

Full Length Research Paper

Anti-inflammatory capacity of the aqueous leaf extract of *Senna mimosoides* on inhibition of rat oedema, platelet aggregatory activity and prostaglandin synthase activity

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The effect of aqueous extract of *Senna mimosoides* leaves on systemic acute inflammation was evaluated in Wistar albino rats. The LD₅₀ of the extract was found to exceed 5 g/kg as no death was recorded. In the systemic oedema of the rat paw, scalar doses of the extract significantly suppressed the development of paw oedema induced by egg albumin, this compares well with a standard anti-inflammatory drug indomethacin which at 100 mg/kg inhibits egg albumin induced rat paw oedema (59.7%). There was also a noticeable decrease in the activity of prostaglandin synthase which was concentration dependent since at 0.1, 0.2 and 0.3 mg/ml, the enzyme activity was 8.64, 6.24 and 3.93 iu, respectively. Also, at 0.2 and 0.3 mg/ml of indomethacin, the enzyme activity was 6.10 and 3.60 iu. Similarly, the extract significantly inhibited platelet aggregation in a dose and time dependent manner. At 0.1, 0.2, 0.4, 0.6 and 0.8 mg/ml the percentage inhibition of platelet aggregation at 2 min was 75, 63, 46.8, 40.6 and 31.3%, respectively. Moreover, 0.1 mg/ml of the extract at different time intervals (2, 4, 6 and 8 min) inhibited platelet aggregation; thus 75, 81.1, 82.4 and 83.3. These results indicate that the extract produced significant ($P < 0.05$) anti-inflammatory activity when compared with the standard drug indomethacin and untreated control. They also revealed that, the mechanism of action of the drug may be by inhibiting prostaglandin synthase, and platelet aggregation.

Key words: Inflammation, prostaglandin synthase, platelet aggregation, indomethacin.

INTRODUCTION

Most researches on herbal medicine have hinged on traditional folklore medicine. From different parts of the world natural products of some plants, fungi, bacteria and other organisms will continue to be used in pharmaceutical preparations either as pure compounds or as extracts (Araujo and Leon, 2001). There are hundreds of medicinal plants with long history of curative properties against various diseases and ailments have been identified. The plant kingdom has long served as a prolific source of useful drugs, food, additives, flavouring agents, colourants, binders and lubricants. Many herbs also possess anti-inflammatory (also known as

antiphlogistic) characteristics. Herbs can be used as the sole therapy in autoimmune disease or as complementary corticosteroid-sparing therapies allowing patients to take smaller doses or shorter courses of corticosteroids. The screening of the plants for their biological activity is done on the basis of either chemotaxonomic investigation or ethnobotanical knowledge, in relation to a particular disease (Juneja et al., 2007). The importance of a plant lies in its primary and secondary metabolites.

Some of these metabolites exert significant physiologic effects on the mammalian system. *Senna mimosoides* formerly known as *Cassia mimosoides* belongs to the family Caesalpinaceae and the genus *Senna* (Young, 2000). It is known as sensitive *Senna* and its leaves, pods and seeds are edible (Bruce, 2006). The genus *Senna* is a large and diverse group of annual and

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perennial herbs, shrubs, woody vines and trees found in the tropics and subtropics (Flory et al., 1992). Inflammation is a complex pathophysiologic response of vascularised tissue to injury arising from various stimuli including thermal, chemical or physical damage, ischemia, infectious agents, antigen-antibody interactions and other biologic processes (Clark, 2002). No matter the initiating stimulus, the classic inflammatory response is characterised by five clinical signs: calour (warmth), dolour (pain), rubour (redness), tumour (swelling) and functio laesa (loss of function) (Rigler, 1997). The redness is caused by a great increase of blood volume in the inflamed part; the swelling is as a result of increased blood flow and presence of other substances which exude from the blood vessels into the surrounding tissues. The heat results from the increased flow of blood. The pain is often attributed to increased pressure on nerve endings, but the irritating effects of toxic products and certain mediators of the process may be significant (Rigler, 1997). There are three distinct phases of inflammation, 1) an acute phase, 2) a delayed sub-acute phase and 3) a chronic proliferative phase (Rozas and Freitas, 2007). Soluble, diffusible molecules, that can act both locally and systemically, are released during inflammation to intensify and propagate inflammatory response (Mukaida et al., 1998). These mediators are derived from plasma and injured tissue cells or white blood cells.

Platelets are important in inflammation and the role they play in inflammatory processes is being increasingly recognised, in addition to their function in haemostasis and thrombosis (Risa et al., 2007). Platelets accumulate in inflammatory sites concomitantly with leukocytes and regulate a variety of inflammatory responses by secreting or activating adhesion proteins, growth factors, chemokines, cytokine-like factors, and coagulation factors. These proteins induce widely differing biological activities, including cell adhesion, chemotaxis, cell survival and proliferation, all of which accelerate inflammatory processes. Indeed the involvement of platelet has been demonstrated in the pathomechanism of inflammatory disorders, including asthma and arthritis (Risa et al., 2007). Platelet induced chronic inflammatory processes at the vascular wall result in the development of arterosclerotic lesions and arterothrombosis. At the site of vascular lesions, extracellular matrix proteins such as von Willebrand factor, (vWF) and collagen are exposed to the blood. Platelet adhesion to the exposed matrix is considered to be the initial step in thrombus formation. Platelets adhere to vWF via the membrane adhesion receptor glycoprotein [Ib/lx/v (GPIb/lx/x),] (Ruggeri, 2002) and to collagen via GPVI (Nieswandt and Inatson, 2003). This results in platelet activation and transformation of the integrin receptor $\alpha\text{IIb}\beta_3$ (GP11b/111a fibrinogen receptor) (Gawaz et al., 2005) and $\alpha_2\beta_1$ (collagen receptor) (Kahn, 2004), which firmly

binds to the respective extracellular cell matrix component.

Many mechanisms are involved in the promotion and resolution of inflammatory processes (Serhan and Chiang, 2004; Kyriakis and Avruch, 2001). Although earlier studies focused on the promotion of migration of cells out of the microvasculature, recent work has focused on adhesive interactions, including the E-, P- and L- selectins, ICAM-I, VCAM-1 and leukocyte integrins, in the adhesion of leukocytes and platelets to endothelium at sites of inflammation (Meager, 1999). The use of anti-inflammatory agents for the symptomatic relief of infection dates back to the use of aspirin to reduce fever (Mayer and Johnson, 2000).

During inflammation, early intervention with selective anti-inflammatory therapy or with a combination of the appropriate agent at different times will reduce inflammation, preserve organ function and result in an increase in survival rate.

Despite decades of research, corticosteroids and non-steroidal anti-inflammatory drugs (NSAIDs) remain the main pharmacological weapons to control inflammation in the clinic (Miele, 2003). Generally, the class, NSAIDs is applied to drugs that inhibit one or more step in the metabolism of arachidonic acid AA (Brunton et al., 2006). NSAIDs have the potential to relieve pain and inflammation without the immunosuppressive and metabolic side effects associated with corticosteroids. NSAIDs inhibit the enzyme prostaglandin H2 synthase (also called cyclooxygenase or COX) which catalyses an early step in the pathway from arachidonate to prostaglandins and thromboxanes (Miele, 2003). There are two isoforms of COX namely COX-1 and COX-2. The isoform COX-1 expressed in virtually all tissues of the body catalyzes the formation of constitutive PGs, which mediate a variety of normal physiologic effects including homeostasis, gastrointestinal mucosal protection, and protection of the kidney from hypertensive insult (Seibert et al., 1997).

For example PGI₂ released by the endothelial cell participates in the regulation of vascular tone, while PGE₂ and PGI₂ released by gastric mucosa are cytoprotective. The activation of COX-1 also leads to the production of prostacyclin which when released by the vascular endothelium is anti-thrombogenic (Botting, 2006) and when released by the gastric mucosa is cytoprotective.

It is also COX-1 in the platelet that leads to thromboxane A₂ production, causing aggregation of the platelets to prevent inappropriate bleeding (Funk et al., 1991). The other isoform COX-2 is activated in damaged and inflamed tissues and catalyzes the formation of inducible PG, including PGE₂, associated with intensifying inflammatory response (Topper et al., 1996). COX-2 is also involved in thermoregulation and the pain response to injury.

Therefore, COX-2 inhibition by NSAIDs is thought to be responsible for the antipyretic, analgesic, and

anti-inflammatory actions of NSAID (Breden et al., 1995). However, concurrent inhibition of COX-1 and COX-2 may result in many of the unwanted effects of NSAIDs including gastric ulceration and renal toxicity. The leaves of *S. mimosoides* are used in folk medicine to treat swelling of legs during pregnancy. This work is therefore aimed at the pharmacological testing for the anti-inflammatory activity of the leave extract of *S. mimosoides* by comparing its activity with that of a well-known anti-inflammatory drug, using experimental animal model.

Moreover, the probable mechanism of action of the extract will also be determined to know how it exerts its action.

MATERIALS AND METHODS

The plant material used was *S. mimosoides* leaves. They were collected from Ibagwa Roadside Nsukka in Enugu State of Nigeria, during the months of July to August. The plant was characterized and identified by Mr. Ugwuozor of Botany Department of the University of Nigeria Nsukka. Wistar albino rats of either sex weighing between 130 and 250 g, and fed on standard Top Feeds, were obtained from the Animal House, Faculty of Biological Sciences, University of Nigeria Nsukka.

Mice of either sex weighing 13 to 35 g were also obtained from the same source. These animals were given Top Feeds for at least one week after purchase to acclimatise them before using them for the study.

Beef testes were purchased from the local slaughter house in Nsukka main market Nsukka, Nigeria and used as the seminal vesicles, the source of prostaglandin synthase. The blood samples used were collected from healthy adult volunteers from the University of Nigeria Nsukka community. These volunteers were free from all drug treatments for at least two weeks. Egg albumin used in rat paw oedemogenesis was obtained from newly laid chicken egg from Animal science Poultry, University of Nigeria, Nsukka.

Extraction procedure

A known amount (300 g) of *S. mimosoides* leaves was macerated with 1200 ml of distilled water at room temperature. It was then filtered first with calico, and subsequently with glass wool and finally (Whatmann No.4) filter paper (Nwodo, 1981). The filtrate was concentrated by lyophilisation at -30 to -35°C. A brown slurry-like substance was obtained and stored in the refrigerator at -20°C for further analysis.

Assay of biological activity

Acute toxicity and lethality determination

Investigation on the acute toxicity of the extract with estimation of the lethal dose (LD₅₀) was carried out using Lorke's method (1983). Thirteen mice weighing 20 to 30 g were used for the test. In the investigation, three groups of mice containing three mice each were administered 10, 100 and 1000 mg/kg respectively of the aqueous extract intraperitoneally (ip). They were observed closely for 24 h for lethality and other behavioural responses. Based on the results, further increased doses of 1500, 2000, 3000 and 5000 mg/kg were

administered ip to four other mice respectively. They were also observed for 24 h for any death or behavioural changes.

Effect of extract on egg albumin induced rat paw oedema

The rat paw oedema method of Winter et al. (1962) was used for the test. Then increase in the right hind paw volume induced by the sub-plantar injection of fresh egg albumin was used as a measure of acute inflammation. Adult Wistar albino rats of either sex were divided into four groups of five animals each. The animals were allowed to acclimatise for 7 days before the experiment. The animals were then fasted and deprived of water for 18 h before the experiment, to ensure uniform hydration and to minimise variability in oedematous response.

After the deprivation, the right hind paw volume of the rats were measured at time zero (t=0) by water displacement. Then the first two groups received different doses of the extracts (200 and 400 mg/kg) in normal saline administered intraperitoneally. The third group received 100 mg/kg of indomethacin while the last group (control group) received equivalent volume (0.32 ml/kg) of normal saline.

One hour after the administration of test substances, acute inflammation was induced by injecting 0.1 ml of undiluted fresh egg albumin into the sub-plantar of the right hind paw of the rats. The volumes of the paw were measured at 0.5, 1, 2, 3, 4 and 5 h after egg albumin injection. Oedema formation was assessed in terms of the difference in the zero time paw volume of the injected paw and its volume at the different times after egg albumin injection. For each dose of the extract, average inflammation, % inflammation and % inhibition of inflammation were calculated using the following relation.

$$i) \text{ Average inflammation} = (V_T - V_0)$$

where

V_0 = Volume of oedema at time zero (initial time)

V_T = Volume of oedema at time T (0.5, 1, 2, 3, 4, 5 h)

ii)

$$\% \text{ inflammation} = \frac{\text{Average inflammation of treated groups at time T}}{\text{Average inflammation of control at time T}} \times \frac{100}{1}$$

Then

$$\% \text{ inflammation} = \frac{(V_T - V_0)_{grp\ 1,2,3,4,5}}{(V_T - V_0)_{Control}} \times \frac{100}{1}$$

$$\% \text{ inflammation} = \frac{(V_T - V_0)_{control} - (V_T - V_0)_{treated\ grps}}{(V_T - V_0)_{Control}} \times \frac{100}{1}$$

That is,

$$\frac{\text{Average inflammation of control} - \text{Average inflammation of treated grp}}{\text{Average inflammation of control}} \times \frac{100}{1}$$

Effect of extract on prostaglandin synthase activity

The method of assay was a modification of both methods of Yoshimoto et al. (1970) and Flower et al. (1973). Beef seminal vesicle was used as the source of prostaglandin synthase. Nugteren et al.'s (1966) method was used in isolating the enzyme from beef seminal vesicle. The beef seminal vesicle was frozen, partially thawed and freed of fat and connective tissues. A 50 g

quantity of the tissue was weighed out, sliced, allowed to chill in a freezer and then homogenised in 40 ml of 0.02 M Tris-HCl buffer, pH 7.6 for 2 min at 4°C with a blender. The homogenate was centrifuged at 6,000 g for 10 min. The supernatant from the centrifuged homogenate was decanted, centrifuged again at 15,000 g for 10 min at 4°C. The supernatant was decanted and centrifuged at 18,000 g for 10 min and used as the crude enzyme preparation.

A cofactor solution was prepared by mixing 33 mM hydroquinone, 21 mM glutathione and 40 µM haemoglobin in the ratio 1:1:8. Seven milligrams of the crude prostaglandin synthase preparation was weighed into each set of test tubes, 1.5 ml of cofactor solution was added and the mixture was allowed to pre-incubate for 2 min at 37°C. The reaction was then started by the addition of 0.2 ml of substrate (arachidonic acid) and allowed to proceed for 2 min at 37°C. Varying concentrations of extract and buffer were also added to give a total volume of 2.5 ml. The reaction mixture was incubated for 2 min after which the reaction was terminated by the addition of 0.5 ml of citric acid (0.2 M). The tube containing the reaction mixture was extracted twice with 5 ml ethyl acetate and centrifuged at 2,500 x g for 10 min. Each time, 10 ml aliquot of the top organic layer was pipetted out into a clean test tube. The combined ethyl acetate extract was evaporated to dryness on a sand bath under a stream of nitrogen. The residue was dissolved in 2 ml methanol. Then 0.5 ml of 3 M KOH solution was added to the solution and allowed to stand for 15 min. The absorbance of the tests against blanks at 37°C were read at 278 nm. The blanks contained exactly the same substances as in the test solution except that, prostaglandins of the blanks were boiled in cofactor solution and cooled before use (Nwodo, 1981). The experiments were repeated with indomethacin. Enzyme activity was calculated using the following relationship

$$\text{Enzyme activity} = \frac{\Delta A_{278} \text{ min}^{-1} \times 10 \times 2.5 \times 1000}{25.6 \times 9 \times \text{mg enzyme test}}$$

Determination of anti-platelet aggregatory activity

A modification of the method of Born and Cross (1963) was used to determine anti-platelet activity. Blood samples were taken from healthy volunteers. Fresh blood samples (5 ml) were drawn intravenously using 5 ml plastic syringe into plastic tubes containing 0.01 ml of 1% EDTA as an anticoagulant. The tubes were centrifuged at 3000 rpm for 10 min and the supernatant was collected, diluted twice with normal saline and then used as the platelet rich plasma (Nwodo, 1981). Changes in absorption of the platelet rich plasma (PRP) were determined. PRP (0.2 ml), 0.4 ml of 2 M CaCl₂, varying concentrations of normal saline and extract were incubated. The absorbances of the solutions were measured at 520 nm. Changes in absorption at 520 nm were taken at intervals of 2 min for 8 min.

RESULTS

Extraction of *S. mimosoides*

When subjected to cold aqueous extraction, 300 g of *S. mimosoides* (fresh leaves) yielded 5.30% of brown and slurry-like extract. This extract was used in all biological activity determinations.

Biological effects

In this study, the solvent extract was prepared in

physiological solutions and used in the following biological activity determination.

***LD*₅₀**

In the investigation, there was no lethality or behavioural change in the three groups of mice that received 10, 100 and 1000 mg/kg of the extract. Based on this result, further increased doses of 1500, 2000, 3000 and 5000 mg/kg of the extract were administered. There was no change in behaviour or death in animals that received 1500 and 2000 mg/kg of the extract; those that received 3000 and 5000 mg/kg showed weakness and drowsiness but no death was recorded within 24 h of administration.

Effect of extract on egg-albumin induced rat paw oedema

(Figure 1) shows that at zero time the paw volume of the rats before any different treatments were in the following order, indomethacin > Normal saline > 200 mg/kg > 400 mg/kg. This order was maintained at 30 min, 1 and 2 h after the injection of egg albumin. At the 3 h, the paw volume of the control (Normal saline) continued to increase while that of the different doses of the extract showed a decrease. It is also deduced from the table that the aqueous extract of *S. mimosoides* reduce the increases in the paw volume of rats produced by egg albumin. This reveals that it inhibits oedema formation, hence it inhibits inflammation. The percentage inhibition of the first dose which is 200 mg/kg at the third, fourth and fifth hour were 20.25, 56.25 and 78.45% and those of the second dose (400 mg/kg) was 53.16, 91.25 and 96.20% respectively. This observation reveals that the inhibition of the oedema by the extract is dose dependent. The extract significantly ($P < 0.05$) inhibited the rat paw oedema. The percentage inhibition of the second dose is comparable with that of 100 mg/kg indomethacin (66.62, 90.00 and 94.94%) a standard non-steroidal anti-inflammatory drug (NSAID).

Effect of the extract on prostaglandin synthase activity

Assaying prostaglandin synthetase activity with the microsomal fraction of bovine testis and using bamba oil as the substrate gave high absorbance values. (Figure 2) shows that the enzyme activity was high before the introduction of the extract and indomethacin. This activity decreases drastically when the extract and indomethacin was introduced. For instance, at 0.1, 0.2 and 0.3 mg/ml, the enzyme activity was 8.68, 6.24 and 3.93 respectively. The result obtained compares well with that of indomethacin an NSAID which decreased enzyme activity from 6.10 to 3.60 at 0.2 and 0.3 mg/ml.

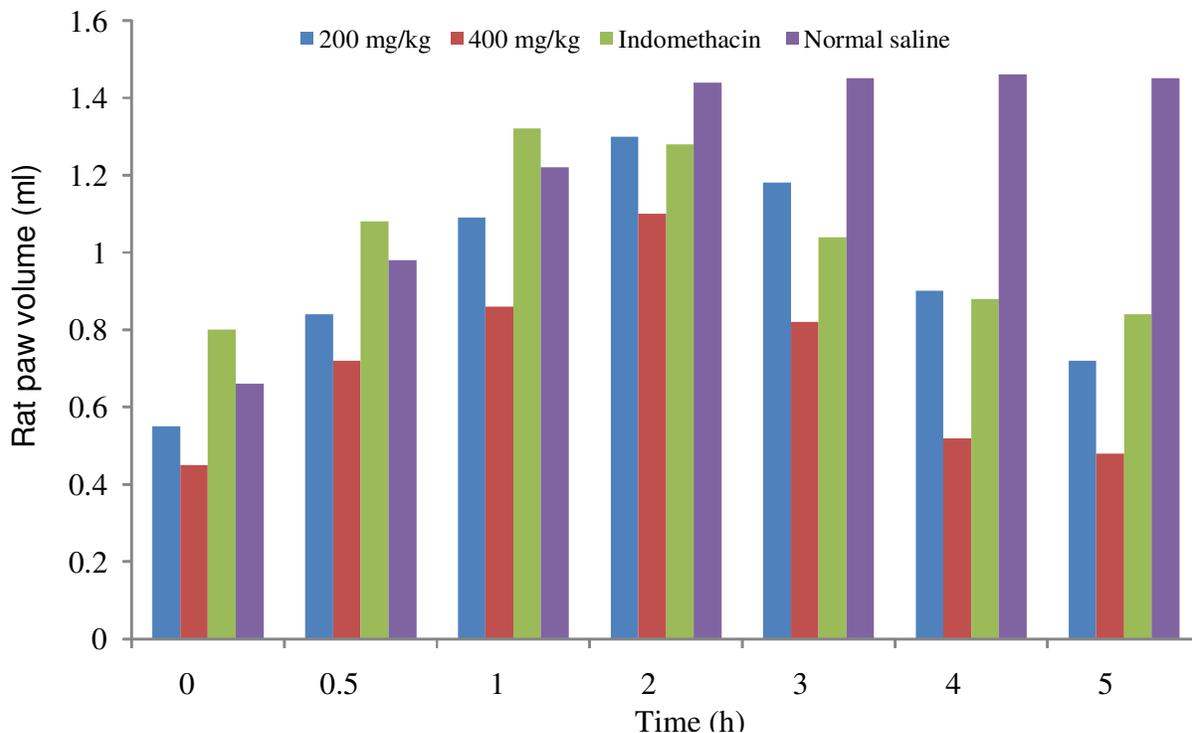


Figure 1. Graph of paw volume against different time for different treatments.

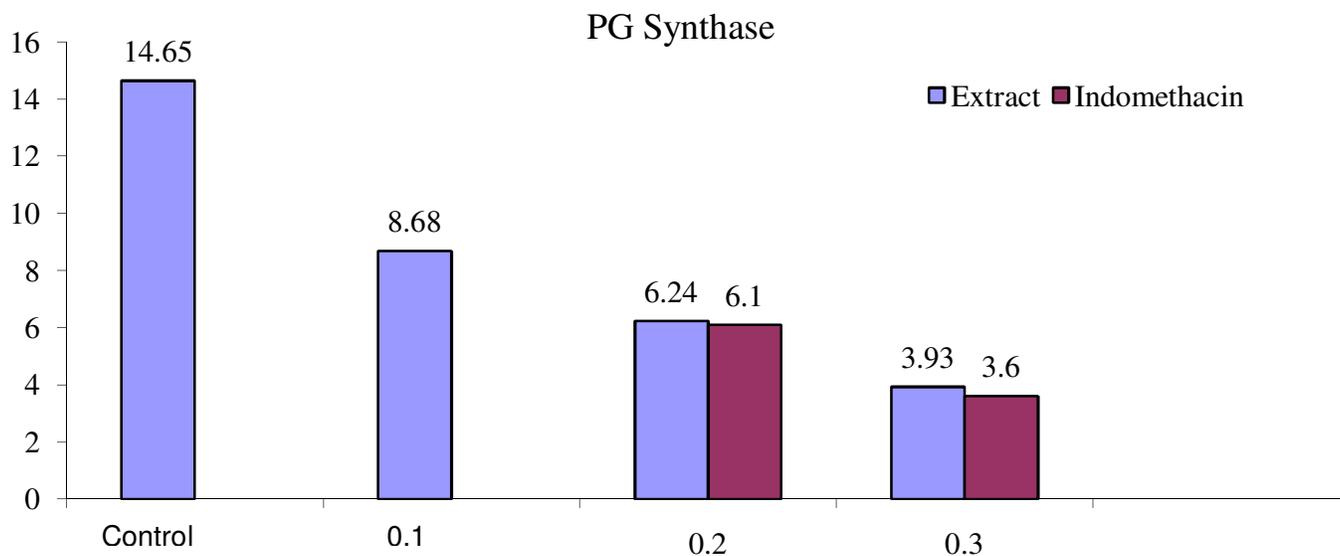


Figure 2. Graph of enzyme activity against different concentration of the extract and indomethacin.

Effect of extract on platelet aggregation

Table 1 shows that the different concentrations of the extract inhibited platelet aggregation induced by CaCl_2 (2 M). The maximum platelet aggregatory activity was attained at the 8th min. The inhibition of platelet by the

extract corresponds with that of indomethacin. For example, 0.2 mg/ml of the extract gave a percentage inhibition of 63.0, 69.7, 70.6 and 72.22% at different time interval (2, 4, 6 and 8 min). As the concentration of the extract increases, platelet aggregation decreases. For instance, at 4 min 0.1, 0.2, 0.4 and 0.6 mg/ml inhibited

Table 1. Effect of extract on platelet aggregation.

T.T	Pla (ml)	CaCl ₂ (ml)	N.S (ml)	Extract (mg/ml)	Indo (mg/ml)		Δ O.D			
1	0.2	0.4	1.9	-	-	0	0.030±0.001 ✦100	0.033±0.001 ✦100	0.034±0.000 ✦100	0.036±0.000 ✦100
2	0.2	0.4	1.8	0.1	-	0	0.024±0.001 ✦ 75	0.027±0.001 ✦81.8	0.028±0.001 ✦82.4	0.029±0.001 ✦83.3
3	0.2	0.4	1.7	0.2	-	0	0.020±0.000 ✦63.0	0.023±0.001 ✦ 69.7	0.024±0.001 ✦ 70.6	0.025±0.000 ✦ 72.2
4	0.2	0.4	1.5	0.4	-	0	0.015±0.001 ✦46.8	0.017±0.001 ✦ 51.5	0.019±0.002 ✦ 55.8	0.020±0.001 ✦58.3
5	0.2	0.4	1.3	0.6	-	0	0.013±0.001 ✦40.6	0.015±0.001 ✦ 45.45	0.017±0.001 ✦ 50.0	0.018±0.001 ✦ 52.8
6	0.2	0.4	1.1	0.8	-	0	0.010±0.002 ✦31.3	0.014±0.000 ✦ 42.2	0.015±0.001 ✦ 44.1	0.016±0.000 ✦ 44.4
7	0.2	0.4	1.7	-	0.2	0	0.013±0.000 ✦ 40.6	0.016±0.000 ✦ 48.5	0.017±0.001 ✦ 50.0	0.018±0.001 ✦ 50.0
8	0.2	0.4	1.5	-	0.4	0	0.009±0.001 ✦28.1	0.012±0.0002 ✦36.4	0.014±0.002 ✦ 41.2	0.015±0.001 ✦ 41.7

P<0.05, n = 5. T.T = Test tube, Indo = indomethacin and N.S = normal saline, pla = platelet
✦ = % Platelet aggregation.

platelet aggregation as follows; 81.1, 69.7, 51.5 and 45.45%, respectively.

DISCUSSION

Even though herbs are claimed to provide relief of pain and inflammation, the therapeutic reputation has to be verified in a scientific manner. In this study, the leaves of such herb, *S. mimosoides* were used. The aqueous extract of *S. mimosoides* was used in assaying for the different biological activities of the plant. The possession of anti-inflammatory properties by the extract would depend on its ability to affect patho-physiological changes accompanying inflammatory diseases. The acute toxicity studies of oral doses of aqueous extract of *S. mimosoides* leaves in mice revealed that it has a high safety profile, as the extract was tolerated by the animals up to 5000 mg/kg. Indigenous drug systems can be a source of a variety of new drugs which can provide relief in inflammation. The most widely used primary test to screen anti-inflammatory agents measures the ability of a compound to reduce local oedema induced in the rat paw

by injection of an irritant agent (Omkar et al., 2007). This oedema depends on the participation of kinins and polymorphonuclear leukocytes with their pro-inflammatory factors including prostaglandins (Damas et al., 1986).

Carageenan induced inflammation is a useful model to detect the oral action of anti-inflammatory agents (Illavarasan, 2005). The assay is a good method for the comparative bioassay of anti-inflammatory agents. The use of carageenan can be said to be synonymous to the use of undiluted egg albumin, also as a model to detect anti-inflammatory agents. In rat paw injected with egg albumin, there was a progressive increase in volume, that is, oedema which peaked at approximately 4 h post injection. (Figure 1) shows that egg albumin is capable of increasing paw volume in rats. The paw volume taken immediately after fasting the animals and soon before the injection of egg albumin was assumed to be at time zero. At this time, none of the rats have received any treatment and the paw volume of the rats that received indomethacin was greater than the ones that received 200 mg/kg of the extract. The rats that received 400 mg/kg of the extract had the least paw volume. This was

Table 2. Effect of extract on platelet aggregation

Parameters	Δ Optical Density (O.D) or Absorbance				
	0 min	2 min	4 min	6 min	8 min
Normal Saline	0	0.030±0.001 * 100	0.033±0.001 * 100	0.034±0.000 * 100	0.036±0.000 * 100
Extract 0.1 mg/ml	0	0.024±0.001 * 75	0.027±0.001 * 81.8	0.028±0.001 * 82.4	0.029±0.001 * 83.3
Extract 0.2 mg/ml	0	0.020±0.000 * 63.0	0.023±0.001 * 69.7	0.024±0.001 * 70.6	0.025±0.000 * 72.2
Extract 0.4 mg/ml	0	0.015±0.001 * 46.8	0.017±0.001 * 51.5	0.019±0.002 * 55.8	0.020±0.001 * 58.3
Extract 0.6 mg/ml	0	0.013±0.001 * 40.6	0.015±0.001 * 45.45	0.017±0.001 * 50.0	0.018±0.001 * 52.8
Extract 0.8 mg/ml	0	0.010±0.002 * 31.3	0.014±0.000 * 42.2	0.015±0.001 * 44.1	0.016±0.000 * 44.4
Indometacin 0.2 mg/ml	0	0.013±0.000 * 40.6	0.016±0.000 * 48.5	0.017±0.001 * 50.0	0.018±0.001 * 50.0
Indometacin 0.4 mg/ml	0	0.009±0.001 *28.1	0.012±0.0002 *36.4	0.014±0.002 * 41.2	0.015±0.001 * 41.7

* = % Platelet aggregation.

Data were presented as mean \pm standard error of the mean (n = 5). *S.E.M.: Standard error \pm mean. Post-hoc LSD test

the order, indomethacin>Normal saline>200 mg/kg > 400 mg/kg. The result showed that 3 h after the injection of the extract and egg albumin, the paw volume of the control (Normal saline) continue to increase while that of the indomethacin and the different doses of the extract showed decrease.

The development of oedema in the paw of the rat after the injection of the egg albumin has been described as a biphasic event (Omkar et al., 2006). The initial phase observed around 1 h, is attributed to the release of histamine, 5-hydroxytryptamine and kinins while the second phase which is the accelerating phase of swelling is due to the release of prostaglandin-like substances. Egg albumin induces paw oedema by inducing protein rich exudates containing a large number of neutrophils (Nitha et al., 2007). These chemical substances produce increased vascular permeability thereby promoting accumulation of fluid in tissues that accounts for the oedema (Umukoro and Ashorobi, 2006; Williams and Morley, 1973; White, 1999). The increase in the paw volume of the rats after the injection of egg albumin correlates with the work of Ilavarasan (2005), that carageenan induces paw oedema in rats.

The present result indicates the potential of *S. mimosoides* leaves as an efficient therapeutic agent in acute inflammatory conditions. With the knowledge that *S. mimosoides* extract inhibits inflammation, the mechanism of action of the extract was studied using different parameters. This *in vitro* study also showed that, the extract inhibited prostaglandin synthase activity implying that the extract is efficacious as an anti-inflammatory substance. The evidence (shown in Table 2) that prostaglandins are potent mediators of inflammation. NSAIDs exert their effect by inhibition of prostaglandin production (Moncada and Vane, 1979; Meade et al., 1993). The pharmacological target of NSAIDs is cyclooxygenase also known as prostaglandin H synthase, which catalyses the first committed step in arachidonic acid metabolism. From (Figure 2), the activity of the enzyme decreased with increase in the concentration of the extract. Since the extract inhibited the sheep seminal enzyme preparation significantly and dose dependently; it is evident that it (this extract) produced its anti-inflammatory effect, at least in part, inhibiting the synthesis of prostaglandins. The concentration of the extract which inhibits the enzyme is almost synonymous

to that of the indomethacin that is at 0.2 mg/ml of the extract, enzyme activity is 6.24 iu, while that of the indomethacin at 0.2 mg/ml is 6.10 iu. This goes a long way to establish the fact that, the extract has the ability to suppress the last phase of inflammation.

The role of platelets in the inflammatory processes is being increasingly recognized, in addition to their function in haemostasis and thrombosis (Risa et al., 2007). Platelets accumulate in inflammatory sites concomitantly with leukocytes (Schmitt-sody et al., 2005) and regulate a variety of inflammatory response by secreting or activating adhesion proteins, growth factors, chemokines, cytokine-like factors and coagulation factors. These proteins induce widely differing biological activities, including cell adhesion chemotaxis, cell survival, and proliferation, all of which accelerate inflammatory process (Gawaz et al., 2005). (Table 1) indicates that the extract inhibited significantly ($P < 0.05$) calcium induced platelet aggregation *in vitro*. Maximum platelet aggregation was attained at the 8th min. The percentage inhibition of platelet aggregation increases with increase in time. This shows that the inhibition of platelet aggregation by the extract is dose dependent implying that as the extract increases, its ability to inhibit platelet aggregation increases too.

Moreover, the inhibition of platelet aggregation by the extract might be due to its ability to inhibit PLA₂ which is the first enzyme needed in the synthesis of thromboxanes from AA. Thromboxanes are known to induce blood vessel constriction and platelet aggregation. Platelet aggregation is brought about by binding of an agonist to a specific receptor on platelet surface. This leads to a release of lipase which converts arachidonic acid to thromboxane A₂ (TXA₂). TXA₂ increases intracellular ionised calcium (Ca²⁺), which promote fusion of dense and alpha granules with the platelet membrane releasing their contents. This result in activating binding sites for specific peptide sequences found on the fibrinogen and von Willebrand factor, vWF. Multiple platelets bind to the same fibrinogen molecule forming a molecular bridge that result in aggregation.

In conclusion, the plant extract reduced the level of inflammatory activities in systemic inflammation. At present, the exact chemical constituent of the extract that is/are specifically responsible for the anti-inflammatory activity of the extract has not been investigated. Identification of the active agent and its effect on the 2 isoforms of COX are still speculative. Therefore, further studies have to be done to identify the active component(s) of the plant. Further investigation should also be carried out to determine if the extract inhibits the two isoforms of COX or if it is a COX selected herb.

REFERENCES

Araujo CAC, Leon LL (2001). Biological activities of *Curcuma longa L.* Memórias do Instituto Oswaldo Cruz., 95: 723-728.

- Born GVR, Cross MJ (1963). Inhibition of aggregation of blood platelets by substances related to adenosine diphosphate. *J. Physiol.*, 166: 29-30.
- Botting RM (2006). Inhibitors of cyclooxygenases: mechanisms, selectivity and uses. *J. Physiol. Pharmacol.*, 57: 113-124.
- Breden CD, Dewith D, Kraig RP (1995). Characterization of inducible cyclooxygenase in rat brain. *J. Compr. Neurol.*, 355: 296-315.
- Bruce RF (2006). Food composition table for food plants in Papua Guinea. Burnie publishers, pp. 1-72.
- Brunton LL, Lazo JS, Parker LK (2006). *The Pharmacological Basis of Therapeutics*. Medical publishers. London, pp. 671-712.
- Clark R (2002). Leucocyte function in inflammation and immunity McGraw Hill Publishers, pp. 1-8.
- Damas J, Remacle VG, Deflandre E (1986). Further studies of the mechanism of counter irritation by turpentine. *Arch. Pharmacol.*, 332: 196-200.
- Flory W, Spainhour B, Colvin B, Herbert CD (1992). The toxicologic investigation of a feed grain contaminated with the seeds of the plant species cassia. *J. Vet. Diagn. Invest.*, 4: 65-69.
- Flower RJ, Cheung HS, Cushman DW (1973). Quantitative determination of prostaglandins and malondialdehyde formed by the arachidonate oxygenase (prostaglandin synthase) system of the bovine seminal vesicle. *Prostaglandins*, 4: 325-341.
- Funk CD, Funk LB, Kennedy ME, Pong AE, FitzGerald GA (1991). Human platelet/erythrocyte cell prostaglandin G/H synthase. cDNA cloning, expression and gene chromosomal assignment. *FASEB J.*, 5: 2304-2312.
- Gawaz M, Langer H, May AE (2005). Platelet in inflammation and atherogenesis. *J. Clin. Invest.*, 115: 3378-3384
- Illavarasan R, Mallika M, Venkataraman S (2005). The anti-inflammatory and anti-oxidant activity of Cassia fistula. *Afr. J. Tradit. CAM*, 2: 70-85
- Juneja D, Shrivastava PN, Guha MK, Saxena RC (2007). Preliminary phytochemical screening of some folklore medicinal plant for their anti-inflammatory activity. *Pharmacogn. Mag.*, 11: 201-203.
- Kyriakis JM, Avruch J (2001). Mammalian mitogen activated protein kinase signal transduction pathways activated by stress and inflammation. *Physiology*, 81: 807-869.
- Mayers I, Johnson D (2000). Septic shock: treating more than just blood pressure. *Can. Med. Assoc. J.*, 162(3): 369-370.
- Meade EA, Smith WC, Dewitt DL (1993). Differential inhibition of prostaglandin endoperoxide synthetase (COX) by non-steroidal anti-inflammatory drugs. *J. Biol. Chem.*, 268: 6610-6614.
- Meager A (1999). Cytokine regulation of cellular adhesion molecule expression in inflammation. *Growth Factor Revolut.*, 9: 27-39.
- Miele L (2003). New weapons against inflammation: dual inhibitors of phospholipase A₂ and transglutaminase. *J. Clin. Invest.*, 111: 19-21
- Moncada S, Vane JR (1979). Mode of action of aspirin-like drugs. *Adv. Int. Med.*, 24: 1-22.
- Mukaida N, Hirada A, Natsushima K (1998). IL-8 and monocyte chemotactic and activating factor. *Cytokine Growth Factor Revolut.*, 9: 9-23.
- Nitha B, Meera CR, Janardhanan KK (2007). Anti-inflammatory and anti-tumour activities of cultured mycelium of morel mushroom, *Morchella esculenta*. *Curr. Sci.*, 92(2): 235-239.
- Nugteren DH, Beerthuis RK, Van DDA (1966). Enzymic conversion of all cis-8, 11, 14-eicosatrienoic acid into prostaglandin E. *Rec. Trav. Chim. Pays-Bas*, 85: 405.
- Nwodo OFC (1981). Elucidation of the nature of some pharmacologically active substances extractible from the seeds of *Abrus precatorius*. A Ph.D Thesis submitted to the Department of Biochemistry, University of London, 40p.
- Omkar A, Jeeja T, Chhaya G (2007). Evaluation of anti-inflammatory activity of *Nyctanthes arbour-tristis* and *Onosma echioides*. *Pharmacogn. Mag.*, 2(8): 258-260.
- Rigler DJ (1997). *Inflammation and Repair*. Veterinary pathology. Williams and Wilkins Publishers, pp. 113-157.
- Risa T, Norito K, Eiichiro U, Hideya Y, Masakazu K, Saburo K (2007). The role of platelet in leukocyte recruitment in chronic contact hypersensitivity induced by repeated elicitation. *Am. J. Pathol.*, 170(6): 219-229.

- Rozas E, Freitas JC (2007). Anti-inflammatory activity of the apolar extract from the sea weed *Galaxaura marginata* (Rhodophyta nemaliales). *J. Venom. Anim. Toxins incl. Trop. Dis.*, 13(2): 544-548.
- Schmitt SM, Klose A, Gottschalk D, Metz P, Gebhard H, Zysk S, Eichhorn ME, Hernandez RTM, Jansson V, Veihelmann A (2005). Platelet endothelial cell interactions in murine antigen-induced arthritis. *Rheumatology*, 44: 885-889.
- Seibert K, Zhang T, Lealy K (1997). Distribution of COX-1 and COX-2 in normal and inflamed tissues. *Adv. Exp. Med. Biol.*, 4000A: 167-170.
- Serhan CN, Chiang N (2004). Novel endogenous small molecules as the checkpoint controller in inflammation and resolution: entrée for resolvin. *Rheum. Infect. Dis. Clin. North*, 30: 69-95.
- Topper JN, Cai F, Falb D, Gimbrone MA (1996). Identification of muscular endothelial genes differentially responsive to fluid mechanical stimuli: Cyclooxygenase-2, manganese superoxide dismutase, and endothelial cell nitric oxide synthase are selectively up-regulated by steady laminar shear stress. *Proc. Nat. Acad. Sci. USA*, 93: 10417-10422.
- Umukoro S, Ashorobi RB (2006). Evaluation of anti-inflammatory and membrane stabilizing property of aqueous leaf extract of *Mormodica charantia* in rats. *Afr. J. Biomed. Res.*, 9: 119-124.
- Winter CA, Risely EA, Nuss GW (1962). Carrageenin induced oedema in hind paw of the rat as an assay for anti-inflammatory drugs. *Pro. Soc. Exp. Biol. Med.*, 111: 544-547.
- Yoshimoto A, Ito H, Tomita K (1970). Cofactor requirement of the enzyme synthesizing prostaglandin in bovine seminal vesicles. *J. Biochem.*, 68: 487-499.