Original Research Article

Attenuation of the effect of 100mg aspirin on platelet aggregation in regular kava drinkers

ABSTRACT

Background and purpose: Numerous interactions between herbal medicines and conventional drugs have been documented. While the significance of many interactions is uncertain, several others may have serious clinical consequences. Kava (*Piper methysticum*) is an ancient crop of the western Pacific. Kava preparation and extracts are very popular in the Pacific 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. Management of cardiovascular disease in Fiji includes anti-platelet drugs, especially aspirin which is prescribed routinely. **Aim**: Our study aimed to assess the effect of two different doses of aspirin (100mg and 300mg) on the degree of platelet aggregation induced by collagen amongst healthy non kava drinkers and kava drinkers. The objective of this study was to examine potential effect of concomitant aspirin on platelet aggregation (PA) in kava drinkers. **Methods**: platelet aggregation was measured using whole blood platelet aggregometer (Chronology Corp) using collagen as an aggregating agent in two main ethnic groups, Fijians and Indo-Fijians, before and after the intake of a single dose of 100mg and 300mg of aspirin. The Fijian and Indo-Fijian volunteers were divided into three groups, non-kava drinkers (NKD), occasional-kava drinkers (OKD) and regular-kava drinkers (RKD). **Results:** The results were found to be non-significant in NKD, OKD and RKD Fijians and Indo-Fijians before aspirin intervention, the intake of kava did not have any effect on platelet aggregation. Overall PA remained within the normal range (15-27 ~~ohms~~ ~~(~~Ω). After a single dose

of 100 mg of aspirin, a large number of participants in both ethnic groups of RKD were found to have decreased aspirin sensitivity. The decreased aspirin sensitive participants had their PA within the normal range (15-27 Ω) even after administration of aspirin. All the participants 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). The most important finding of this study is that 100mg aspirin had significantly less inhibitory effect on PA in both Fijian and Indo-Fijian RKD (p < 0.001). **Conclusion**: Our studies show a reduced effect of aspirin on platelet aggregation in regular kava drinkers (RKD).

KEY WORDS

Platelet aggregation(PA), Kava, Aspirin, Fijian, Indo-Fijian.

INTRODUCTION

Fiji is a Pacific island country located in the southwest Pacific. Kava (*Piper methysticum,* also called the pepper plant or kava kava) is an ancient crop of the western Pacific. It is found in Polynesia, Melanesia, and Micronesia. Kava has a peppery taste and has long been a part of religious, political, and cultural life throughout the Pacific. The kava root is ground to a powder and the brownish powder is then mixed with water and drunk, without being fermented. Kava also, is effective as a pain reliever (9), 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. Kavalactones (kava pyrones), are the biologically active compounds present in the kava drink. Kavain, one of the six major kavalactones present in the kava roots, was determined to have antithrombotic action on human platelets. One *in vitro* study showed that, kawain, appears to decrease thromboxane 2 production and inhibit cyclo-oxygenase, indicating that kava may have significant inhibitory effect on platelet aggregation.(5). The kavalactones have been shown to demonstrate better or similar COX-1 inhibition activities as compared to ibuprofen, aspirin and naproxen (4).

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 (Ischaemic Heart Diseases) has lead to the highest mortality rate in Fiji amongst the Pacific Island nations (15).

Management of cardiovascular disease in Fiji includes anti-platelet drugs, 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.

Non-steroidal anti-inflammatory drugs (NSAIDs), particularly aspirin, have the potential to interact with herbal supplements that are known to possess antiplatelet activity. While there is a considerable body of knowledge on the use of herbal medicines, there is limited evidence on the concurrent use of herbals with conventional medicines (1)

This study was therefore undertaken to analyse the effect of aspirin on platelet aggregation in kava drinkers and to determine the interaction between kava and aspirin.

Methodology

This study was carried out in Suva city, Fiji Islands.

The volunteers for this research work were selected randomly. 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. Apparently healthy Fijian and Indo-Fijian volunteers were selected. Both males and females between the age group of 21-50yrs were included in the study**. (**The age group of the participants was decided based on the observation that many people above the age of 50 were on some kind of medication or suffering from diseases or disorders like Diabetes and Blood Pressure. The percentage of menopausal women after the age of 50 was also found to be high). Informed consent was obtained from all the participants.

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. 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.

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).

Verification regarding the kava drinking habits was obtained from the spouse, friends or colleague (whosoever could be contacted). Aspirin 100mg (Cartia coated) and 300mg (generic, uncoated), were purchased from the local pharmacy.

Instrumentation:

The chronolog model 591whole blood aggregometer was used for platelet function testing of whole blood specimens using impedance aggregometry. Sterile physiological saline was used for dilution of the whole blood specimen. 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 4^0C and not frozen. The experiments were performed as mentioned in the Chronolog Corporation manual for Whole Blood Platelet Aggregometer, (2006).

There was a gap of about one week between the intake of 100mg and 300mg of aspirin, termed as the washout period. Blood sample was collected and analysed for PA

Sample analysis

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 5minutes in the incubation well. Lintless wipes 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. The results of the whole blood aggregation test were measured in ohms (Ω) by the instrument with its digital readout.

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.

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.

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.

The data were subjected to the following statistical tests as described by Tabachnick and Fidell (2007). One way ANOVA and post hoc Scheffe test for inter and intravariability between the groups and within the groups. Two sample independent t-test for comparing two groups.

RESULTS

I. Fijian volunteers: Comparison within the group

The ANOVA results indicated that there was a significant difference between mean platelet aggregation due to the different aspirin dosages in NKD, OKD and RKD ($p < 0.001$). Further, the pairwise comparisons revealed that the mean platelet aggregation before aspirin (control group) was significantly higher than the mean platelet aggregation after with 100mg aspirin and 300mg aspirin. (Table 1, Fig.1)

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

A. 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. (Table 1, Fig.1)

B. 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 Scheffe test (using α =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. (Table 1, Fig.1)

C. After 300mg of aspirin

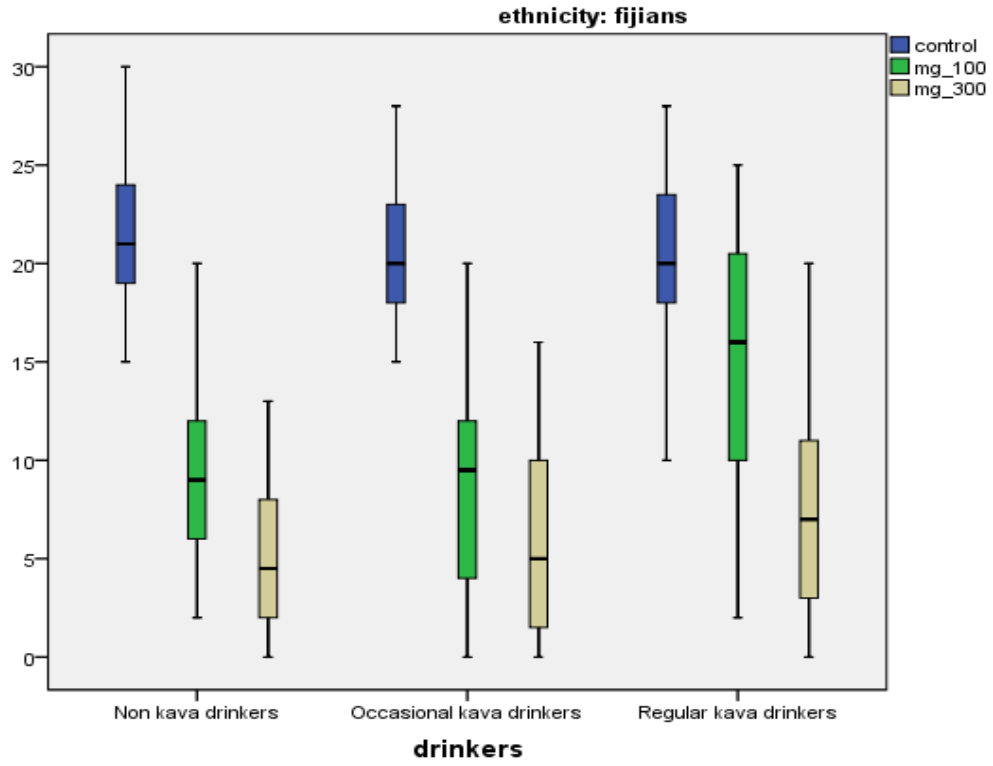
The ANOVA was not statistically significant F (2171) = 2.073, p=0.129, indicating that there is no significant difference in platelet aggregation among three groups, after giving 300 mg aspirin.

All the participants in the NKD group showed a decrease in platelet aggregation after a single dose of 300mg of aspirin (Table 1, Fig.1)

Table1 : Platelet aggregation (Ω,mean±sd) in Fijian population

Kava category	Control (15-27 Ω)	100mg aspirin	300mg aspirin
NKD (n=58)	21.4 ±3.9	9.1 ±3.9	5.4 ±3.6
OKD (n=60)	20.3 ±3.5	8.4 ±4.8	5.8 ±4.5
RKD (n=56)	20.6 ±3.9	15.3 ±6.0	6.9 ±4.7
Overall (n=174)	20.8 ±3.8	10.8 ±5.8	6.0 ±4.3

Fig 1: Overall comparison of PA in NKD, OKD and RKD before and after 100mg and 300mg of aspirin in the Fijian population.



III. Indo-Fijian volunteers: Comparison within the group

The ANOVA results, $p < 0.001$ 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 was significantly higher than the mean platelet aggregate after 100mg aspirin and 300mg aspirin.(Table 2, Fig.2)

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

A. Before aspirin

A comparison of platelet aggregation was done between the non kava drinkers, occasional kava drinkers and regular kava drinkers before aspirin. 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. (Table 2, Fig.2)

B. 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 α =0.05) revealed that the platelet aggregation was significantly different between NKD (mean 10.27) and RKD (mean=15.71 Ω), and OKD (mean=10.26 Ω) and RKD (mean=15.71 Ω) after giving 100mg aspirin. However, there was no significant difference in the platelet aggregation of non kava drinkers and occasional kava drinkers. (Table 2, Fig.2)

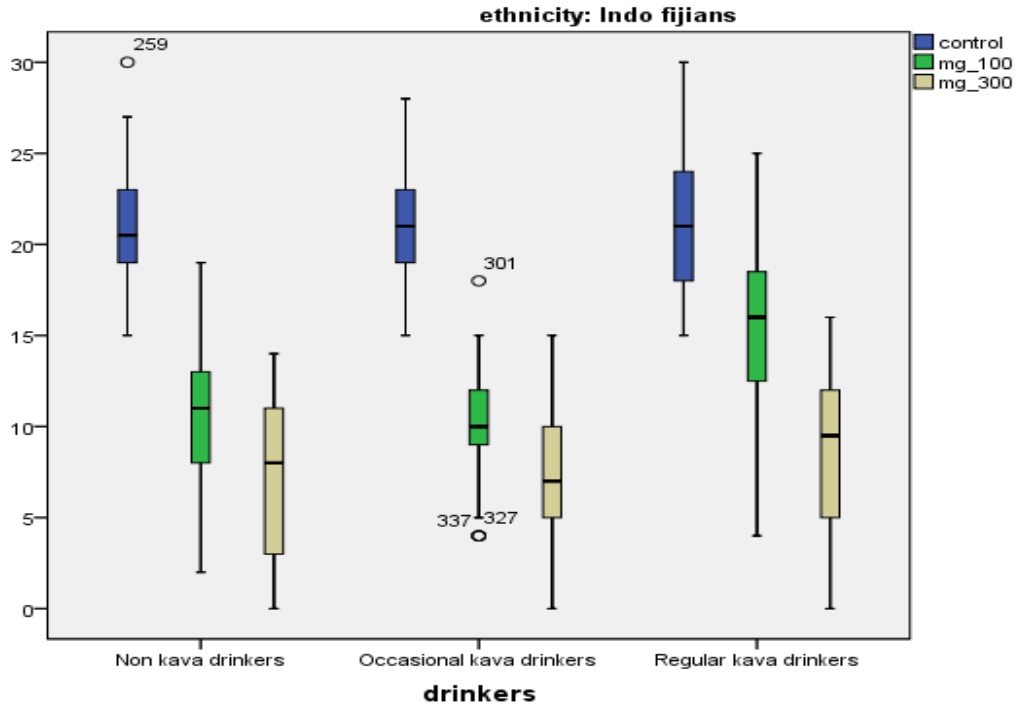
C. After 300mg of aspirin

The ANOVA was not statistically significant, F(2,165)=2.315, p=.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. (Table 2, Fig.2)

Table 2 : Platelet aggregation (Ω, mean±sd) in Indo-fijian population

Kava category	Control (15-27 Ω)	100mg aspirin	300mg aspirin
NKD (n=58)	20.86 ±3.19	10.28 ±3.92	7.12 ±4.07
OKD (n=58)	20.88 ±3.11	10.26 ±3.07	7.05 ±3.25
RKD (n=52)	20.87 ±3.62	15.71 ±4.45	8.52 ±4.61
Overall (n=174)	20.87 ±3.29	11.95 ±4.57	7.53 ±4.02

Fig 2: Overall comparison of PA in NKD, OKD and RKD before and after 100mg and 300mg of aspirin in the Indo-Fijian population.



DISCUSSION

Though interactions between herbal medicines and conventional drugs have been documented, the significance of many interactions is uncertain and may have serious clinical consequences (7). Since all herbal medicines are mixtures of more than one active ingredient, such combinations of many substances obviously increase the likelihood of interactions taking place (6). Potential herb-drug interaction is a clinical concern because of co-morbidities and slower clearance of pharmacologically active compounds (1). 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 (8, 10). 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. 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.

Mathews (1998) 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. 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.

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 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 3minutes 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.

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 Indofijian 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 (RKD and OKD). Therefore explanations regarding the reported high incidence of IHD in the Indofijian population can be based upon supposedly biological differences as well as social differences that might be able to explain the differences in disease patterns.

Since this study has been done for the first time and there are no studies to compare with, we can only 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 (16). 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 (2). 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 1enzyme, 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 (14), it is possible that constant bombardment of platelets with 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, (12, 17), 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.

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.

Conclusion

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. 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.

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