.1 Non-kava drinkers

Mean platelet aggregation before aspirin intervention (control) was found to be higher as compared to the mean platelet aggregation after 100mg and 300mg aspirin.

**Fig 5:** PA (Ω, mean±se) before and after aspirin intervention in Fijian NKD

![Platelet aggregation graph](image)

Kolmogorov-Smirnov and Shapiro-Wilk statistics indicated that the assumption of normality was supported, and $F_{\text{max}} = 1.1773$ demonstrating homogeneity of variances. However, Mauchly’s test indicated that the sphericity assumption was violated. Thus the degrees of freedom for ANOVA test needed to be adjusted.

The ANOVA results, $F = 405.608$ with df 1.804, $p < 0.001$ and effect size $\eta^2 = 0.877$, indicated that there was a significant difference between mean platelet aggregates due to the different aspirin dosages. Further, the pairwise comparisons revealed that the mean platelet aggregation before aspirin (control group) (21.4±3.9 Ω) was significantly higher than the mean platelet aggregation after with 100mg aspirin (9.1±3.9 Ω) and 300mg aspirin (5.4±3.6 Ω). The pairwise comparisons also showed that the platelet aggregation after 100mg aspirin was significantly higher than the platelet aggregation after 300mg aspirin.

<table>
<thead>
<tr>
<th>NKD (Fijians)</th>
<th>Control (mean+sd)</th>
<th>100mg (mean+sd)</th>
<th>300mg (mean+sd)</th>
</tr>
</thead>
<tbody>
<tr>
<td>n=58</td>
<td>21.4 ±3.9</td>
<td>9.1 ±3.9</td>
<td>5.4 ±3.6</td>
</tr>
</tbody>
</table>

Taking the number of participants into consideration, after a single dose of 100mg of aspirin, 94.82% (55/58) showed a decrease in platelet aggregation (range<15Ω) whereas in 5.17%, (3/58) the platelet aggregation was found to be within the normal range (15-27 Ω). These
participants were classified as aspirin resistant. On administration of 300 mg of aspirin, a decrease in platelet aggregation was seen in all the 58 participants.

### 4.2.2 Occasional kava drinkers

Mean platelet aggregation before aspirin intervention (control) was found to be higher as compared to the mean platelet aggregation after 100mg and 300mg aspirin.

**Fig 6:** PA (Ω, mean±se) before and after aspirin intervention in Fijian OKD

<table>
<thead>
<tr>
<th></th>
<th>Control (mean±sd)</th>
<th>100mg (mean±sd)</th>
<th>300mg (mean±sd)</th>
</tr>
</thead>
<tbody>
<tr>
<td>OKD (Fijians) n=60</td>
<td>20.3 ±3.5</td>
<td>8.4 ±4.8</td>
<td>5.8 ±4.5</td>
</tr>
</tbody>
</table>

Kolmogorov-Smirnov and Shapiro-Wilk statistics indicated that the assumption of normality was supported, and $F_{\text{max}}=1.895$ demonstrating homogeneity of variances. However, Mauchly’s test indicated that the sphericity assumption was violated. Thus the degrees of freedom for ANOVA test needed to be adjusted.

The ANOVA results, $F=271.451$ with df 1.814, $p < 0.001$ and effect size $\eta^2 = 0.822$, indicated that there was a significant difference between mean platelet aggregates due to the different aspirin dosages. Further, the pairwise comparisons revealed that the mean platelet aggregation before aspirin (control group) (20.3±3.5 Ω) was significantly higher than the mean platelet aggregation after 100mg aspirin (8.4±4.8 Ω) and 300mg aspirin (5.8±4.5 Ω). The pairwise comparisons also showed that the platelet aggregation after 100mg aspirin was significantly higher than the platelet aggregation after 300mg aspirin.

Taking the participant numbers into consideration, after a single dose of 100mg of aspirin, 93.33% (56/60) showed a decrease in platelet aggregation (range<15Ω), whereas in 6.66%, (4/60) the platelet aggregation was found to be within the normal range (15-27 Ω). These
participants were classified as aspirin resistant. On administration of 300mg of aspirin, 98.33% (59/60) a decrease in platelet aggregation was seen in all the 59 participants and 1.66% (1/60) was found to be aspirin resistant or aspirin non-responder.

### 4.2.3 Regular Kava drinkers

Mean platelet aggregation before aspirin intervention (control) was found to higher as compared to platelet aggregation after 100mg and 300mg aspirin. The mean platelet aggregation after 100mg aspirin was found to be within the normal range, whereas, a significant reduction in platelet aggregation was seen only after 300mg aspirin.

**Fig 7:** PA (Ω, mean±sd) before and after aspirin intervention in Fijian RKD

<table>
<thead>
<tr>
<th>RKD (Fijians) n=56</th>
<th>Control (mean±sd)</th>
<th>100mg (mean±sd)</th>
<th>300mg (mean±sd)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>20.6±3.9</td>
<td>15.3±6.0</td>
<td>6.9±4.7</td>
</tr>
</tbody>
</table>

Kolmogorov-Smirnov and Shapiro-Wilk statistics indicated that the assumption of normality was supported, and $F_{max}=2.454$ demonstrating homogeneity of variances. However, Mauchly’s test indicated that the sphericity assumption was violated. Thus the degree of freedom for ANOVA test was adjusted.

The ANOVA results, $F=140.012$ with df 1.857, $p < 0.001$ and effect size $\eta^2 = 0.929$, indicated that there was a significant difference between mean platelet aggregates due to the different aspirin dosages. Further, the pairwise comparisons revealed that the platelet aggregation before aspirin (control group) (20.6±3.9Ω) was significantly higher than the platelet aggregation after 100mg aspirin (15.3±6.0Ω) and 300mg aspirin (6.9±4.7Ω). The pairwise comparisons also showed that the platelet aggregation after 100mg aspirin was significantly higher than the platelet aggregation after 300mg aspirin.
Taking the participant numbers into consideration, after a single dose of 100mg of aspirin, only 41.07% (23/56) showed a decrease in platelet aggregation (range<15Ω), whereas in 58.92%, (33/56) the platelet aggregation was found to be within the normal range (15-27 Ω). This showed that a high number of participants (58.92%) in the regular kava drinking group were found to be aspirin resistant. On administration of a higher dose of aspirin i.e. 300mg, 96.42% (54/56) showed a decrease in platelet aggregation, whereas, 3.57% (2/56) were found to aspirin resistant or aspirin non-responder.

4.3 Fijians volunteers: Comparison between the NKD, OKD and RKD groups

4.3.1 Before Aspirin

A comparison of platelet aggregation was done between the non kava drinkers, occasional kava drinkers and regular kava drinkers before aspirin. Platelet aggregation was found to be within the normal range (15-27 Ω) in all the three groups. No significant difference in platelet aggregation was observed among the three groups, $F(2,171) = 1.434, p=0.241$.

Fig 8: PA (Ω, mean±sd) before aspirin intervention in Fijians volunteers

<table>
<thead>
<tr>
<th></th>
<th>Control (mean±sd)</th>
<th>One way ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>NKD (n=58)</td>
<td>21.4 ±3.9</td>
<td>Non significant</td>
</tr>
<tr>
<td>OKD (n=60)</td>
<td>20.3 ±3.5</td>
<td></td>
</tr>
<tr>
<td>RKD (n=56)</td>
<td>20.6 ±3.9</td>
<td></td>
</tr>
<tr>
<td>Overall (n=174)</td>
<td>20.8 ±3.8</td>
<td></td>
</tr>
</tbody>
</table>

4.3.2 After 100mg of aspirin

The ANOVA was statistically significant, indicating that there is a significant difference in platelet aggregation among the three groups, $F (2,171) = 33.006, p<0.0001$. Post hoc analysis with Scheffé test (using $\alpha=0.05$) revealed that after administration of a single dose of aspirin, the platelet aggregation was significantly different between non-kava drinkers and
regular kava drinkers, and occasional kava and regular kava drinkers. However, no difference in platelet aggregation was seen between non kava drinkers and occasional kava drinkers.

**Fig 9:** PA (Ω, mean±sd) after a single dose of aspirin intervention in Fijian volunteers

<table>
<thead>
<tr>
<th>Comparison of PA after 100mg aspirin</th>
<th>After 100mg aspirin (mean±sd)</th>
<th>One way ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>NKD (n=58)</td>
<td>9.1^a ±3.9</td>
<td>Significant at 5%</td>
</tr>
<tr>
<td>OKD (n=60)</td>
<td>8.4^a ±4.8</td>
<td></td>
</tr>
<tr>
<td>RKD (n=56)</td>
<td>15.3^b ±6.0</td>
<td></td>
</tr>
<tr>
<td>Overall (n=174)</td>
<td>10.8 ±5.8</td>
<td></td>
</tr>
</tbody>
</table>

Different superscripts differ significantly (post hoc Scheffe test)

Taking the participant numbers into consideration, in the regular kava drinking group, 58.92% (33/56) participants showed no inhibition of platelet aggregation after 100mg of aspirin. These participants were classified as aspirin resistant. 94.58% (55/58) in the NKD group and 93.33% (56/60) in the occasional kava drinking group showed inhibition of platelet aggregation. The results showed a significant difference between the NKD group and RKD group and the OKD group and RKD group.

**4.3.3 After 300mg of aspirin**

The ANOVA was not statistically significant \( F (2, 171) = 2.073, p=0.129 \), indicating that there is no significant difference in platelet aggregation among three groups, after giving 300 mg aspirin.

Taking participant numbers into consideration, all the participants in the NKD group showed a decrease in platelet aggregation after a single dose of 300mg of aspirin, whereas 98.33% (59/60) in the OKD group showed a decrease in platelet aggregation and 96.42% (54/56) in the RKD group showed a decrease in platelet aggregation.
**Fig 10:** PA (Ω, mean±sd) after a single dose of aspirin intervention in Fijian volunteers

<table>
<thead>
<tr>
<th>Kava category</th>
<th>Control</th>
<th>100mg aspirin</th>
<th>300mg aspirin</th>
</tr>
</thead>
<tbody>
<tr>
<td>NKD (n=58)</td>
<td>21.4 ±3.9</td>
<td>9.1 ±3.9</td>
<td>5.4 ±3.6</td>
</tr>
<tr>
<td>OKD (n=60)</td>
<td>20.3 ±3.5</td>
<td>8.4 ±4.8</td>
<td>5.8 ±4.5</td>
</tr>
<tr>
<td>RKD (n=56)</td>
<td>20.6 ±3.9</td>
<td>15.3 ±6.0</td>
<td>6.9 ±4.7</td>
</tr>
<tr>
<td>Overall (n=174)</td>
<td>20.8 ±3.8</td>
<td>10.8 ±5.8</td>
<td>6.0 ±4.3</td>
</tr>
</tbody>
</table>

**Table 10: Platelet aggregation (Ω, mean±sd) in Fijian population**

**Fig 11:** Overall comparison of PA in NKD, OKD and RKD before and after 100mg and 300mg of aspirin in the Fijian population.

**4.4 Indo-Fijian volunteers: Comparison within the group**

**4.4.1 Non-kava drinkers**

Mean platelet aggregation before aspirin intervention (control) was found to be higher as compared to the mean platelet aggregation after 100mg and 300mg aspirin. The platelet aggregation was found to be within the normal range (15-27 Ω) in the control group i.e.
before the administration of aspirin. Platelet aggregation was reduced after aspirin intervention.

**Fig 12:** PA (Ω, mean±sd) before and after a single dose of aspirin intervention in Indo-Fijian volunteers.

<table>
<thead>
<tr>
<th>Control (mean±sd)</th>
<th>100mg (mean±sd)</th>
<th>300mg (mean±sd)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NKD n=58</td>
<td></td>
<td></td>
</tr>
<tr>
<td>20.9</td>
<td>10.3</td>
<td>7.1</td>
</tr>
<tr>
<td>±3.2</td>
<td>±3.9</td>
<td>±4.1</td>
</tr>
</tbody>
</table>

Kolmogorov-Smirnov and Shapiro-Wilk statistics indicated that the assumption of normality was supported, and $F_{\text{max}}=1.629$ demonstrating homogeneity of variances. Mauchly’s test indicated that the sphericity assumption had not been violated.

The ANOVA results, $F=331.634$ with df 2, $p < 0.001$ and effect size $\eta^2=0.853$ indicated that there was significant difference between at least two of the mean platelet aggregates due to the different dosages of aspirin. Further the pair wise comparisons revealed that the mean platelet aggregate in the control group (20.9±3.2) was significantly higher than the mean platelet aggregate after 100mg aspirin (10.3±3.9) and 300mg aspirin (7.1±4.1). The pairwise comparisons also showed that the platelet aggregation with 100mg aspirin was significantly higher than the platelet aggregation with 300mg aspirin.

Taking the participant numbers into consideration, after a single dose of 100mg of aspirin, 93.10% (54/58) showed a decrease in platelet aggregation (range<15Ω) whereas in 6.89% (4/58) the platelet aggregation was found to be within the normal range (15-27 Ω). These participants were classified as aspirin resistant. On administration of 300mg of aspirin, a further decrease in platelet aggregation was observed in all the 58 participants.
4.4.2 Occasional kava drinkers

With a mean platelet aggregation of 20.88 Ω, the control group appeared to have more platelet aggregation than after 100mg and 300mg aspirin.

Fig 13: PA (Ω, mean+sd) before and after a single dose of aspirin intervention in Indo-Fijian volunteers.

<table>
<thead>
<tr>
<th></th>
<th>Control (mean+sd)</th>
<th>100mg (mean+sd)</th>
<th>300mg (mean+sd)</th>
</tr>
</thead>
<tbody>
<tr>
<td>OKD n=58</td>
<td>20.9 ±3.1</td>
<td>10.3 ±3.1</td>
<td>7.1 ±3.3</td>
</tr>
</tbody>
</table>

Kolmogorov-Smirnov and Shapiro-Wilk statistics indicated that the assumption of normality was supported and $F_{\text{max}} = 1.122$ demonstrating homogeneity of variances. Mauchly’s test indicated that the sphericity assumption had been violated thus the degree of freedom for ANOVA test needed to be adjusted.

The ANOVA results, $F=342.976$ with df 1.797, $p < 0.001$ and effect size $\eta^2 = 0.857$, indicated that there was a significant difference between at least two of the mean platelet aggregates due to the different dosages of aspirin. Further the pair wise comparisons revealed that the platelet aggregation before aspirin (control group) (20.9±3.1) was significantly higher than the mean platelet aggregation after 100mg aspirin (10.3±3.1) and 300mg aspirin (7.1±3.3). Pairwise comparisons also showed that the mean platelet aggregation after 100mg aspirin was significantly higher than the mean platelet aggregation after 300mg aspirin.

Taking participant numbers into consideration, after a single dose of 100mg of aspirin, 93.10% (54/58) participants showed a decrease in platelet aggregation (range<15Ω) whereas in 6.89% (4/58) the platelet aggregation was found to be within the normal range(15-27 Ω).
These participants were classified as aspirin resistant. On administration of 300mg of aspirin, 98.27% (57/58) participants showed a further reduction in platelet aggregation, whereas 1.72% (1/58) was found to be aspirin resistant or aspirin non-responder.

4.4.3 Regular Kava drinkers

With a mean platelet aggregation of 8.5 Ω, the 300mg group appeared to have decreased platelet aggregation as compared to the control group (before aspirin) and 100mg aspirin.

**Fig 14:** PA (Ω, mean±sd) before and after a single dose of aspirin intervention in Indo-Fijian volunteers.

<table>
<thead>
<tr>
<th></th>
<th>Control (mean±sd)</th>
<th>100mg (mean±sd)</th>
<th>300mg (mean±sd)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RKD</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n=52</td>
<td>20.9a ±3.6</td>
<td>15.7a ±4.5</td>
<td>8.5b ±4.6</td>
</tr>
</tbody>
</table>

Different superscripts differ significantly.

Kolmogorov-Smirnov and Shapiro-Wilk statistics indicated that the assumption of normality was supported, and $F_{\text{max}}=1.616$ demonstrating homogeneity of variances. Mauchly’s test indicated that the sphericity assumption had not been violated.

The ANOVA results, $F=122.309$, with df 2, $p < 0.000$ and effect size $\eta^2 =0.706$, indicated that there was a significant difference between at least two of the mean platelet aggregates due to the different dosages of aspirin. Further the pair wise comparisons revealed that the platelet aggregation in control group (20.9±3.6) was significantly higher than the mean platelet aggregation after 100mg aspirin (15.7±4.5) and 300mg aspirin (8.5±4.6). Pairwise comparisons also showed that the platelet aggregation after 100mg aspirin was significantly higher than the platelet aggregation after 300mg aspirin.
Taking participant numbers into consideration, after the administration of 100mg of aspirin, a large number of aspirin resistant or aspirin non-responders were found amongst this group. i.e. almost 67.30% (35/52) had their platelet aggregation within the normal range (15-27 Ω). Only 17 out of the 52 participants showed a decrease in PA after a single dose of 100mg of aspirin. After 300mg of aspirin, 96.15% (50/52) showed a decrease in platelet aggregation except 2 participants (3.84%), who exhibited normal aggregation.

4.5 Indo-Fijians: Comparison between the NKD, OKD and RKD groups

4.5.1 Before aspirin

A comparison of platelet aggregation was done between the non kava drinkers, occasional kava drinkers and regular kava drinkers before aspirin.

The PA in non-kava drinkers (20.86 Ω), occasional kava drinkers (20.88 Ω) and the regular kava drinkers (20.87 Ω) was found to be similar. All the participants (n=168) showed normal platelet aggregation (range 15-27 Ω) after the addition of collagen (5μg/ml).

The ANOVA was not statistically significant, indicating that there is no significant difference in platelet aggregation among the three groups. $F(2,165) = 0.752, p=0.473$.

Fig.15: PA (Ω, mean±sd) before a single dose of aspirin intervention in Indo-Fijian volunteers

<table>
<thead>
<tr>
<th>Indo-Fijians</th>
<th>Control (mean±sd)</th>
<th>$p=0.473$</th>
</tr>
</thead>
<tbody>
<tr>
<td>NKD (n=58)</td>
<td>20.9±3.2</td>
<td></td>
</tr>
<tr>
<td>OKD (n=58)</td>
<td>20.9±3.1</td>
<td></td>
</tr>
<tr>
<td>RKD (n=52)</td>
<td>20.9±3.6</td>
<td></td>
</tr>
<tr>
<td>Overall (n=174)</td>
<td>20.9±3.3</td>
<td></td>
</tr>
</tbody>
</table>
4.5.2 After 100mg of aspirin

The ANOVA was statistically significant, indicating that there is a significant difference in platelet aggregation among the three groups i.e. NKD, OKD and RKD, after the administration of 100mg of aspirin, $F(2,165) = 3.696, p=0.027$.

Post hoc analysis with Scheffe test (using $\alpha =0.05$) revealed that the platelet aggregation was significantly different between NKD (10.27 $\Omega$) and RKD (mean=15.71 $\Omega$), and OKD (10.26 $\Omega$) and RKD 15.71 $\Omega$) after giving 100mg aspirin. However, there was no significant difference in the platelet aggregation of non kava drinkers and occasional kava drinkers.

**Fig 16**: PA ($\Omega$, mean±sd) after a single dose of aspirin intervention in Indo-Fijian volunteers

<table>
<thead>
<tr>
<th>Control (mean±sd)</th>
<th>NKD (n=58)</th>
<th>OKD (n=58)</th>
<th>RKD (n=52)</th>
<th>Overall (n=168)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10.3±3.9</td>
<td>10.3±3.1</td>
<td>15.7±4.5</td>
<td>11.9±4.6</td>
</tr>
<tr>
<td>Different superscripts differ significantly</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Taking participant numbers into consideration, after a single dose of 100mg of aspirin, 93.10% (54/58) participants in the NKD group showed a decrease in PA and 6.90% (4/58) participants were found to be aspirin resistance or aspirin non-responders. Similarly, in the OKD group, 93.10% (54/58) participants had a reduced PA and 6.90% (4/58) were aspirin resistant, whereas, in the RKD group 67.31% (35/52) participants were found to be aspirin resistant. The aspirin resistant participants had their PA within the normal range (15-27 $\Omega$) even after administration of aspirin.

4.5.3 After 300mg of aspirin

The ANOVA was not statistically significant, $F(2,165)=2.315, p=0.102$, indicating that there is no significant difference in platelet aggregation among the three groups, after giving
300mg aspirin. All the three groups, NKD, OKD and RKD showed a reduction in platelet aggregation (<15 Ω) after the administration of 300mg of aspirin.

Fig 17: PA (Ω, mean±sd) after a single dose of aspirin intervention in Indo-Fijian volunteers

![Comparison of PA after 300mg aspirin](image)

<table>
<thead>
<tr>
<th>Kava category</th>
<th>Control (mean±sd)</th>
<th>100mg aspirin (mean±sd)</th>
<th>300mg aspirin (mean±sd)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NKD (n=58)</td>
<td>7.1 ±4.1</td>
<td>10.28 ±3.92</td>
<td>7.12 ±4.07</td>
</tr>
<tr>
<td>OKD (n=58)</td>
<td>7.1 ±3.3</td>
<td>10.26 ±3.07</td>
<td>7.05 ±3.25</td>
</tr>
<tr>
<td>RKD (n=52)</td>
<td>8.5 ±4.6</td>
<td>15.71 ±4.45</td>
<td>8.52 ±4.61</td>
</tr>
<tr>
<td>Overall (n=174)</td>
<td>7.5 ±4.0</td>
<td>11.95 ±4.57</td>
<td>7.53 ±4.02</td>
</tr>
</tbody>
</table>

Fig 18: Overall comparison of PA in NKD, OKD and RKD before and after 100mg and 300mg of aspirin in the Indo-Fijian population.

![Box plot comparison recorded uniformly normal PA before aspirin (control) administration and a decrease in PA after a single dose of 100 mg and 300 mg of aspirin. Potential outliers were present on both sides in occasional kava drinkers (after 100 mg aspirin).](image)

Table 11: Platelet aggregation (Ω, mean±sd) in Indo-Fijian population

Box plot comparison recorded uniformly normal PA before aspirin (control) administration and a decrease in PA after a single dose of 100 mg and 300 mg of aspirin. Potential outliers were present on both sides in occasional kava drinkers (after 100 mg aspirin).
4.6 Ethnic Differences in Platelet Aggregation

PA was compared between the Fijian and the Indo-Fijian, NKD, OKD and RKD before and after aspirin intervention (100mg and 300mg) to see if there is any difference in PA between these two major ethnic groups.

Comparing the PA between the Fijian and Indo-Fijian NKD volunteers showed that there was no statistically significant difference in PA before and after 100mg aspirin. A significant difference in PA was seen after 300mg of aspirin where the PA in the Indo-Fijian volunteers was comparatively higher than the Fijian volunteers.

No significant difference in PA was seen between the Fijian and the Indo-Fijian OKD before and after aspirin intervention.

Similarly, comparing the RKD Fijians with RKD Indo-Fijians, no statistically significant difference in PA was seen before and after aspirin.

4.6.1.1 NKD (before aspirin)

An independent \( t \)-test was use to compare the average platelet aggregation in Fijian NKD with the Indo-Fijians NKD before giving aspirin. No difference in PA was found between the two groups. The \( t \)-test was non significant \((t_{114} = 0.865, \ p=0.38, \ two \ tailed)\).

**Fig. 19:** Comparison of platelet aggregation PA \((Ω, \ \text{mean}±\text{sd})\) between the Fijian and Indo-Fijian NKD before aspirin

<table>
<thead>
<tr>
<th>Ethnicity</th>
<th>Fijians</th>
<th>Indo-Fijians</th>
<th>( p )-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean PA in ( Ω )</td>
<td>21.4 ± 3.9</td>
<td>20.9 ± 3.2</td>
<td>0.38</td>
</tr>
</tbody>
</table>

4.6.1.2 NKD (after 100mg)

No significant difference in PA aggregation was seen between the two ethnic groups after a single dose of 100mg aspirin \((t_{114}=1.669, \ p=0.098)\).
Fig 20: Comparison of PA (Ω, mean±sd) between the Fijian and the Indo-Fijian NKD after aspirin intervention

<table>
<thead>
<tr>
<th>Ethnicity</th>
<th>NKD (100mg) (mean±sd)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fijians</td>
<td>n=58</td>
<td>9.1±3.9</td>
</tr>
<tr>
<td>Indo-Fijians</td>
<td>n=58</td>
<td>10.3±3.9</td>
</tr>
</tbody>
</table>

4.6.1.3 NKD (after 300mg)

A significant difference in PA was seen between the two groups. The mean PA in Indo-Fijians (7.12 Ω), was significantly higher as compared to the Fijians (5.4 Ω) ($t_{114} = 2.450$, $p=0.016$).

Fig 21: Comparison of PA (Ω, mean±sd) between Fijian and Indo-Fijian NKD after 300mg aspirin

<table>
<thead>
<tr>
<th>Ethnicity</th>
<th>NKD (300mg) (mean±sd)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fijians</td>
<td>n=58</td>
<td>5.4±3.6</td>
</tr>
<tr>
<td>Indo-Fijians</td>
<td>n=58</td>
<td>7.1±4.1</td>
</tr>
</tbody>
</table>

4.6.2.1 OKD (before aspirin)

An independent t-test was use to compare the average platelet aggregation between Fijian OKD and the Indo-Fijian OKD before giving aspirin. The t-test was found to be statistically
non significant \((t_{116} = -0.948, p=0.345,\text{ two tailed})\). Both the Fijian and the Indo-Fijian OKD groups had similar pattern.

**Fig. 22:** Comparison of PA \((\Omega, \text{ mean} \pm \text{sd})\) between the Fijian and Indo-Fijian OKD before aspirin

| OKD (before aspirin) \((\text{mean} \pm \text{sd})\) |
|-----------------|-----------------|
| Fijians \(n=60\) | Indofijians \(n=58\) |
| 20.3 \(\pm 3.5\) | 20.9 \(\pm 3.1\) |
| \(p=0.345\)     |                  |

**4.6.2.2 OKD (after 100mg)**

With a mean of 10.3\(\Omega\) \((\pm 3.1)\) in the Indo-Fijians and 8.4 \(\Omega\) \((\pm 4.8)\) in the Fijians, the PA in the Indo-Fijians was found to be higher than the Fijians. A significant difference in PA aggregation was seen between the two ethnic groups \((t_{116} = 2.510, p = 0.013)\).

**Fig 23:** Comparison of PA \((\Omega, \text{ mean} \pm \text{sd})\) between the Fijian and the Indo-Fijian OKD after aspirin intervention

| OKD (100mg) \((\text{mean} \pm \text{sd})\) |
|-----------------|-----------------|
| Fijians \(n=60\) | Indo-Fijians \(n=58\) |
| 8.4 \(\pm 4.8\)  | 10.3 \(\pm 3.1\) |
| \(p=0.013\)      |                  |
4.6.2.3 OKD (after 300mg)

No significant difference in PA was seen between the two groups \( t_{116} = 1.767, p = 0.080 \).

**Fig 24**: Comparison of PA (Ω, mean±sd) between Fijian and Indo-Fijian OKD after 300mg aspirin

<table>
<thead>
<tr>
<th>PA (Ω, mean±sd)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>OKD (300mg)</strong></td>
<td></td>
</tr>
<tr>
<td><strong>(mean±sd)</strong></td>
<td></td>
</tr>
<tr>
<td>Fijians</td>
<td>Indo-Fijians</td>
</tr>
<tr>
<td>n=60</td>
<td>n=58</td>
</tr>
<tr>
<td>5.8</td>
<td>7.1</td>
</tr>
<tr>
<td>±4.5</td>
<td>±3.3</td>
</tr>
</tbody>
</table>

4.6.3.1 RKD (before aspirin)

An independent \( t \)-test was used to compare the average platelet aggregation between the Fijian RKD and the Indo-Fijian RKD before giving aspirin. The \( t \)-test was found to be statistically non-significant \( t_{106} = 0.358, p=0.721 \), two tailed. Both ethnic groups had similar PA.

**Fig. 25**: Comparison of PA (Ω, mean±sd) between the Fijian and Indo-Fijian RKD before aspirin

<table>
<thead>
<tr>
<th>PA (mean±sd)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>RKD (before aspirin)</strong></td>
<td></td>
</tr>
<tr>
<td><strong>(mean±sd)</strong></td>
<td></td>
</tr>
<tr>
<td>Fijians</td>
<td>Indo-Fijians</td>
</tr>
<tr>
<td>n=56</td>
<td>n=52</td>
</tr>
<tr>
<td>20.6</td>
<td>20.9</td>
</tr>
<tr>
<td>±3.9</td>
<td>±3.6</td>
</tr>
</tbody>
</table>

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4.6.3.2 RKD (after 100mg)

With a mean of 15.7 Ω (±4.4) in the Indo-Fijians and 15.3 Ω (±6.0) in the Fijians, the PA in the Indo-Fijians was found to be higher than the Fijians. A significant difference in PA aggregation was seen between the two ethnic groups ($t_{106} = 0.449$, $p = 0.654$).

**Fig 26**: Comparison of PA (Ω, mean±sd) between the Fijian and the IndoFijian RKD after aspirin intervention

<table>
<thead>
<tr>
<th>RKD (100mg) (mean±sd)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Fijians n=56</td>
<td></td>
</tr>
<tr>
<td>IndoFijians n=52</td>
<td></td>
</tr>
<tr>
<td>15.3 ±6.0</td>
<td></td>
</tr>
<tr>
<td>15.7 ±4.4</td>
<td></td>
</tr>
</tbody>
</table>

4.6.3.3 RKD (After 300mg)

No significant difference in PA was seen between the two ethnic groups ($t_{106} = 1.763$, $p = 0.081$).

**Fig 27**: Comparison of PA (Ω, mean±sd) between Fijian and Indo-Fijian RKD after 300mg aspirin

<table>
<thead>
<tr>
<th>RKD (300mg) (mean±sd)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Fijians n=56</td>
<td></td>
</tr>
<tr>
<td>Indo-Fijians n=52</td>
<td></td>
</tr>
<tr>
<td>6.9 ±4.7</td>
<td></td>
</tr>
<tr>
<td>8.5 ±4.6</td>
<td></td>
</tr>
</tbody>
</table>
4.7 Effect of Gender on platelet aggregation

The results were analyzed to determine if there is any difference in the PA between the males and the females of different groups before and after aspirin (100mg and 300mg) intervention.

Results showed that when PA in the females was compared with the PA in males, in the NKD Fijian group before aspirin, the females had a higher mean PA as compared to the males and the difference was statistically highly significant. No significant difference in PA was seen after aspirin intervention.

Similarly, comparing the PA in males and females of the OKD Fijian group, showed that there was a highly significant difference before aspirin, whereas, no significant difference in PA was seen between the males and the females after aspirin intervention.

In the RKD Fijian group, no statistically significant difference in PA was seen between the males and the males before and after aspirin intervention.

A highly significant difference was seen in the PA between the males and the females in the NKD Indo-Fijian group before and after 100mg and 300mg aspirin.

In the OKD Indo-Fijian group, the difference in PA between the males and females was significant before aspirin intervention only. The difference was non-significant after aspirin intervention.

There was no statistically significant difference in PA between males and females in the RKD Indo-Fijian group before and after aspirin intervention.

4.7.1. Fijians

4.7.1.1 NKD (before aspirin)

Though the PA was within the normal range in both the males and the females, the females had higher values (mean=23.5 Ω) as compared to the males (mean=19.6 Ω). This difference was found to be statistically highly significant ($t_{56} = 4.430$, two tailed, $p<0.001$).
Fig 28: Comparison of PA (Ω, mean±sd) between the male and female NKD Fijian group before aspirin intervention

![Box plot showing comparison of PA (Ω, mean±sd) between male and female NKD Fijian groups before aspirin intervention](image)

Fijian NKD (before aspirin) (mean±sd)

<table>
<thead>
<tr>
<th>Gender</th>
<th>Female</th>
<th>Male</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>27</td>
<td>31</td>
</tr>
<tr>
<td>23.5</td>
<td>19.6</td>
<td></td>
</tr>
<tr>
<td>±3.3</td>
<td>±3.4</td>
<td></td>
</tr>
</tbody>
</table>

p<0.001

4.7.1.2 NKD (After 100mg)

The PA in the females was comparatively higher than the males. The difference was statistically non-significant (t_{56} = -2.580, two tailed, p=0.13).

Fig 29: Comparison of PA (Ω, mean±se) between the male and female NKD Fijian group after 100mg aspirin

![Bar chart showing comparison of PA (Ω, mean±se) between male and female NKD Fijian groups after aspirin intervention](image)

Fijian NKD (100mg aspirin) (mean±sd)

<table>
<thead>
<tr>
<th>Gender</th>
<th>Female</th>
<th>Male</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>27</td>
<td>31</td>
</tr>
<tr>
<td>10.4</td>
<td>7.9</td>
<td></td>
</tr>
<tr>
<td>±4.2</td>
<td>±3.2</td>
<td></td>
</tr>
</tbody>
</table>

p=0.13
4.7.1.3 NKD (After 300mg)

There was no difference in PA between the male NKD and female NKD after 300mg aspirin. The results were statistically non-significant ($t_{56} = 0.644$, two tailed, $p=0.522$).

**Fig 30** Comparison of PA (Ω, mean±sd) between the male and female NKD Fijian group after 300mg aspirin

![Bar chart showing comparison of PA between male and female NKD Fijians after 300mg aspirin]

4.7.1.4 OKD (before aspirin)

With a mean PA of 18.6 Ω in males and 22.5 Ω in females, the PA was higher in females than males and result was highly statistically significant ($t_{58} = 5.063$, two tailed, $p<0.000$).

**Fig 31:** Comparison of PA (Ω, mean±sd) between the male and female OKD Fijians before aspirin

![Bar chart showing comparison of PA between male and female OKD Fijians before aspirin]
4.7.1.5 OKD (after 100mg)

Females had a higher PA as compared to the males. The difference was statistically non-significant ($t_{58} = -1.703$, two tailed, $p=0.094$).

**Fig 32:** Comparison of PA ($\Omega$, mean±sd) between the male and female OKD Fijians after 100mg aspirin

<table>
<thead>
<tr>
<th></th>
<th>Fijian OKD (100mg aspirin) (mean±sd)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Female $n=26$</td>
</tr>
<tr>
<td></td>
<td>9.6 ± 4.7</td>
</tr>
</tbody>
</table>

4.7.1.6 OKD (after 300mg)

The difference in PA between the males and the females of this group was statistically non-significant ($t_{58} = 0.808$, two tailed, $p=0.422$).

**Fig 33:** Comparison of PA ($\Omega$, mean±sd) between the male and female OKD Fijians after 300mg aspirin

<table>
<thead>
<tr>
<th></th>
<th>Fijian OKD (300mg aspirin) (mean±sd)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Female $n=26$</td>
</tr>
<tr>
<td></td>
<td>6.3 ± 4.2</td>
</tr>
</tbody>
</table>
4.7.1.7 RKD (before aspirin)

PA was compared between the females and the males of the RKD group. It was seen that the mean PA in the females (mean=20.10 Ω) was lower as compared to the PA in males (mean=21.91 Ω). The difference was found to be statistically non-significant ($t_{54} = -1.165$, $p=2.49$).

**Fig 34:** Comparison of PA (Ω, mean±sd) between the male and female RKD Fijian group before aspirin

<table>
<thead>
<tr>
<th></th>
<th>Fijian RKD (before aspirin)</th>
<th>p=2.49</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Female</td>
<td>Male</td>
</tr>
<tr>
<td>Male</td>
<td>n=21</td>
<td>n=35</td>
</tr>
<tr>
<td></td>
<td>20.1</td>
<td>20.9</td>
</tr>
<tr>
<td></td>
<td>±2.9</td>
<td>±4.3</td>
</tr>
</tbody>
</table>

4.7.1.8 RKD (after 100mg)

The difference in PA between the males and females in this group was found to be statistically non-significant ($t_{54} = 0.147$, two tailed, $p=0.884$).

**Fig 35:** Comparison of platelet aggregation PA (Ω, mean±sd) between the male and female RKD Fijian group after 100mg aspirin

<table>
<thead>
<tr>
<th></th>
<th>Fijian RKD (100mg aspirin)</th>
<th>p=0.884</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Female</td>
<td>Male</td>
</tr>
<tr>
<td>Male</td>
<td>n=21</td>
<td>n=35</td>
</tr>
<tr>
<td></td>
<td>15.1</td>
<td>15.3</td>
</tr>
<tr>
<td></td>
<td>±5.4</td>
<td>±6.4</td>
</tr>
</tbody>
</table>
4.7.1.9 RKD (after 300mg)
Mean PA was higher in the males as compared to the females in this group. The difference was statistically non-significant ($t_{54} = 1.491$, two tailed, $p=0.142$).

**Fig 36**: Comparison of PA (Ω, mean±sd) between the male and female RKD Fijian group after 300mg aspirin

<table>
<thead>
<tr>
<th></th>
<th>Fijian RKD (300mg aspirin) (mean±sd)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Female</strong></td>
<td><strong>Male</strong></td>
</tr>
<tr>
<td>n=21</td>
<td>n=35</td>
</tr>
<tr>
<td>5.8</td>
<td>7.7</td>
</tr>
<tr>
<td>±4.3</td>
<td>±4.8</td>
</tr>
</tbody>
</table>

p=0.142

4.7.2 Indo-Fijian

4.7.2.1 NKD (before aspirin)

The mean PA was comparatively higher in the females (22.2 Ω) as compared to the males (19.8 Ω). A significant difference in PA was found between the females and the males of this group ($t_{56} = 3.168$, p=0.002).

**Fig 37**: Comparison of PA between the male and female NKD Indo-Fijian group before aspirin

<table>
<thead>
<tr>
<th></th>
<th>NKD (before aspirin) (mean±sd)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Female</strong></td>
<td><strong>Male</strong></td>
</tr>
<tr>
<td>n=26</td>
<td>n=32</td>
</tr>
<tr>
<td>22.2</td>
<td>19.8</td>
</tr>
<tr>
<td>±2.8</td>
<td>±3.1</td>
</tr>
</tbody>
</table>

p=0.002
4.7.2.2 NKD (after 100mg aspirin)

With mean PA of 11.5 Ω, the females in this group had a higher PA than the males (9.3 Ω). The difference was statistically significant ($t_{56} = -2.214$, two tailed, $p=0.013$).

**Fig 38:** Comparison of PA between the male and female of NKD Indo-Fijian group after aspirin intervention

<table>
<thead>
<tr>
<th></th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>9.3 ± 3.1</td>
<td>11.5 ± 3.6</td>
</tr>
<tr>
<td>SD</td>
<td>5.6</td>
<td>3.9</td>
</tr>
<tr>
<td>N</td>
<td>32</td>
<td>26</td>
</tr>
</tbody>
</table>

4.7.2.3 NKD (after 300mg aspirin)

The females had a higher PA as compared to the males and the difference was statistically highly significant ($t_{56} = 3.544$, two tailed, $p=0.001$).

**Fig 39:** Comparison of PA between the male and female of NKD Indo-Fijian group after 300mg aspirin

<table>
<thead>
<tr>
<th></th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>5.6 ± 3.5</td>
<td>9.0 ± 3.9</td>
</tr>
<tr>
<td>SD</td>
<td>3.5</td>
<td>3.9</td>
</tr>
<tr>
<td>N</td>
<td>32</td>
<td>26</td>
</tr>
</tbody>
</table>
4.7.2.4 OKD (before aspirin)
The females had a higher PA than the males in this group and the results were statistically highly significant ($t_{56} = -5.173$, two tailed, $p=0.000$).

**Fig 40**: Comparison of PA between the male and female OKD Indo-Fijian group before aspirin

<table>
<thead>
<tr>
<th>OKD (before aspirin) (mean±sd)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Female</td>
<td>Male</td>
</tr>
<tr>
<td>n=23</td>
<td>n=35</td>
</tr>
<tr>
<td>23.0</td>
<td>19.5</td>
</tr>
<tr>
<td>±2.9</td>
<td>±2.4</td>
</tr>
</tbody>
</table>

4.7.2.5 OKD (after 100mg aspirin)
Mean PA in the females (11.0 Ω) was higher than the males (9.7) in this group. The difference was statistically non-significant ($t_{56} = -1.600$, two tailed, $p=0.115$).

**Fig 41**: Comparison of PA between the male and female OKD Indo-Fijian group after aspirin

<table>
<thead>
<tr>
<th>OKD (100mg aspirin) (mean±sd)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Female</td>
<td>Male</td>
</tr>
<tr>
<td>n=23</td>
<td>n=35</td>
</tr>
<tr>
<td>11.0</td>
<td>9.7</td>
</tr>
<tr>
<td>±2.3</td>
<td>±3.4</td>
</tr>
</tbody>
</table>
4.7.2.6 OKD (after 300mg aspirin)

In this group, mean PA in the females (6.87 Ω) was lower as compared to the males (7.17Ω). The difference was statistically non-significant.

**Fig 42:** Comparison of PA between the male and female OKD Indo-Fijian group after aspirin

<table>
<thead>
<tr>
<th>Gender</th>
<th>OKD (300mg aspirin) (mean±sd)</th>
<th>p &gt;0.05</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female</td>
<td>n=23</td>
<td>6.87±3.31</td>
</tr>
<tr>
<td>Male</td>
<td>n=35</td>
<td>7.17±3.26</td>
</tr>
</tbody>
</table>

4.7.2.7 RKD (before aspirin)

The PA values were higher in the males (21.6 Ω) as compared to the females (19.8 Ω). No statistically significant difference in PA was seen between the males and the females of this group ($t_{50} = -1.792, p=0.079$).

**Fig 43:** Comparison of PA between the male and the female of the RKD Indo-Fijian group before aspirin

<table>
<thead>
<tr>
<th>Gender</th>
<th>RKD (before aspirin) (mean±sd)</th>
<th>p=0.079</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female</td>
<td>n=20</td>
<td>19.8±3.5</td>
</tr>
<tr>
<td>Male</td>
<td>n=32</td>
<td>21.6±3.6</td>
</tr>
</tbody>
</table>
4.7.2.8 RKD (after 100mg aspirin)
The mean PA in the females (15.6 Ω) was almost similar to the PA in the males (15.8 Ω). No reduction in PA was seen after aspirin intervention. The PA was within the normal range (15-27 Ω). The results were statistically non-significant ($t_{50} = 0.142$, two tailed, $p=0.888$).

Fig 44: Comparison of PA between the male and the female of the RKD Indo-Fijian group after aspirin.

<table>
<thead>
<tr>
<th></th>
<th>RKD (100mg aspirin) (mean+sd)</th>
<th>$p$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female n=20</td>
<td>15.6 ± 3.7</td>
<td>0.888</td>
</tr>
<tr>
<td>Male n=32</td>
<td>15.8 ± 4.9</td>
<td></td>
</tr>
</tbody>
</table>

4.7.2.9 RKD (after 300mg aspirin)
PA was inhibited in both the males and the females of this group. No statistically significant difference was seen between the males and the females after 300mg aspirin ($t_{50} = 0.577$, two tailed, $p=0.567$).

Fig 45: Comparison of PA between the male and the female of the RKD Indo-Fijian group after 300mg aspirin.

<table>
<thead>
<tr>
<th></th>
<th>RKD (300mg aspirin) (mean+sd)</th>
<th>$p$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female n=20</td>
<td>8.1 ± 3.9</td>
<td>0.567</td>
</tr>
<tr>
<td>Male n=32</td>
<td>8.8 ± 4.9</td>
<td></td>
</tr>
</tbody>
</table>
4.8. Body Mass Index (BMI) and Platelet aggregation

The height and weight of the all the participants were recorded and BMI was calculated to rule it out as a confounding factor affecting PA. The participants in each group (NKD, OKD and RKD) were divided into 3 groups based on their BMI. PA was measured in both the Fijians and the Indo-Fijians before aspirin intervention.

Table 12: BMI reference values

<table>
<thead>
<tr>
<th>BMI</th>
<th>Weight Status</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>group I</td>
<td>Below 18.5</td>
<td></td>
</tr>
<tr>
<td>group II</td>
<td>18.5 – 24.9</td>
<td></td>
</tr>
<tr>
<td>group III</td>
<td>25.0 – 29.9</td>
<td></td>
</tr>
</tbody>
</table>

Ref: CDC manual

4.8.1 Fijians

The participants in each group (NKD, OKD and RKD) were divided into three groups based on their BMI as mentioned in the table above. A one way between groups ANOVA was used to investigate any significant difference in PA between the three BMI groups.

The ANOVA was not statistically significant indicating that there is no significant difference in PA among the three groups of BMI. The PA in all the three groups of BMI in each category (NKD, OKD and RKD) was within the normal range (15-27 Ω). Thus PA was found to be similar in the different BMI groups.

4.8.1.1 NKD

There was statistically no significant difference in PA between the different BMI groups. All the groups had normal PA (15-27 Ω).

Table 13: PA in different BMI groups of the NKD

<table>
<thead>
<tr>
<th>BMI</th>
<th>Mean±Sd</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>group I (n=21)</td>
<td>20.7±3.8</td>
<td></td>
</tr>
<tr>
<td>group II (n=17)</td>
<td>20.7±3.7</td>
<td></td>
</tr>
<tr>
<td>group III (n=20)</td>
<td>22.9±3.8</td>
<td></td>
</tr>
<tr>
<td>Total (n=58)</td>
<td>21.4±3.9</td>
<td>0.108</td>
</tr>
</tbody>
</table>

PA in NKD (Fijians)

![Graph showing PA in different BMI groups of the NKD]
4.8.1.2 OKD

Statistically no significant difference in PA was seen between the different BMI groups.

**Table 14: PA in different BMI groups of OKD**

<table>
<thead>
<tr>
<th>BMI</th>
<th>Mean ± Sd</th>
<th>p=0.629</th>
</tr>
</thead>
<tbody>
<tr>
<td>group I (n=12)</td>
<td>20.4±3.8</td>
<td></td>
</tr>
<tr>
<td>group II (n=20)</td>
<td>20.9±4.1</td>
<td></td>
</tr>
<tr>
<td>group III (n=28)</td>
<td>19.9±2.9</td>
<td></td>
</tr>
<tr>
<td>Total (n=60)</td>
<td>20.3±3.5</td>
<td></td>
</tr>
</tbody>
</table>

4.8.1.3 RKD

Statistically no significant difference in PA was seen between the BMI groups.

**Table 15: PA in different BMI groups of RKD**

<table>
<thead>
<tr>
<th>BMI</th>
<th>Mean ± Sd</th>
<th>p=0.097</th>
</tr>
</thead>
<tbody>
<tr>
<td>group I (n=18)</td>
<td>20.7±4.4</td>
<td></td>
</tr>
<tr>
<td>group II (n=19)</td>
<td>21.9±3.6</td>
<td></td>
</tr>
<tr>
<td>group III (n=19)</td>
<td>19.2±3.3</td>
<td></td>
</tr>
<tr>
<td>Total (n=56)</td>
<td>20.6±3.9</td>
<td></td>
</tr>
</tbody>
</table>

4.8.2 Indo-Fijian

The participants in each group (NKD, OKD and RKD) were divided into three groups based on their BMI as mentioned in table 15 above. A one way between groups ANOVA was used to investigate any significant difference in PA between the three BMI groups. The ANOVA was not statistically significant indicating that there is no significant difference in PA among the three groups of BMI. The PA in all the three groups of BMI in each category (NKD, OKD and RKD) was within the normal range (15-27 Ω). Thus PA was found to be similar in the different BMI groups.
4.8.2.1 NKD

No significant difference in PA was seen between the different BMI groups.

**Table 16**: PA in different BMI groups of NKD

<table>
<thead>
<tr>
<th>BMI</th>
<th>Mean±Sd</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>group I  (n=20)</td>
<td>20.7±3.2</td>
<td>0.928</td>
</tr>
<tr>
<td>group II (n=15)</td>
<td>21.1±3.3</td>
<td></td>
</tr>
<tr>
<td>group III (n=23)</td>
<td>20.9±3.3</td>
<td></td>
</tr>
<tr>
<td>Total    (n=58)</td>
<td>20.9±3.2</td>
<td></td>
</tr>
</tbody>
</table>

4.8.2.2 OKD

No difference in PA was seen between the different BMI groups.

**Table 17**: PA in different BMI groups of OKD

<table>
<thead>
<tr>
<th>BMI</th>
<th>Mean±Sd</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>group I  (n=10)</td>
<td>20.6±2.2</td>
<td>0.733</td>
</tr>
<tr>
<td>group II (n=28)</td>
<td>21.3±3.4</td>
<td></td>
</tr>
<tr>
<td>group III (n=19)</td>
<td>20.6±3.3</td>
<td></td>
</tr>
<tr>
<td>Total    (n=57)</td>
<td>20.9±3.1</td>
<td></td>
</tr>
</tbody>
</table>
4.8.2.3 RKD

No difference in PA was seen between the BMI groups.

**Table 18:** PA in different BMI groups of RKD

<table>
<thead>
<tr>
<th>BMI</th>
<th>Mean+SD</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>group I</td>
<td>22.3±3.3</td>
<td>0.276</td>
</tr>
<tr>
<td>group II</td>
<td>20.6±4.4</td>
<td></td>
</tr>
<tr>
<td>group III</td>
<td>20.3±2.6</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>20.9±3.6</td>
<td></td>
</tr>
</tbody>
</table>

4.9 Smoking/Non-smoking status and platelet aggregation

PA was analysed in all the participants (smokers and non-smokers) before aspirin intervention to see if there is any difference in PA between smokers and non-smokers and to rule out smoking as a variable affecting PA in this study.

4.9.1 Fijians

Each group (NKD, OKD and RKD) of the Fijian participants were divided into two groups of smokers and non-smokers. PA was analysed in each group (NKD, OKD and RKD) between the smokers and non-smokers before aspirin intervention. Results showed that there is no difference in PA between the smokers and non-smokers belonging to the three groups. The results were found to be statistically non-significant.

4.9.1.1 NKD

**Table 19:** Comparison of PA between the smokers and non-smokers belonging to the NKD group

<table>
<thead>
<tr>
<th>Smoker (ns)/non-smoker (s)</th>
<th>Mean+SD</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>ns (n=46)</td>
<td>21.1±4.0</td>
<td>0.186</td>
</tr>
<tr>
<td>s (n=12)</td>
<td>22.8±2.8</td>
<td></td>
</tr>
</tbody>
</table>
4.9.1.2 OKD

Table 20: Comparison of PA between the smokers and non-smokers belonging to the OKD group

<table>
<thead>
<tr>
<th>Smoker (ns)/non-smoker (s)</th>
<th>Mean+Sd</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>ns (n=45)</td>
<td>20.0±3.6</td>
<td>0.254</td>
</tr>
<tr>
<td>s (n=15)</td>
<td>21.2±3.3</td>
<td></td>
</tr>
</tbody>
</table>

4.9.1.3 RKD

Table 21: Comparison of PA between the smokers and non-smokers belonging to the RKD group

<table>
<thead>
<tr>
<th>Smoker (ns)/non-smoker (s)</th>
<th>Mean+Sd</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>ns (n=21)</td>
<td>20.2±3.5</td>
<td>0.536</td>
</tr>
<tr>
<td>s (n=35)</td>
<td>20.9±4.1</td>
<td></td>
</tr>
</tbody>
</table>

4.9.2 Indo-Fijians

Each group (NKD, OKD and RKD) of the Indo-Fijian participants was divided into two groups of smokers and non-smokers. PA was analysed in each group (NKD, OKD and RKD) between the smokers and non-smokers before aspirin intervention. A slightly significant \((p \leq 0.05)\) difference in PA was seen between the smokers and non-smokers of the NKD and RKD group. Mean PA in the smokers was found to be slightly higher than the non-smokers. The results of both these groups were statistically significant. No difference in PA was seen between the smokers and the non-smokers belonging to the OKD group and the results were statistically non-significant.
4.9.2.1 NKD

Table 22: Comparison of PA between the smokers and non-smokers belonging to the NKD group

<table>
<thead>
<tr>
<th>Smoker (ns)/non-smoker (s)</th>
<th>Mean±Sd</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>ns (n=35)</td>
<td>20.2±3.1</td>
<td>0.05</td>
</tr>
<tr>
<td>s (n=23)</td>
<td>21.9±3.2</td>
<td></td>
</tr>
</tbody>
</table>

4.9.2.2 OKD

Table 23: Comparison of PA between the smokers and non-smokers belonging to the OKD group

<table>
<thead>
<tr>
<th>Smoker (ns)/non-smoker (s)</th>
<th>Mean±Sd</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>ns (n=33)</td>
<td>20.3±2.8</td>
<td>0.126</td>
</tr>
<tr>
<td>s (n=25)</td>
<td>21.6±3.4</td>
<td></td>
</tr>
</tbody>
</table>

4.9.2.3 RKD

Table 24: Comparison of PA between the smokers and non-smokers belonging to the RKD group

<table>
<thead>
<tr>
<th>Smoker (ns)/non-smoker (s)</th>
<th>Mean±Sd</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>ns (n=21)</td>
<td>19.5±3.4</td>
<td>0.021</td>
</tr>
<tr>
<td>s (n=31)</td>
<td>21.8±3.5</td>
<td></td>
</tr>
</tbody>
</table>
4.10 Correlation between age and platelet aggregation study.

4.10.1 Fijian Volunteers:
Pearson correlation showed that age is not correlated with PA in Fijian NKD before aspirin \((r=0.150, p\geq 0.05)\), after 100mg \((r = -0.192, p\geq 0.05)\), and after 300mg \((r = -0.096, p\geq 0.05)\).

Pearson correlation showed that age is not correlated with PA in Fijian OKD before \((r=0.134, p\geq 0.05)\), after 100mg aspirin \((r = 0.012, p\leq 0.05)\) and after 300mg \((r = -0.124, p\geq 0.05)\).

Pearson correlation showed that age is not correlated with PA in Fijian RKD before \((r=0.262, p\geq 0.05)\), after 100mg aspirin \((r = 0.200, p\geq 0.05)\), and after 300mg \((r = 0.182, p\geq 0.05)\).

4.10.2 Indo-Fijian Volunteers:
Pearson correlation revealed that age is highly correlated with PA in Indo-Fijian NKD before aspirin \((r=0.566, p\leq 0.01)\). However there is no correlation after 100mg \((r = 0.110, p\geq 0.05)\), and after 300mg aspirin \((r = 0.179, p\geq 0.05)\).

Pearson correlation showed that age is significantly correlated with PA in Indo-Fijian OKD before \((r=0.403, p\leq 0.01)\), and significantly negatively correlated after 100mg aspirin \((r = -0.286, p\leq 0.05)\). However, no correlation was found after 300mg aspirin \((r = -0.243, p \geq 0.05)\).

Pearson correlation exhibited that age is significantly correlated with PA in Indo-Fijian RKD before aspirin \((r=0.313, p\leq 0.05)\), however, no correlation is seen after 100mg aspirin \((r = -0.129, p\geq 0.05)\), and after 300mg aspirin \((r = -0.058, p\geq 0.05)\).

These results are now discussed in the next chapter in relation to the available literature.
CHAPTER 5: DISCUSSION AND CONCLUSION

5.1 Introduction

This chapter describes the results considering following aspects:
1) Effect of kava on platelet aggregation
2) Effect of two different doses of aspirin on platelet aggregation in non-kava drinkers and kava drinkers
3) Aspirin resistance
4) Ethnic variations in platelet aggregation
5) Gender difference in platelet aggregation
6) Correlation between age and platelet aggregation

5.2 Effect of kava on platelet aggregation

In this study, PA was analysed in the non-kava drinkers and kava drinkers (Fijians and Indofijians) before and after the intake of a single dose of 100mg and 300mg of aspirin. Results showed that the intake of kava by the Fijians and Indofijians did not have any effect on platelet aggregation. Platelet aggregation was within the normal range in the KD and NKD. A decrease in PA was seen after the ingestion of 100mg of aspirin in NKD, occasional kava drinkers (OKD) and regular kava drinkers (RKD), assessed by the whole blood platelet aggregometer using collagen as the aggregating agent. The most important finding of this study is that 100mg aspirin had a significantly less inhibitory effect on PA in both Fijian and Indo-Fijian RKD, and these subjects have been referred to as aspirin resistant or aspirin non-responders.

The platelet aggregation was reduced to the same extent in all the groups after the administration of 300mg of aspirin.

5.2.1 Before ingestion of 100mg of aspirin

Herbal drugs have been increasingly popular among patients and physicians. This is due to the perception that drugs derived from plants are generally safe and devoid of side effects. However numerous studies have reported adverse effects by plants containing alkaloids that
are finally biotransformed to toxins. Because a combination of herbs are administered together in many cases, the identification of the crucial compound that is either therapeutic or causes toxicity almost impossible and this seems to be a constant problem in herbal medicine in particular traditional Chinese and Indian herbal medicine (Beckert et al., 2007). Numerous interactions between herbal medicines and conventional drugs have been documented. While the significance of many interactions is uncertain, several interactions may have serious clinical consequences. Kava (Piper methysticum) increases the clearance of chlorzoxazone (a CYP2E1 substrate) and may interact with alprazolam, levodopa and paroxetine (Izzo and Ernst, 2009).

Kava root (Piper methysticum rhizome) is a popular tradition remedy used for its psychotropic effects in Hawaii, Polynesia and Fiji Islands. In industrialized countries kava containing preparations are marketed for the treatment of anxiety disorders and depression. The potency of the kava drink can vary greatly depending on the proportions and potency of kava lactones in the plant variety used, the method of preparation, and the degree of dilution in the preparation process (Kelly, 2006; Jones, 2004). The degree of dilution affects the potency of kava preparation. The effects of kava may also depend on how it is consumed in terms of whether it is used concomitantly with other drugs, food alcohol or physical activity. In our study the kava drinkers were classified based on the frequency of kava consumption as occasional and regular kava drinkers. The type of kava consumed by the participants or its preparation was not taken into consideration in our studies. Though the history of any other drug use was noted and those participants were excluded from the study.

Kava is a complex mixture of substances referred to as kava lactones and although its chemistry and pharmacology have been well studied, the physiological effects of the individual constituent pyrones and alkaloids are not well understood. The most symptomatic effect seen in chronic kava drinkers is the appearance of dry and scaly skin with yellow or white discoloration known as kani (Stickel et al., 2003). This condition develops in people who regularly i.e. almost daily consume of kava and it takes from a few months to a year to develop. It is readily reversible by decreasing kava intake. In this study, presence of kani was observed in most of the regular kava drinking volunteers.

Kava is also one of the most widely used botanical supplements in the United States and Europe. The reasons for its popularity are its reported health benefits, which include effects
on cognitive function in older adults and peripheral blood circulation among patients with PAD (Thompson et al., 2004; Cairney et al., 2002). Several mechanisms have been postulated for these health benefits, all of which are considered to be due to the kava lactones. Some of the potential actions of kava lactones include mild sedation, a slight numbing of the gums and mouth, and vivid dreams. Kava has been reported to improve cognitive performance and promote a cheerful mood (Thompson et al., 2004). Kava has similar effects to benzodiazepine medications including muscle relaxant, anaesthetic, anticonvulsive and anxiolytic effects. They are thought to result from direct interactions of kavalactones with voltage-gated ion channels (Cairney et al., 2002). Research currently suggests that kava lactones potentiate GABA A activity but do not alter levels of dopamine and serotonin in the CNS (Hunter, 2006; Cairney et al., 2002).

The line of investigation that is particularly relevant to our current study, regarding mechanisms, has linked kava to a decrease in platelet aggregation. However, we are not aware of the doses of kava constituents required to decrease platelet aggregation in whole blood studies. Our study did not analyse the kava constituents or the effect of individual kava lactones on platelet aggregation.

Mathews (1988) undertook research which was perhaps the first rigorous study into the health effects of kava. The paper reported a systematic survey of the physical health of heavy users of kava and matched control subjects in a coastal community in Arnhem Land. Indigenous people living in Arnhem Land were interviewed regarding their levels of kava consumption. The community health workers confirmed the participants’ reports of kava consumption. A variety of measurements were used to determine the effects of the long-term usage of large doses of kava. The findings demonstrated that the majority of the participants who experienced a number of health effects were either heavy or very heavy users of kava. According to this study, increased kava consumption was associated with an increased red cell volume (normal 76-96fl) and mean corpuscular hemoglobin (normal 27-32pg/RBC) and with decreased platelet volume (normal 7.4-10.4 fl) however all the values observed were within the range of normal variation. As compared to the study described above, in our study, the packed cell volume and the platelet count was estimated in each of the participants and was found to be within the normal range. Consumption of kava had not affected the red cell count and the platelet count. The participants who showed a decrease in the platelet and red
cell count were excluded from this study (2 participants). Platelet aggregation was also found to be within the normal range in the NKD, OKD and RKD, Fijian and Indo-Fijian participants.

The mechanisms of the effect of kava on platelet aggregation can only be speculated due to paucity of research done on this subject. We can compare our results with only one study carried out by Gleitz et al. (1997). In their study the kava pyrone, kavain was investigated for its possible antithrombotic action on platelets. This study showed that an addition of arachidonic acid to human platelets induced an aggregation of almost 90% within about 3 minutes which was detected turbidimetrically by an increase in light transmission. Arachidonic acid applied exogenously to the platelets was reportedly metabolized by COX to prostaglandins and thromboxane A2. Binding of TXA2 to its receptors causes an increase of cytosolic Ca2+ which triggers exocytosis of inducers like ATP and PAF thus amplifying aggregation of platelets. In this study kavain dose dependently suppressed aggregation of human platelets, the release of endogenous ATP, and the formation of PGE2 and TXB2, which are usually detected to estimate the activity of COX and TXS.

In contradiction to the study by Gleitz et al. (1997) results of our study showed that kava did not affect platelet aggregation. The difference might be due to the method employed and the aggregating agent used. In our study measurement of platelet aggregation was done by using the whole blood platelet aggregometer and the aggregating agent used was collagen. Gleitz et al. (1997) used turbidimetric method on washed platelets with arachidonic acid as a platelet aggregation aggregating. Measurement of platelet aggregation by using the whole blood platelet aggregometer is more physiological in clinical set up, as it takes into consideration the other components of blood like erythrocytes leucocytes and other biomolecules which are known to affect platelet function.

A case control study by Clough et al. (2004) was undertaken to determine the association between kava use and IHD in aboriginal communities in Eastern Arnhem Land (Northern Australian territory). Results showed that there is no clear evidence for an association between kava use and IHD. In Fiji the incidences of IHD is high especially amongst the Indo-Fijian population. As platelet reactions contribute to thrombus formation and inhibition of platelets is important for preventing IHD, platelet aggregation was therefore estimated in this study and it was found to be normal in the kava drinkers (both RKD and OKD). Therefore,
explanations regarding the reported high incidence of IHD in the Indo-Fijian population could be based upon supposedly biological as well as social differences that might be able to explain the differences in disease patterns.

5.2.2 Post Aspirin Platelet Aggregation

After a dose of 100mg of aspirin, platelet aggregation was inhibited in both the non-kava drinking and the kava drinking groups (OKD, RKD) in Fijian and Indo-Fijian population. Our results showed that a large number of participants in the Fijian RKD population did not have a reduced platelet aggregation after 100mg of aspirin (33/56). Similar results were seen in the Indofijian RKD group (35/50). Platelet aggregation was found to be within the normal range in these participants even after the ingestion of a single dose of 100mg of aspirin. Since this study has been done for the first time and there are no studies to compare with, we can only hypothesize and speculate the mechanisms which might have lead to this result.

Literature shows that regular consumption of certain nonsteroidal anti-inflammatory drugs (NSAIDs), such as ibuprofen appear to antagonize the antiplatelet effects of aspirin (Sanderson et al., 2005). Aspirin irreversibly acetylates a serine residue at position 529 in COX-1, preventing arachidonic acid from reaching the binding site. However, certain NSAIDs can block the access of aspirin to the COX-1 binding site by occupying the nearby catalytic site, thus preventing aspirin from gaining access to its target serine (Catella-Lawson, 2001). This interaction is potentially important because many patients taking aspirin may also take NSAIDs for other conditions. Similarly, since kava lactones have been known to have so many different effects on the body, it might be possible that there may be some interaction between kava and platelets which might then prevent aspirin from inhibiting the COX 1 enzyme, an action similar to NSAIDs due to which aspirin is unable to inhibit the platelet aggregation in the regular kava drinkers even after the ingestion of aspirin. Because aspirin has a short half life (15-20minutes) in the human circulation and since only 10% of the platelet pool is replenished each day (Patrignani et al., 1999), it is possible that constant exposure of platelets to kava would lead to a strong kava-platelet interaction preventing the action of aspirin on platelets in the regular kava drinkers.

The studies conducted by Mathews (2002) elucidate that the constituents of kava may interact synergistically, and therefore help explain and predict interactions with drugs with which kava is taken concomitantly. Their studies show that some of the kava pyrones block several
subtypes of the enzyme cytochrome P450, (Mathews et. al., 2002), which can result in adverse interactions with other drugs used concomitantly. These data indicate that kava has a high potential for causing drug interactions through inhibition of P450 enzymes responsible for the majority of the metabolism of pharmaceutical agents.

After administration of a single dose of 300mg of aspirin to the participants who did not respond to 100mg or showed normal platelet aggregation, an inhibition of platelet aggregation was seen. Though we are not sure of the exact mechanism, probably this can be explained on the basis of competitive binding mechanism. A stronger dose of aspirin would be capable of displacing kava and binding to that site and inhibiting the COX 1 enzyme. Various studies using two doses of aspirin (Pederson and Fitzgerald, 1984; Benedek et al., 1995; Cox et al., 2006) have shown that in some patient’s dose higher than 100mg is more effective in inhibiting platelet aggregation. Finally, it is theoretically possible that polymorphisms and/or mutations in the COX-1 gene affecting Ser529 may represent the structural basis for aspirin resistance seen in the RKD, although this hypothesis also remains to be tested. Non-steroidal anti-inflammatory drugs, particularly aspirin, have the potential to interact with herbal supplements which possess antiplatelet activity. Health-care professionals should be aware of the potential adverse interactions between herbal supplements and analgesic drugs. Further research is needed to confirm and assess the clinical significance of these potential interactions (Abebe, 2002).

**Conclusion:** Further research is also needed to discover whether there is a direct link between the biochemical or laboratory aspirin resistance and clinical outcome especially in the regular kava users. At present there is no indication of the biochemical pathways by which kava might be influencing platelet function.

Based on the results this study definitely raises the concern about the dose of aspirin required to be administered in kava drinkers with cardiovascular diseases. Intense study needs to be done is to discover the mechanisms of the effect of kava on the platelets and whether these effects are reversed when the usage of kava ceases. Kava extracts or their components have important implications for both purported benefits and adverse effects on health. Kava preparation and extracts are very popular in the Pacific as well as western society. An
important conclusion is that the potential remains for herbal medicines like kava to interact negatively with other drugs in vivo which needs to be thoroughly explored.

**Limitations:** A weak point of our study is that we did not know the type of kava which the participants have consumed. There are currently dozens of different types of kava that are commercially available, and among these different products there can be substantial compositional differences as well as differences in bioavailability. Our study also did not quantitate accurately the levels of kava that were consumed by the subjects. The wide variation in the estimated consumption within each category reflects the difficulties of estimation and individual variations in daily consumption as well as real difference in long term consumption.

The results and conclusions are limited to the specific methodology using the whole blood aggregometer and platelet aggregation tests that were conducted with a selected agonist used. But there are other measures of platelet function and coagulation not assessed in this study. The tests we used, however, are commonly employed in clinical practices designed to investigate platelet aggregation.

### 5.3 Effect of two different doses of aspirin (100mg and 300mg) on platelet function

Our studies showed that ingestion of a single dose of aspirin did not completely inhibit platelet aggregation in the NKD and OKD of both the groups, Fijian and Indofijian. A further reduction in platelet aggregation was seen after the administration of 300mg of aspirin. A large number of participants in the RKD group were found to be aspirin resistant after 100mg of aspirin. Platelet aggregation was inhibited in them after a single dose of 300mg of aspirin. Cardiovascular diseases are a common cause of death and morbidity in western countries. Aspirin is the single most important drug prescribed for the secondary prevention of atherothrombotic disease. Its effectiveness has been well established through a number of meta-analyses and clinical trials. It is an antiplatelet agent that inhibits platelet COX-1 and, as a result, prevents the formation of TXA₂. Aspirin irreversibly acetylates COX-1 in platelets, thereby preventing arachidonic acid from reaching the enzyme's binding and catalytic sites (Hoogendijk and Cate, 1990). This eventually results in reduced prostaglandin biosynthesis for the platelet's lifetime of about 8 to 10 days and therefore reduces the production of
thromboxane A2. Since COX-1 inhibition in platelets is irreversible, regular low doses of aspirin lead to more than 95% suppression of thromboxane A2 generation. As platelets are anucleate and cannot resynthesize COX-1, after a single dose of aspirin, platelet COX-1 activity recovers by about 10% per day, in line with platelet turnover.

The discussion regarding the correct dosage of aspirin for platelet inhibition seems to be never ending. Meta-analysis of randomized trials of antiplatelet therapy did not reveal any greater benefit from doses which were greater than 350 mg as compared with lower doses. Therefore, it has been widely accepted that high doses of 500-1500 mg aspirin daily are no more effective than medium doses of 160-325 mg/day or low doses of 75-150 mg/day (Anonymous 2002). However, there are a large scale of randomized clinical studies comparing the medium (160-325) and low dose (75-150) aspirin regimens. Low dose of aspirin is preferred over medium or larger doses because of fewer gastrointestinal side effects and undesired cyclooxygenase inhibition of the vascular wall (Weksler et. al., 1983 and Hampton et. al., 1990). Although the results of the previous studies demonstrate the preventive effects of low or medium doses of aspirin, there may be no ideal dose of aspirin for all patients. If an optimal dose of aspirin for a particular patient can be known, a greater proportion of patients may actually benefit from aspirin because the sensitivity of platelets to aspirin differs between patients. Whether individualized aspirin dosage is superior remains an open question and, there is no study to detect the difference between individualized aspirin dosage and the fixed low dose aspirin. On the other hand platelet function tests may be used only to differentiate those patients most likely to benefit from a higher aspirin dosage.

Our results showed that aspirin at 100 mg, single dose, was effective in the inhibition of platelet function as assessed by the whole blood platelet aggregometer in majority of the volunteers. However, there was a further increase in platelet inhibition with higher aspirin in subjects, who have not achieved the desired level of antiplatelet effects from 100 mg aspirin daily. Our results support the study done by Abacia et al. (2005), who measured the effect of increasing doses of aspirin on platelet function by PFA in patients with diabetes. In the study by Alp et. al. (2010) the overall prevalence of incomplete platelet inhibition (22%) was consistent with previous data (24-28%) (Morimoto et al., 2007), and there were significant correlations between incomplete inhibition of platelets and aspirin dose. Their study was designed to measure the effectiveness of two widely used aspirin regimens (100 mg vs. 300 mg) by the PFA-100 and the authors found that 300 mg was more effective to inhibit
platelets. In a study using the Verify Now test (test to measure platelet aggregation), a higher incidence of incomplete platelet inhibition was found in patients taking lower doses of aspirin (≤100 mg). In the ASPECT study, Gurbel and Bliden, 2007, investigated the effect of different doses of aspirin (81, 162, and 325 mg/day) on platelet responsiveness to aspirin in 120 patients with stable coronary artery disease using light transmittance aggregometry, Verify Now, PFA-100, and levels of urinary 11-dehydro-thromboxane B2. Statistically significant differences were observed between different aspirin dose groups. In a subgroup analysis of the ASPECT study, DiChiara and Bliden, 2007, demonstrated that diabetic patients with coronary artery disease had a higher prevalence of incomplete platelet inhibition during therapy with 81 mg and that increasing the dose of aspirin in diabetic patients reduced platelet resistance. Recently in the JPAD trial, low-doses of aspirin were not effective for primary prevention of atherosclerotic events in patients with type 2 diabetes mellitus (Mori et al., 1992).

Helgason et al. (1994) showed that inhibition of platelet function is dose dependent in patients taking aspirin for stroke prevention and increase of aspirin dose resulted in complete inhibition of platelet function in more patients.

Tohgi et al. (1992) showed that with higher doses, platelet aggregability and thromboxane A2 production were inhibited more conspicuously and in a greater proportion of stroke patients. Recent studies have also shown that aspirin inhibited platelet function in a dose dependent manner in patients with stable coronary artery disease or stroke. To our knowledge, there is no study comparing two different doses of aspirin on platelet function in kava drinkers and non kava drinkers. In our study, although 100 mg aspirin was effective for the majority of non kava drinking and occasional kava drinking volunteers, greater statistically significant platelet inhibition was seen with the use of 300 mg aspirin. In accordance with the results of our study, Watala et al. (2004) have recently shown that the inhibitory effect of 150 mg aspirin a day on platelet function is less profound in diabetic patients compared to non-diabetic individuals and have suggested that at least some patients with diabetes might require higher aspirin doses. Indeed, the results of the primary prevention project trial suggested that low-dose aspirin might be less effective in primary prevention of cardiovascular disease in diabetic patients as compared to non-diabetics. In our study we used collagen as an aggregating agent, however, the measurement of platelet deposition on
collagen monomers might be influenced by the fact that it is mostly GP Ia/IIa-dependent and thus does not entirely correspond to the normal function of platelets. The large individual variability in platelet functions could also partly explain differences in responses to antiplatelet medication. Individual variability in platelet functions has been postulated to be associated with increased risk for atherothrombotic events (Michelson, 2004).

Despite aspirin being proven as an effective drug in the prevention of acute thrombotic events, its mechanism of action remains controversial. The evidence on its inability to inhibit in vitro platelet aggregation to a variety of stimuli were recorded by Oates et al. (1988) and Taylor et al. (1992). The results demonstrated that while aspirin at all concentrations inhibited aggregation induced by arachidonic acid, it failed to inhibit thrombin-induced aggregation. Data from comparable studies demonstrates the poor efficacy of aspirin in inhibiting platelet aggregation induced by collagen and platelet activating factor (PAF) (Taylor et al., 1992). The observed delay in thrombin induced aggregation in the presence of aspirin may be due to a reduction in thromboxane production. Since our study used collagen as an aggregating agent, probably it is due to the use of this aggregating agent that incomplete inhibition was seen on administration of low dose aspirin. The weak effect of acetylsalicylic acid on collagen induced aggregation has also been described previously (Taylor et al., 1992) and is believed to be due to the ability of the impedance method to detect even very small platelet aggregates which may still form in the presence of acetylsalicylic acid. In our study we used the impedance method for measurement of platelet aggregation.

It has been also postulated that since aspirin blocks only one of the several pathways by which platelet aggregation can occur, aspirin is a weak antiplatelet agent because platelet aggregation can be stimulated via another pathway. There is ongoing debate regarding the minimum effective dose of aspirin required for antithrombotic efficacy and the suggestion that part of the antithrombotic effect of aspirin maybe unrelated to inhibition of platelet TXA₂ (Patrono et al., 2001).

Though the results of our study showed that low dose aspirin (100mg) did not completely inhibit platelet aggregation in most of the participants, there are both theoretical and practical reasons to choose the lowest effective dose of aspirin. Increasing dosage of aspirin leads to a number of GI (gastrointestinal) side effects which need to be considered as in many clinical situations, treatment with aspirin is indicated for an indefinite period. Therefore use of the
lowest effective dose (i.e. 50 to 100 mg daily for long-term treatment) is probably the most sensible strategy to maximize efficacy and minimize toxicity.

In contrast to our studies, it has been suggested that dose-dependent effects for aspirin is indirect and is inconsistent with the failure to show a dose effect in randomized clinical trials and in the overview analysis of the Antithrombotic Trialists. Failure to show a dose effect has always been a point of debate and discussion because it correlates with the satureability of the effect of aspirin on the platelet COX-1. In studies with purified enzyme using isolated platelets, nanomolar concentrations of aspirin completely blocked prostaglandin synthesis within 20 min after exposure (Roth et al., 1975). So it has been suggested that higher concentrations of aspirin and longer exposures will not alter the inhibitory effect of aspirin on prostaglandin synthesis. Maximal inhibition of platelet aggregation at low doses has been seen in clinical trials with aspirin as an antithrombotic agent. When the dose of aspirin is increased, no further or additional effect can be seen because the maximal inhibition of platelet TXA2 synthesis has already occurred (Burch et al., 1979). Thus, the consistency of dose requirements and satureability of the effects of aspirin in inhibiting TXA2 production (Patrono et al., 1994) and preventing atherothrombotic complications (Patrono et al., 1994; Roth and Calverley, 1994).

Hjemdahl and colleagues (1994) observed platelet aggregation in platelet-rich plasma and in whole blood. They measured the urinary excretion of thromboxane and prostacyclin metabolites as well as thromboxane B2 (TXB2) generation in whole blood clotting. Additionally, the researchers examined the time dependence of the effects of different aspirin doses to determine if there was significant recovery of COX-dependent platelet function during the normal dosing interval of 24 hours. According to Professor Paul Hjemdahl, 10% of platelets in circulation are normally renewed each day, but the number can be higher if there is increased platelet turnover. Therefore one daily dose of aspirin may be insufficient for patients with increased platelet turnover. Though aspirin suppressed serum TXB2 by more than 98%, patients had significant recovery of platelet function 24 hours after dosing. It has been shown that one needs virtually complete COX-1 inhibition for full blockade of thromboxane mediated platelet aggregation. According to Professor Hjemdahl’s study, aspirin provides only a weak inhibition of platelet aggregation in whole blood with normal calcium levels, despite effective platelet cyclooxygenase (COX) inhibition.
An earlier study by Harker and Fuster (1996), demonstrated suboptimal inhibition of platelet aggregation in baboons who were fitted with Teflon vascular implants with a single dose of aspirin, daily complete inhibition of excessive platelet removal by the implants required two to three daily doses of aspirin.

Hjemdahl's study highlights the difficulty in correlating *in vitro* and *in vivo* observations of platelet activation, platelet aggregation tests are done *in vitro* using platelet-rich plasma or whole blood in different anticoagulants and under non-physiological stirring conditions (very low shear rates). Moake *et al.* (1988) have stressed on the fact that, *in vivo* platelet aggregation in small arteries occurs in blood flowing at high shear (flow) rates and is dependent on large vWF forms to induce the platelet aggregation process. High shear, vWF-mediated platelet aggregation is not inhibited by acetyl salicylic acid. Other proteins may be involved in hemostasis and thrombosis acetylated and altered by acetyl salicylic acid in addition to COX-1 that generates thromboxane A2. It has been theorized that platelet behavior may be largely governed by plasma factors. An alpha globulin that has recently been found in normal plasma inhibits collagen induced platelet aggregation. Most probably, a lack of this factor result in the platelet hyperaggregability to collagen seen in patients of IHD. It has therefore been hypothesized a low dose aspirin may not bring about the required effect and higher dose of aspirin may be required.

Regular aspirin is rapidly absorbed from the acidic environment of the stomach. Enteric coated aspirin releases into the alkaline environment of the small bowel, where it is hydrolyzed. As a result, enteric-coated aspirin has lower bioavailability than regular aspirin. The antiplatelet effects of full-dose (>300 mg) enteric-coated aspirin are similar to those of uncoated formulations (Hawthorne *et al.*, 1991 and Stampfer *et al.*, 1986). However, the efficacy of low-dose (<100 mg) enteric-coated preparations has not been clearly established, and it is possible that such doses may result in inadequate platelet inhibition. Thus, if coated aspirin is prescribed, larger doses may be necessary to obtain the desired antiplatelet effect. In our studies we used coated 100mg aspirin (Cartia) whereas 300mg was uncoated (generic). Based on the explanation provided above, we can say that the complete effect of 100mg of aspirin may have not been seen because of the lower bioavailability of the drug.
Conclusion: Although a single dose of 100 mg aspirin effectively inhibited platelet function in majority of our subjects, a considerable proportion of them showed a greater platelet inhibition with the use of higher doses. The whole blood platelet aggregometer may be used to differentiate between those patients who require a higher dose of aspirin to get a desired antiplatelet effect. In this study, we evaluated incomplete platelet inhibition using the whole blood platelet aggregometer. Although an effective inhibitor of platelet TX production, aspirin is often considered a weak platelet inhibitor because of its limited effects on aggregation by high concentrations of ADP or collagen. This may account for some of the variability in individual responses to the various agonists, which, in contrast to AA, activate platelets through both thromboxane dependent and -independent pathways. From the above explanations we can conclude that treatment with higher doses of aspirin could probably reduce biochemical aspirin resistance. Most of the platelet aggregation studies done so far have been done using platelet rich plasma by the turbidometric method utilizing the optical aggregometer and is based on the turbidometric method of Born. This method has many disadvantages. Preparation of sample by centrifugation removes the other cellular elements like RBCs and WBCs and also a proportion of heavier platelets which may influence platelet aggregation. To minimize these disadvantages platelet aggregation has recently been studied using the whole blood platelet aggregometer.

5.4 Aspirin Resistance

In our studies, aspirin resistance was found to be prevalent in all the three groups, NKD, OKD and RKD (Fijian and Indo-Fijian) after the ingestion of 100mg of aspirin. PA values remained within the normal range after a single dose of 100mg of aspirin were not inhibited after the intake of a single dose of 100mg of aspirin. Aspirin resistance was found to be predominant in the RKD (Fijian and Indo-Fijian group). The participants who were aspirin resistance exhibited a decrease in PA after administration of 300mg of aspirin.

Aspirin resistance can be defined as a biochemical phenomenon that may have clinical consequences. An important area of research in relation to aspirin therapy is the concept of aspirin resistance. Aspirin resistance has been variably defined and the response to aspirin has been characterized in a variety of methods as well as differences in the severity of atherothrombotic diseases in the patients studied. In addition variable doses have been used. The difficulty has been to convincingly demonstrate the clinical relevance of the various in
vitro tests of platelet function and as of now it seems no test has emerged as a clear gold standard for identifying patients resistant to aspirin. Therefore it is not surprising that the reported incidence of aspirin resistance varies widely from 5 to 75% (Bhatt, 2004). Based on more than 500 publications on the topic, it has been proposed that aspirin “resistance” represents a true clinical diagnosis, requiring a change in antiplatelet therapy.

A variety of methods have been used to detect aspirin resistance and a large number of studies have been published during the past few years in a bid to unify the terminology and methodology (Cattaneo, 2004; Hankey and Eikelboom, 2004). Through some of these studies it has been suggested that aspirin resistance being measured by TxA2-dependent methods should be defined as pharmacological or true aspirin resistance whereas methods which measure platelet activity inducible also via TxA2-independent pathways should be addressed as functional or unproven aspirin resistance (Cattaneo, 2004 and Wong et al., 2004).

Aspirin resistance has been determined by several different methods, which were either independent of or dependent on TxA2 production. In our study, aspirin resistance has been determined by using the whole blood platelet aggregometer and the aggregating agent used is collagen.

Therefore it seems that classification of aspirin resistance into at least two different types is justified. The type which is directly related to aspirin’s pharmacological mechanism of action, i.e. inhibition of TxA2 formation, can be referred to as pharmacological resistance as suggested by Wong et al. (2004). Even though the best possible options at this time is measuring AA-induced aggregation and formation of thromboxane A2, both seem to have limitations leaving clinicians without a definitive test for pharmacological aspirin efficacy. The limitation with the measurement of thromboxane levels ignores the fact that while aspirin might block COX-1-induced thromboxane formation in platelets, it could also be formed in other cells or in platelets by COX-1-independent mechanisms, thus making a patient appear aspirin resistant which might not be the case. In addition, increased levels of thromboxane B2, which is a stable metabolite of thromboxane A2, could also reflect enhanced F2-isoprostanooid function and not specifically formation of thromboxane A2 from arachidonic acid via COX enzymes (Cipollone et al., 2000). Findings by Frelinger et al. (2006) also supported the role of COX-independent thromboxane formation in aspirin resistance as in aspirin treated patients; arachidonic acid induced aggregation could not be inhibited.
Numerous trials have been conducted which show variability in the baseline response to aspirin by a variety of *in vitro* laboratory based, assays of platelet function including flow cytometric analyses of markers of platelet activation (Valettas *et al.*, 1997) optical platelet aggregometry (Benedek *et al.*, 1995; Pappas *et al.*, 1994; Poggio *et al.*, 1999), whole blood aggregometry and bleeding time (Buchanan *et al.*, 1995). The variability in the prevalence of aspirin resistance in various studies can be explained on the basis that a variety of methods, different doses of aspirin used as well as differences in severity of atherothrombotic disease in the patients studied. Studies using PFA-100 or combination of different agonists are more common. Likewise, arachidonic acid induced aggregation has rarely been used to detect aspirin resistance, instead PFA-100, ADP and collagen-induced aggregations are more commonly used though they are not specific to the mechanism of action of aspirin, but measure general platelet activity (Cattaneo, 2004; Hankey and Eikelboom, 2006).

Despite aspirin’s proven effectiveness in treating and preventing atherosclerotic disease, it is considered a relatively weak antiplatelet agent as it produces only partial inhibition of platelet aggregation. Aspirin does not completely inhibit TXA₂ synthesis, (Tohgi *et al.*, 1992) and other non-TXA₂-dependent activators of platelet aggregation (e.g., thrombin, ADP, and collagen) can bypass the aspirin-inhibitory effect and result in thrombosis. Additionally, some of the patients appear to be relatively resistant to the antiplatelet effects of aspirin, even when it is administered in large doses (Helgason *et al.*, 1994). Platelet aggregation studies have demonstrated incomplete inhibition of aggregation in 25% of patients with prior ischemic stroke who were receiving long-term aspirin therapy (minimum dose 325 mg/d). Though some of the patients demonstrate improved platelet inhibition when administered a higher aspirin dose yet, about 8% of patients taking 1300 mg of aspirin per day may still be aspirin resistant (Helgason *et al.*, 1994).

Gum *et al.* (2003) reported that patients with stable coronary disease (n=325) who were treated chronically with 325 mg of aspirin showed incomplete inhibition of platelet function in 5.5% of the patients using classical platelet aggregometry, whereas 23.8% of the subjects were found to be semi-responders using PFA-100, which simulates primary hemostasis in whole blood samples. With this device, 9.5% of patients were found to be resistant to the effects of aspirin. The results of over 2 years of follow-up of these patients indicated that
those initially found to be resistant to aspirin by platelet aggregometry were at an increased risk of death, MI, or stroke. However, there was no relationship between aspirin resistance as measured by the PFA-100 and subsequent adverse events (12.9% aspirin-sensitive, 15.1% aspirin-resistant, \( P = 0.4 \)) (Topol, 2003). This apparent disconnection may be explained by subsequent work which has demonstrated that platelet aggregation in the PFA-100 cartridge is sensitive to serum von Willebrand factor levels which were not measured in the study by Gum et al. (Chakraoun et al., 2004).

Several reasons have been suggested for the phenomenon of aspirin resistance, such as drug interactions, non-compliance, inadequate dosing, gender difference, diabetes, genotype and increased TxA2 production.

Recent studies found that 2-3% of CAD patients were non-compliant to aspirin (Tantry et al., 2005 and Frelinger et al., 2006). Enough evidence is not available to assess what proportion of patients taking aspirin and experiencing recurrent vascular events were not taking aspirin as prescribed as many patients do not take medications as prescribed. Nevertheless, the whole phenomenon of aspirin resistance cannot be explained by poor compliance and aspirin resistance has been reported despite supervised aspirin administration (Grundmann et al., 2003). More work is needed to assess the scale of the problem and to develop methods to improve adherence in aspirin-treated patients.

Although many drugs have inhibitory effects on platelet function, none of them inhibits all of the mechanisms that may be involved in the various forms of thrombosis. Aspirin inhibits the arachidonate pathway in platelets and can only be expected to be effective against thromboembolic events in which the generation of thromboxane A2 plays a major part. The co-administration of aspirin and other NSAIDs can lead to pharmacodynamic interactions between the two, which leads to attenuation of the antiplatelet effect of aspirin (De Gaetano et al., 2003) and may worsen outcomes in patients who are also prescribed aspirin. Certain NSAIDs, like ibuprofen, if taken just before aspirin or in multiples doses each day, can reduce the anti-platelet effects of aspirin and theoretically render aspirin less effective in preventing heart attacks and ischemic strokes. The ibuprofen molecule is believed to adhere to the COX-1 enzyme, thus keeping aspirin from reaching the enzyme. Hence possible drug interactions should also be considered (Harker and Fuster, 1986). No studies to date have
examined the proportion of patients experiencing recurrent vascular events who are taking NSAIDs as well as aspirin.

In light of current limited information it seems that some of the patients could benefit from increased dosing, but inadequate dosing is unlikely to explain the whole phenomenon. Patients with apparent states of increased platelet turnover such as during post-operative periods could be the ones to benefit from increased and more frequent dosing (Zimmermann et al., 2003).

Individual variability in platelet activity is vast and thus might explain some of the variability seen in responses to antiplatelet medication. A genetic basis for explaining individual variations in response to aspirin, such as single-nucleotide polymorphisms of COX-1, may represent an intriguing mechanism that deserves further investigation (Halushka et al., 2003). The finding of an association between COX-1 polymorphism and aspirin resistance suggests that genotype influences individual’s drug response. COX-1 polymorphisms might modulate the generation of TxA2 in platelets and increased levels of TxA2 have been suggested to influence aspirin resistance (Davi et al., 1997; Weber et al., 1999; Rocca et al., 2002; Ziegler et al., 2002). It must be emphasized, however, that the reduced sensitivity to aspirin treatment was more evident using collagen as an agonist; this could be dependent on the sensitivity of the assay, as aspirin inhibits collagen-induced platelet aggregation potently but has only a modest effect on ADP-induced platelet aggregation (Wittke et al., 2000).

Another interesting possibility is provided by a recent study which demonstrates that under physiologic conditions, platelets express a negligible amount of COX-2, whereas newly formed platelets have a detectable amount of COX-2, which may contribute to TXA2 biosynthesis (Rocca et al., 2002). Presently, there is no strong evidence to support the hypothesis that platelets become less sensitive to aspirin as a consequence of COX-2–dependent TXA2 production. Though the mechanism of decreased efficacy of aspirin in some patients is not well understood, it probably may reflect its limited potency as an inhibitor of COX-2, the expression of which has recently been demonstrated in human platelets. Such a hypothesis needs further investigation.

Factors like smoking, unstable angina, hyperlipidemia and diabetes may also interfere with aspirin's effect on platelet activation by increasing the production of prostaglandin F2–like
compounds, known as isoprostanes (Sanderson et al., 2005). Isoprostanes are produced from arachidonic acid, primarily through a non-COX process of lipid peroxidation catalyzed by oxygen free radicals, so their synthesis is unaffected by aspirin.

Weber et al. (1999) reported that the circulating platelets from healthy subjects express COX-2 protein and messenger RNA, and they suggested that this may represent a factor in aspirin resistance. This finding has been disputed by Patrignani et al. (1999). However in some patients treated with low-dose aspirin, despite > 95% suppression of platelet COX-1 activity (Vejar et al., 1990). COX-2 induction in monocytes, macrophages or activated endothelial cells may contribute to aspirin-insensitive TXA2 biosynthesis by generating PGH2 as a substrate for the TX-synthase of the same cell or by providing PGH2 to the TX-synthase of aspirinated platelets (Maclouf et al., 1998).

There is an increasing concern about the number of individuals who exhibit aspirin resistance, non-responsiveness or hypo-responsiveness, to the effects of aspirin. Three possible mechanisms have been offered to explain the phenomenon of aspirin resistance:

1) platelets might become activated by pathways which are not blocked by aspirin.
2) patients may require a higher dose of aspirin to produce an antiplatelet effect.
3) patients generate thromboxane A2 by other mechanisms despite aspirin therapy (Price, 2002). Early data suggests that aspirin resistance may be dose related and resistance is found more often during low-dose therapy (< 100 mg daily) than at higher doses (> 300 mg daily).

The study by Roller and associates (2002) found that the response to aspirin could be improved by increasing the dosage from 100 mg/d to 300 mg/d in one fifth of patients with biochemical aspirin resistance. Tarjan and colleagues (1999) found that 11% of patients with biochemical aspirin resistance were not adherent, based on baseline assessment of urinary salicylate. However, no clear mechanism has been proposed for this effect, which could also be related to other factors, such as adherence.

The phenomenon of aspirin resistance varies widely in the literature and depends on how it is measured. In a prospective study, using light transmission aggregometry which is regarded as the gold-standard technique compared with point-of-care assays, Pharand and colleagues
(2007) observed aspirin resistance (defined as ≥20% residual aggregation) in only 4% of their 200 patients with stable CAD who were taking the medication daily. These patients who were aspirin resistance were on aspirin at a lower mean dosage and had higher platelet counts as compared to the other participants who were aspirin sensitive. The researchers suggest that poor antiplatelet responses to aspirin may be due to inadequate dosing in patients with high platelet counts or that aspirin resistance may simply be a matter of abundant platelets overwhelming the effects of circulating aspirin levels.

**Conclusions:** Despite treatment failures, aspirin is currently the single most cost-effective drug for the secondary prevention of cardiovascular disease. There are many reasons like nonadherence, variable response to different doses, comorbid conditions, and drug interactions as to why aspirin resistance may be present despite being prescribed aspirin in therapeutic doses (biochemical aspirin resistance). This in turn may increase risk for recurrent vascular events.

Research in this area is currently hampered as there seems to be no standardized definition, measurement, and epidemiology of aspirin resistance key challenges for future research are to standardize a definition of aspirin resistance.

The term *aspirin resistance* has been used to describe not only an absence of the expected pharmacologic effects of aspirin on platelets but also poor clinical outcomes, such as recurrent vascular events, in patients treated with aspirin but, it is not clear as to the extent to which biochemical aspirin resistance translates into clinical events. We can only speculate that if aspirin resistance could be reliably identified by using tests of platelet function (or, in the future, genetic tests), patients might benefit from alternative or additional antiplatelet drugs. To optimize its clinical effectiveness, clinicians should be aware of the potential causes of aspirin treatment failure, prescribe aspirin in appropriate doses, and encourage patients to take aspirin, stop smoking, and avoid regular use of NSAIDs.

Platelet aggregation is one event in the complex process of atherothrombosis and measurement of aggregation has its limitations. Given the seriousness and consequences of atherothrombotic events and the low cost, safety and efficacy of aspirin in the prevention of the same it is important to address the issue of aspirin resistance.
The goal of this area of study was to determine whether phenomenon of aspirin resistance is present in the Fijian and Indofijian, non-kava drinking and kava drinking population. Identifying these patients who might at a risk of thromboembolic conditions and more importantly understand the mechanisms behind the increased risk would help to tailor individual antithrombotic treatment to these patients.

Laboratory tests that provide information regarding the effect of aspirin or people who are aspirin resistance, could help in the identification of persons who might be at a risk of experiencing recurrent cardiovascular events. Individual variability to aspirin can be due to medications, laboratory methodology, atherosclerotic burden, suboptimal dosage, genetic polymorphisms, or a combination of these and other factors.

While research scientists are increasingly convinced that aspirin resistance exists, there are no reliable and standardized tests that physicians in clinical practice can use to diagnose this condition.

**Limitations:** Limitations hindered a more comparative review of the articles. Some of the studies had small data sets and short-term follow-up. The studies used different methods of laboratory analysis and different definitions for aspirin responsiveness.

### 5.5 Gender differences in platelet aggregation

In this study involving healthy Fijians and Indo-Fijians (NKD and KD), the aggregability of platelets in response to a dose of commonly used agonist, collagen, was found to be higher in women than men in the NKD and OKD (F and IF) group and the difference was found to be statistically significant. The platelet aggregation in the females was found to be slightly lower than the males in the RKD (F and IF) group, though the difference was not statistically significant.

The finding of our study contradicts with the study done by Meade *et al.* (1985) and Johnson *et al.*, (1975) who found that no significant difference in platelet aggregation was seen in response to agonist like collagen or arachidonic acid but platelets from women were found to be more reactive than male platelets when ADP and adrenaline were used as aggregating agents. It has been suggested that the sex difference in platelet aggregation seen on addition
of ADP and arachidonic acid, may be an artifact probably because of the lower packed cell volume or haematocrit in females which results in a larger volume of distribution of the anticoagulant and therefore consequently, higher ionized calcium concentrations. However, the more recent observations of Meade et al. (1985) have shown that the sex difference in the aggregation responses to ADP remains statistically significant even after correction for the haemoglobin concentration and the haematocrit. In another study performed by Kasjanovova et al. (1993) the effects of gender on platelet aggregation and the relationship with hematocrit were investigated. According to results of this study, there were no differences between men and women in the platelet aggregation and thromboxane B2 production with similar hematocrit values. The results of these studies contradict with our findings whereas agree with the findings by Gader et al. (1990) who found that platelet aggregation in females was greater than in males, upon using a wide range of commonly used agonist like ADP, adrenaline, collagen and ristocetin.

Our results using the impedance method (whole blood platelet aggregometer) are in agreement with the above studies which showed a gender related differences in aggregation induced by collagen. However, the disagreement between the studies and the studies conducted by Mead et al. (1985) using collagen could be due to differences in methodology since whole blood aggregometry (impedance method) and the turbidimetric method differ widely when employed to detect the same biological change.

There are several studies investigating the effects of gender differences on platelet functions. The well-controlled platelet aggregation study performed at Tokai University School of Medicine with ten female and ten male participants, using the light scattering aggregometry, revealed that the female platelets respond to ADP more than male platelets. These authors suggested that females had greater aggregability than males due to the lack of testosterone, but the exact mechanism was not established (Haque et al., 2000).

Most of the studies, which have been done to examine the effects of sex hormones on platelet function, have used platelets from animals with manipulation of hormone levels.

Torres Duarte et al. (1986) investigated the effect of sex on platelet aggregation in mice. In this study, male platelets exhibited a greater response than female platelets to both ADP and
arachidonate in PRP. Contrary to this finding, Leng et al. (2004) reported that platelets of female mice were intrinsically more sensitive to agonists than platelets of males. In this study, female platelets also demonstrated greater aggregation in response to ADP and collagen-related peptide.

The data published and available to date regarding several human studies are also controversial. While Beyan et al. (2006) demonstrated that sex difference has no effect on platelet aggregation, Taylor et al. (1987) found that the response of platelets to agonists was greater in females than males. Some authors suggest that platelet aggregability may change with sexual maturity differently in females and males.

Jayachandran et al. (2004) reported sex-related differences in platelet aggregation and secretion with sexual maturation in pigs. In this study, platelet aggregation and ATP secretion decreased in females but increased in males with maturity. Results indicate that changes in platelet aggregation and secretion change with sexual maturity differently in females and males. The postulated mechanism put forth is that sex hormones are known to be absorbed at platelet membranes modifying their surface properties and inducing potential and permeability changes that may result in altered aggregability. It was reported that testosterone increases human platelet thromboxane A2 receptor density and makes platelets more prone to aggregation which may contribute to thrombotic cardiovascular disease in males (Ajayi et al., 1995). In fact, castration reduces platelet aggregability in males. It was also reported that estrogen increases the aggregability of platelets, accelerating the formation of clots among young women on oral contraceptives. (Ajayi and Halushka, 2005).

Haque et al., (2000) from the study on the effects of sex hormones on platelet aggregation concluded that females had greater aggregability than males due to the presence of estrogen that is elevated in the follicular phase after menstruation. In fact, estrogen inhibits the aggregation of platelets, but such inhibition is dependent on the presence or absence of the polymorphism of glycoprotein IIIa . The study determined whether sex difference occurs in platelet aggregation by using the laser light scattering method. This method has greater sensitivity and can detect small aggregates (i.e. 2-3 platelets). The researchers investigated the effects of estradiol or testosterone on platelet aggregation. The study suggests that the female platelets are more conductive to aggregation than the male platelets with a physiologic
concentration of the agonist ADP, but both estradiol (10 nM) and testosterone (40 nM) seem to have antiaggregative effects at the same physiological condition. Therefore, the reason why females have greater aggregability than males may partly be explained by their lack of testosterone, though the mechanism still remains to be determined. The study by Haque et. al. (2000) considered the short-term effect of sex hormones (30 min) with a single dose. It was also limited to in vitro data which may not reflect in vivo state.

It seems that sex hormones have an impact on platelets, although, many published studies report conflicting results of sex differences on various platelet function.

In our study males and females of two different ethnic groups, Fijian and Indofijian have been considered and a significant difference in platelet aggregation was seen in response to collagen as an agonist. No significant difference in PA of males and females was seen in the regular kava drinking group. The precise role of kava on altered properties of platelets could not be elucidated.

Results from the study by Becker et al. (2006) supports the role of gender on platelet function, identifying a significant difference between platelet aggregability of females and males with different ethnicity. They found that women had consistently more reactive platelets compared to men with multiple agonists. The greatest platelet response to agonists observed in women confirmed that estrogen, but not testosterone, may be a promoting factor in platelet aggregation. This observation may help explain a greater thromboembolic risk in women than men and their different response to non-steroidal anti-platelet medication.

However, additional studies are needed to further investigate the effect of kava, aspirin and estrogen on platelets that may lead to new ways of identifying women at risk for adverse thrombotic events.

Taking into consideration the gender differences in platelet aggregation among different ethnic groups, in the study done by Otabachi et al. (2010) platelet aggregation was measured in 36 healthy men and women free of any antiplatelet medication, aged 22–36 years, of Caucasian (White not of Hispanic origin), Hispanic, and African-American not of Hispanic origin. In this ex-vivo study, platelet aggregation was investigated, using a platelet ionized calcium aggregometer (Chronolog Co.) in response to commonly used agonist like
adenosine-50-diphosphate (ADP), epinephrine (EPI), arachidonic acid (AA) and collagen (COL). Platelet aggregation response to all the tested agonists was found to be higher in females than in males regardless of ethnicity. The most significant differences were observed with collagen. Among the ethnic groups, Caucasian women were most prone to platelet aggregation. The group most prone to overall aggregation was Caucasian women, followed by Caucasian men. Both sexes of Hispanic origin, as compared to Caucasian subjects, had lower platelet aggregation in response to ADP, AA and EPI and had similar responses to COL. Although platelet aggregation to tested agonists was higher in women than men, Hispanic females had lower platelet response to AA and EPI compared to Hispanic males. African-American men’s overall response to all tested agonists revealed a marked aggregation inhibition as compared to all other tested groups. This work provided evidence that the female platelet response from three ethnic groups (Caucasian, Hispanic and African-American) combined was greater than the male response. Females scored slightly higher with EPI. While female and male platelets responded similarly to AA. This study suggested gender influences on platelet aggregation.

In the study conducted by Beyan et al. (2006) did not observe sex difference in platelet aggregation in human subjects. The differences between this study and the others might be the differences of the final concentrations of aggregation inducers used and the performance of the study on human beings. According to this study by Beyan et al. (2006) results show that sex does not affect platelet aggregation in healthy adults. For this reason, it is possible to suggest that results in animals do not reflect the condition present in humans.

Taking into consideration the above studies, it can be concluded that sex difference does affect platelet aggregation. Our result supports that there is a need for gender differentiation while composing control groups in platelet aggregation studies.

**Conclusion:** In conclusion, the finding of our study in healthy males and females (Fijian and Indo-Fijian) shows that gender does affect platelet aggregation in response to collagen which is a commonly used aggregating agent. Although many authors have reported on the effect of gender on platelet aggregability, the underlying mechanisms that contribute to the observed differences remain unsolved and the results are controversial. While some studies have shown increased platelet aggregability in males others indicate that aggregability is more in
females, while some report no difference in platelet responses between the sexes. Based on these observations, we postulate that antiplatelet medication strategies should also focus on gender. The impact of variability of platelet aggregation among different ethnicities on therapeutic decisions also requires consideration in Fiji, where kava, the culture drink, is normally taken by Fijian and Indo-Fijian females.

**Limitations:** Our study employed only one method and a commonly used aggregating agent, collagen to study the gender differences in PA in the Fijian and Indo-Fijian participants.

### 5.6 Ethnic differences in platelet aggregation in healthy individuals

This study assessed the ethnic differences in platelet aggregation taking into consideration the Fijian and Indofijian population of Suva Fiji Islands. No significant difference in platelet aggregation was seen between two major ethnic groups of Fiji (Fijians and the Indo-Fijian). Platelet aggregation in the NKD Fijians and Indo-Fijians was compared. Similarly PA in the Fijian OKD was compared with the PA in the Indofijian OKD and Fijian RKD was compared with Indo-Fijian RKD. Unpaired t-test was used to compare these groups and the results were found to be non significant. So far no study is available in literature to compare our results, where we studied kava- aspirin effect on platelet aggregation in two major ethnic populations in Fiji.

According to some studies, ethnic differences seem to have an impact on platelet aggregability in response to various aggregating agents used like ADP, AA, EPI and COL (Meade et al., 1985). Various explanations have been provided for the differences in platelet aggregation between the ethnic groups. Genetic variations between the ethnic groups may affect the platelet function and the hemostatic platelet response may be influenced by the genetic profile of the platelet enzymes and its membrane glycoprotein While the ethnic differences in glycoprotein IIIa gene polymorphism remain to be established (Park, 2005), the cyclooxygenase polymorphisms have been confirmed in the African-American population (Urlich et al., 2002 and Urlich et al., 2005). Thromboxane synthase (TXS) polymorphism (TBXAS1) has particular importance, since TXS, a cytochrome P450 enzyme, converts prostaglandin H2 into thromboxane A2, a potent inducer of platelet aggregation (Urlich et.
Polymorphism could probably indirectly explain the reduced platelet aggregability in response to AA in 40% of Hispanics and 50% of African-Americans.

Results of studies by Otahbachi et al. (2010) demonstrate that, ethnicity might also influence platelet response to agonists. Caucasians had more aggregation induced by ADP, AA and EPI as compared to the Hispanics and the effect of COL was similar in the tested ethnic groups. Gader et al. (1991) observed significant differences in the aggregation responses between the ethnic groups. While Saudi Arabs and Westerners (Europeans/Americans) had greater aggregation responses to ADP than Asians and Africans, aggregability in response to COL was more pronounced in Saudis and Africans than in Westerners and Asians. A population study in London by Meade et al. (1985) to confirm the higher incidence of IHD in whites as compared to blacks, reported greater aggregability in white men when compared with black men. ADP was used as an aggregating agent. The study was done using a slightly different procedure of ADP dose-response aggregometry. Much greater aggregability in Caucasian than African men was consistent with the higher incidence of ischemic heart disease in whites, as reported by Meade et al. (1985). The study conducted by Gader confirms the observation by Mead when comparing Africans to Westerners. Platelet aggregation was estimated using a slightly different procedure of ADP dose-response aggregometry. However, when comparing Saudi Arabs and Westerners, no obvious differences between the ADP response of Saudi Arabs and Westerners were evident. On the other hand, the responses to ADP in South East Asians, like Africans, were significantly less than both Arabs and Westerners. In a study of disparity in cardiovascular disease rates between Asian Indians and Caucasians, Patel et al. (2007) indicated that there were no differences in platelet aggregation in response to ADP and AA.

There is limited information on the differences in platelet aggregation between Hispanic and other ethnic groups. The reported differences in platelet aggregability between ethnic groups are often linked with genetic and dietary factors. (Salo et al., 1985; Renaud et al., 1981). Racial differences in ristocetin-induced platelet aggregation (RIPA) has been reported in at least other studies as well in which blacks have exhibited markedly inhibited aggregation responses to ristocetin (Buchanan et al., 1981) when compared to whites. The diminished aggregability to ristocetin was also noted, but lesser in magnitude, in other non-Caucasians groups. In a study by Gader et al. (1991), the diminished RIPA (ristocetin induced Platelet
aggregation) in blacks was explained on the basis of a plasma inhibitor against RIPA. However, the authors recently observed and have reported a significant inhibition of RIPA in children with sickle-cell disease (SCD) when compared to normal children (Babikar et al., 1987). Furthermore, West African students in King Saud University in Riyadh, who come from an area where sickle cell haemoglobin is prevalent, demonstrated, inhibited RIPA which was very similar to Saudi patients with Sickle Cell Disease. Therefore, sickle cell haemoglobin may be the cause for the abnormal RIPA seen in blacks. These inhibited aggregation responses to ristocetin may explain the low frequency of thromboembolic disease in Africans (Dupuy et al., 1978) and perhaps non Caucasians at large, since it indicates a diminished tendency of von Willibrand factor-mediated platelets stickiness to the endothelium. Aggregation using arachidonic acid is a test of the integrity of the prostaglandin pathway involved in platelet activation. The higher prevalence of inhibited AA aggregation in Westerners compared to Arabs or Asians remains unexplained. The remarkably higher prevalence (51%) of abnormal aggregation responses to adrenaline in Asians compared to other ethnic groups was also an unexpected finding in the study by Gader et al. (1991).

Most Asian subjects with markedly inhibited aggregation responses to adrenaline showed normal response to AA and ADP. Aggregations in Asians were similar to Arabs. The anomalous adrenaline aggregation seems to be unique to Asians. According to Gader, this could be due to different dietary habits; for example construction workers from South East Asia (mainly Filipinos) living in Riyadh, were shown to consume a considerable amount of fish (different types of fish being served in the three meals every day (Al-Mufarraj et al., 1990). A similar phenomenon i.e., dietary-related anomalous aggregation responses have been reported recently in a Finnish study where platelets collected from farmers were found to be less responsive to adrenaline but have the same sensitivities to other agonists (ADP and thrombin) when compared to platelet responses in semi-urban populations (Salo et al., 1985). These differences were related to the different types of fats consumed by rural versus semi-urban subjects an observation which was confirmed in three farming communities in Britain (Renaud et al., 1981). Similar selective diminution of platelet aggregation responses to adrenaline has also been related to consumption of alcohol (Fenn et al., 1984 and Mikhailidis et al., 1986) but the study by Gader et al. (1991) could not find any evidence that alcohol consumption is the causative factor in the Asian subjects. Their study established ethnic differences in platelet aggregation responses to ADP, adrenaline, arachidonic acid and
ristocetin, with Asians and Africans showing the greatest deviations from other ethnic populations.

Dietary factors such as fish, onions and garlic are known to inhibit platelet function (Ogstun, 1983). These ingredients in addition to a mixture of different spices are used in cooking the ordinary Saudi diet. The study by Bertrand et al. (1987) comparing the platelet aggregation in 50 Ivorian and 50 European showed a lower platelet aggregability in the Ivorian people as compared to the Europeans. The authors have attributed environmental factors in the genesis of such differences. Hypocholesterolaemia, lower smoking and drinking levels and high fish consumption in the Ivorian population has been put forth as the basis for reduced platelet aggregability.

The dietary habits of the Fijians and Indofijians differ in a way that the Indofijian food is rich in spices and contains a lot garlic and onion as compared to the Fijian diet. Despite these differences no difference in PA between the Fijian and Indofijians was seen in our study.

**Conclusion:** The results of this study revealed that there was no difference in platelet aggregation in the Fijian and Indo-Fijian non-kava drinking and kava drinking population. There seem to be wide variation in results regarding the platelet aggregation in different ethnic groups based on the method and aggregating agent used for aggregation. Important factors like, rural versus semi urban, smoking status has been taken into consideration. In this study collagen has been used as the aggregating agent compared to these other studies where different aggregating agents like ADP, adrenaline, ristocetin, have been used. Diet, smoking status, and alcohol consumption of the volunteers has been taking into consideration, yet, no difference in platelet aggregation was seen among the two ethnic groups.

**Study limitations** The results of this study have certain limitations. One is the lack of genotyping to directly determine genetic variation and associated platelet phenotypes in studied subjects. The strict inclusion and exclusion criteria used made the study groups relatively uniform, largely preventing extraneous variables from affecting platelet aggregability. Only one methodology has been employed and only one aggregating agent (collagen) used in the determination of platelet aggregation.
5.7 Correlation between age and platelet aggregation

Our studies showed that there is no correlation between age and PA before and after aspirin intervention in Fijian NKD, OKD and RKD participants.

A highly significant correlation was seen between age and PA in the Indo-Fijian participants (NKD, OKD and RKD). A significant negative correlation was seen between age and PA in the Indo-Fijian OKD after 100mg aspirin dosage.

The correlation seen in the Indo-Fijian participants could be explained based on the study by Goubareva et al. (2007). They demonstrated that age is associated with an increase in incidence of atherothrombotic diseases. Platelet activated nitric oxide is known to inhibit platelet activation. Nitric oxide (NO) is synthesized from l-arginine in a reaction catalyzed by NO synthase (NOS), with l-citrulline being the by-product (Palmer et al., 1989). NO activates soluble guanylyl cyclase (sGC), thereby increasing cyclic guanosine-3',5'-monophosphate (cGMP) in target cells. Endothelium-derived NO causes vasorelaxation, and also inhibits platelet adhesion and aggregation, thus maintaining blood fluidity and preventing thrombosis. In their study (Goubareva et al., 2007) investigated platelet NO biosynthesis and responsiveness in older (>45 years old) as compared with younger (<30 years old) healthy human subject and have suggested that platelet NO production and responsiveness decrease with age. Since O$_2^-$ rapidly combines with NO to produce ONOO$^-$, thereby inactivating NO, their study considered it possible that an increase in platelet O$_2^-$ generation with age might explain the age-related suppression of bioactive NO. The data from their study confirms that, platelets from older subjects produce more O$_2^-$ than do those from younger subjects.

No correlation between age and PA before or after aspirin was found in the Fijian participants, whereas a significant correlation between age and PA was seen in the Indo-Fijian participants. This difference in results between the Fijian and Indo-Fijian participants might be explained on the basis of genetic and environmental differences in PA. According to the Framingham Heart study (Christopher et al., 2001) which is a large population based study, heritable factors play a major role in determining PA. The majority of variation in PA may be due to the cumulative effects of other genetic variants of proteins which may be involved in the cascade of steps mediating platelet adhesion and aggregation.
Specific endogenous and other environmental factors are also known to have an effect on platelet reactivity. Endogenous and environmental determinants of platelet aggregability can only explain the interindividual variability in aggregation responses.

Most of the studies correlating age and PA have included participants > 60 years of age and hence proved that with increase in age, PA also increases. The age group of the participant is upto 50 years in our study which might be one of the reasons why a correlation could not be established especially in the Fijian population (Terres et al., 2001; Lin et al., 1990).

**Conclusion:** Available data suggests that platelet NO production and responsiveness decrease with age which might lead to increase platelet aggregability with age. Endogenous and environmental factors might explain individual variability in aggregation responses.
Bibliography


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Moake, J. L., Turner, N. A., Stathopoulos, N. A., Nolasco, L. and Hellums, J. D. (1988). Shear-induced platelet aggregation can be mediated by vWF released from platelets, as well as by exogenous large or unusually large vWF multimers, requires adenosine diphosphate, and is resistant to aspirin. *Blood.* **71**:1366-1374.


Appendix A

Information Details

EFFECT OF ASPIRIN INTERVENTION ON PLATELET AGGREGATION IN NON-KAVA DRINKING AND KAVA DRINKING FIJIAN AND INDO-FIJIAN HEALTHY VOLUNTEERS AND DETERMINATION OF ASPIRIN RESISTANCE

Principle Investigator – Vaishali Khatri
Supervisors- Dr.Ketan Christi (USP) and Prof. Robert Moulds (FSM)

Introduction

Platelets are a type of blood cells which participate in the formation of blood clots by sticking together, a process called as platelet aggregation. However if the blood clots are formed inside the artery, they can block the flow of blood to the tissue which the artery supplies eventually leading to damage and then death of the cells. So prevention of platelet aggregation is an important part in the treatment of cardiovascular disease, peripheral vascular disease and stroke. Aspirin is the most widely used, cost effective medication for the treatment of such diseases. However even after taking their daily dose of aspirin some patients are not receiving the protection that aspirin gives against cardiovascular events. Researchers call this as aspirin non-responsiveness. Millions of people with heart disease take aspirin and they need to know whether it is working for them. According to available data and ongoing research it has been shown that 5%-60% of the people may be aspirin resistant. This can create a clinical and economic burden on the health care system. It is therefore clear that clinically, aspirin resistance is a major concern and patients who are aspirin resistant are at a greater risk of getting heart attacks and strokes. Kava (Piper methysticum) is used for medicinal, religious, political, cultural and social purposes throughout the Pacific. The active ingredients are the kava lactones. Research shows that Kavain an active ingredient in kava has an effect on platelet aggregation. It inhibits COX (cyclo-oxygenase) enzyme, thus inhibiting the generation of TXA2 (thromboxane A2) which causes platelet aggregation. The aim of this study is to determine the association between aspirin, kava and their effect on platelet aggregation in some kava drinking and non- drinking volunteers. The objective of this study is to evaluate the effect of two different doses of aspirin (100mg and 300mg) in non-kava drinkers and kava drinkers and also to determine if aspirin resistance is present in the participating volunteers. This study will be conducted on two Ethnic groups- Fijians and
Indo-Fijians. This research is clinically important as it will help us to determine the platelet aggregation in volunteers after two different doses of aspirin. Individuals who are aspirin resistant will also be identified. One of the most important research questions that we hope to answer is that does kava affect platelet aggregation.

**Purpose of this research:**
Aspirin is a popular drug which is prescribed by physicians to avoid heart attacks and strokes. People are taking aspirin without actually knowing whether it is benefiting them. We would therefore like to see the effect of aspirin on the platelet aggregation in the kava drinking and the non kava drinking population of Fiji.

Kava is also known to affect platelet aggregation. We would therefore like to see the effect of kava on platelet aggregation. Two ethnic groups (Fijian and Indo-Fijian) will be considered for this study.

**Any Risks:**
Having your blood drawn is a very safe procedure and it is routinely done for various blood tests. A small amount of blood (about 10ml) will be withdrawn which will not have any effect on the daily activities. A single dose of aspirin will be administered once. Adverse effects from a single dose of aspirin are highly unlikely, Dr Robert Moulds, Professor of Medicine at FSM/CWM, a clinician with experience in running clinical trials, will be acting as a consulting physician.

**Voluntary Participation:**
You are under no binding and free to withdrawn from this study whenever you want. You may even refuse to participate in this study.

**Confidentiality:**
Any information you give us and results of this study will be kept confidential. All the forms having this relevant information will be kept locked with restricted access and then the data will be destroyed after approximately 2 years. When using the data or publishing this study none of the participants names will appear anywhere in the publications or presentations.
Appendix B

Informed Consent Statement

EFFECT OF ASPIRIN INTERVENTION ON PLATELET AGGREGATION IN NON-KAVA DRINKING AND KAVA DRINKING FIJIAN AND INDO-FIJIAN HEALTHY VOLUNTEERS AND DETERMINATION OF ASPIRIN RESISTANCE

Principle Investigator: Vaishali Khatri
Supervisors: Dr. Ketan Christi (USP) and Prof. Robert Moulds (FSM)

Having read/heard the description of the project and the requirements for participating in it, and having understood my role as a participant in the research, I freely agree to participate in the study.

I recognize that participation in this research gives the researchers freedom to use and publish the research data. I understand that the data will only to be used for educational and research purposes by the researchers and that the identity of all participants will remain anonymous.
I understand that I can freely withdraw from the study at any time and that this project is for the purposes of research

By signing this form I indicate that I understand my role in the research, and I voluntarily agree to participate.

Signature: ___________________
Name of Participant: ___________________
Email Address: ___________________
Telephone Number: ________________
## Appendix C

### QUESTIONNAIRE

**Study Title:**

**EFFECT OF ASPIRIN INTERVENTION ON PLATELET AGGREGATION IN NON-KAVA DRINKING AND KAVA DRINKING FIJIAN AND INDO-FIJIAN HEALTHY VOLUNTEERS AND DETERMINATION OF ASPIRIN RESISTANCE**

**CONFIDENTIAL**

### Personal information:

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<th>Time:</th>
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<table>
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### Lifestyle Data:

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<th>non vegetarian</th>
<th>Others; Please Specify.</th>
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</table>

If non vegetarian: How many times a week do you consume meat? __________

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<th>Exercise</th>
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<th>Walking</th>
<th>Swimming</th>
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</tr>
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</table>

How many hours a week do you spend exercising? ____________________________

<table>
<thead>
<tr>
<th>Smoking</th>
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<th>No</th>
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</thead>
</table>

How many cigarettes do you smoke per day? _________________________________

When did you start smoking? _____________________________________________

<table>
<thead>
<tr>
<th>Alcohol consumption</th>
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<th>No</th>
</tr>
</thead>
</table>

How many days in a week do you drink alcohol? _____________________________
Kava consumption □ Yes □ No

How many days in a week do you drink kava? _______________________________________________________________________
How much kava do you drink at each occasion? _______________________________________________________________________

**Medical History**

Any recent illness or injury? □ Yes □ No □ Don’t Know
(such as cold or sports related injury)

Type of illness or injury? ______________________________________________

What Medications were you prescribed? __________________________________

Any known disease or disorder? □ Yes □ No □ Don’t Know
(such as diabetes, hypertension)

Type of Disease or Disorder?
What medications were you prescribed? __________________________________
(Medicines you brought for yourself, herbal or traditional remedies.)________

Any kind of emotional stress during the past weeks? □ Yes □ No □ Don’t Know

Depression, anger, anxiety? _____________________________________________

Any general medications taken in the past two weeks? □ Yes □ No □ Don’t Know
(such as for cold, headaches, pain or contraceptives)

What medications were these? (such as paracetamol, aspirin)_____________
## APPENDIX D

Data are grouped into frequency

**Table 1:** Platelet aggregation (% frequency distribution) in Fijian volunteers (before aspirin).

<table>
<thead>
<tr>
<th>PA range</th>
<th>NKD</th>
<th>OKD</th>
<th>RKD</th>
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</tr>
<tr>
<td>27-30</td>
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<td>5.0</td>
<td>7.2</td>
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**Table 2:** Platelet aggregation (% frequency distribution) in Fijian volunteers after 100mg aspirin.

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<th>NKD</th>
<th>OKD</th>
<th>RKD</th>
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<td>6 to 9</td>
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<td>28.6</td>
</tr>
<tr>
<td>&gt; 22</td>
<td>0.0</td>
<td>0.0</td>
<td>14.3</td>
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</tbody>
</table>

**Table 3:** Platelet aggregation (% frequency distribution) in Fijian volunteers after 100mg aspirin(after 300mg aspirin).

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<td>&gt; 15</td>
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Table 4: Platelet aggregation (% frequency distribution) in Indo-Fijian volunteers (before aspirin)

<table>
<thead>
<tr>
<th>Control Indo-Fijian volunteers (%)</th>
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<tbody>
<tr>
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Table 5: Platelet aggregation (% frequency distribution) in Indo-Fijian volunteers (after 100mg aspirin)

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</tr>
<tr>
<td>&gt; 22</td>
</tr>
</tbody>
</table>

Table 6: Platelet aggregation (% frequency distribution) in Indo-Fijian volunteers (after 300mg aspirin)

<table>
<thead>
<tr>
<th>Aspirin 300mg Indo-Fijian volunteers (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PA range</td>
</tr>
<tr>
<td>0 to 2</td>
</tr>
<tr>
<td>3 to 5</td>
</tr>
<tr>
<td>6 to 8</td>
</tr>
<tr>
<td>9 to 11</td>
</tr>
<tr>
<td>12 to 14</td>
</tr>
<tr>
<td>&gt; 15</td>
</tr>
</tbody>
</table>