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Anti-inflammatory and Hepatoprotective Potentials of Methanol Leaf Extract of *Arachis hypogea*

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Authors' contributions

This work was carried out in collaboration between all authors. Author RCI designed the experimental protocol. Authors UIE and GCI performed the plant material preparation. Authors CIN and CJN performed the animal study. Authors FOAI and SOE performed the biochemical analysis. Author EUE performed the statistical analysis. Author CEO wrote the first version of manuscript. Author GSA corrected the first version of manuscript and contributed strongly to writing final version of manuscript. All authors read and approved the final version of manuscript.

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ABSTRACT

The anti-inflammatory and hepatoprotective properties of methanol extract of *Arachis hypogea* leaves were studied. At 400 mg/kg, the extract significantly suppressed the development of hind paw oedema induced by egg albumin, which compares favorably with a standard anti–inflammatory drug aspirin (acetylsalicylate) which at 100 mg/kg inhibits egg-albumin induced rat paw oedema. It was observed that the extract significantly inhibited platelet aggregation in a dose

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dependent fashion. At 0.6 and 0.7 mg/ml the percentage inhibition of platelet aggregation was observed to be 53.13 and 68.75% which compare closely to the standard drug indomethacin at 0.2 and 0.4 mg/ml which was found to be 53.13 and 65.63% respectively. Similarly, there was also an observable decrease in the activity of prostaglandin synthase which was dosage dependent at 0.1, 0.5 and 1 mg/ml, the percentage enzyme activity was 65, 70 and 95% respectively. Also, at 1 and 4 mg/ml of indomethacin, the percentage enzyme activity was 94.75 and 99.25% respectively. There was non-significant difference ($P<0.05$) in the selected liver function parameters assayed in all the groups after 21 days of administration. The study shows that the extract may contain anti-inflammatory agents with hepatoprotective properties and may yet serve as template for the development of more synthetic anti-inflammatory agents of clinical significance.

Keywords: Anti-inflammation; Arachis hypogea; hepatoprotective; egg-albumin; aspirin and platelet.

1. INTRODUCTION

Inflammation is a complex pathophysiologic response of vascularized tissue to injury arising from various stimuli, including thermal, chemical or physical damage, ischemia, infectious agents, antigen-antibody interactions and other biologic processes [1]. Inflammation, a fundamental protective response, can be harmful in conditions such as life-threatening hyper-sensitive reactions to insect bite, drugs, toxins, and in chronic diseases, such as rheumatic arthritis, lung fibrosis and cancer [2]. Inflammatory response is brought about or mediated by inflammatory mediators such as chemokines, cytokines, cell adhesion molecules and extracellular matrix proteins [3], which when in excess, are deleterious [4]. During inflammation, early intervention with selective anti-inflammatory drug 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. Miele [5], reported that despite decades of research, corticosteroids and non-steroidal anti-inflammatory drugs (NSAIDs) remain the main pharmacological targets to control inflammation in the clinic. Generally, according to Brunton et al. [6], the class, NSAIDs is applied to drugs that inhibit one or more step in the metabolism of Arachidonic acid. NSAIDs have the potential to relieve pain and inflammation without the immunosuppressive and metabolic side effects associated with corticosteroids.

Liver has a pivotal role in regulation of physiological processes. It is involved in several vital functions such as metabolism, secretion and storage. Furthermore, detoxification of a variety of drugs and xenobiotics occurs in liver. The bile secreted by the liver has, among other things, an important role in digestion. Liver diseases are among the most serious ailment. They may be classified as acute or chronic hepatitis (inflammatory liver diseases), hepatosis (non-inflammatory diseases) and cirrhosis (degenerative disorder resulting in fibrosis of the liver). Liver diseases are mainly caused by toxic chemicals (certain antibiotics, chemotherapeutics, peroxidized oil, aflatoxin, carbon-tetrachloride, chlorinated hydrocarbons, etc.), excess consumption of alcohol, infections and autoimmune/disorder.

Phytochemicals are natural bioactive compounds found in plants, such as vegetables, fruits, flowers, leaves and roots that work with nutrients and fibers to act as a defense system against diseases or more accurately, to protect against disease. However, phytochemicals can influence various body processes [7].

Ojieh et al. [8] reported that herbal medicine in Nigeria is current enjoying a boost. This may be because the nation is host to hundreds of thousands of plants species, many of which have medicinal values. Many of these plants have been exploited while a host of others remain uninvestigated. Researchers have continued to explore the systemic effects of these plant preparations with the intention to discover new drugs and or increase the potency of existing ones. *Arachis hypogea* is one of such medicinal plants that are being used to treat various ailments.

The widespread use of the leaf extract of the plant for the management of diseases in ethnomedicine coupled with its acclaimed anti-inflammatory potential spurred this study, designed to evaluate the hepatoprotective effect and anti-inflammatory potentials of methanol extract of *Arachis hypogea* leaves.

2. MATERIALS AND METHODS

2.1 Plant Material

The leaves of *Arachis hypogea L. (peanut)* were collected in November 2016 from Umudike in Ikwuano Local Government Area of Abia State. Nigeria. A taxonomist, Prof. I.C Okwulehie of Plant Science and Biotechnology. After identification the leaf was deposited with a voucher no MOUAU/COLNAS/PSB/3471 in the departmental herbarium. Michael Okpara University of Agriculture, Umudike.

2.2 Preparation of Plant Material

The leaves of *Arachis hypogea* were collected, washed with distilled water and dried at 35℃ in a hot air oven for 2 weeks. They were then pulverized into coarse powder with a creston high speed milling machine at 15000 rpm. The powdered sample (500 g) was macerated in 2.5 liters' absolute methanol for 48 hours with regular shaking. After that, the resulting extract was wrung out with muslin cloth before filtering through Whatman no. 1 filter paper. The resulting filtrate was concentrated to dryness using rotary evaporator set at 40℃. The dry extract was stored in a refrigerator at 4℃ until used.

2.3 Liver Function Test

Serum AST and ALT were determined according to the method of Reitman and Frankel [9] using Randox kit supplied by Randox commercial kit. Total protein was determined spectrophotometrically as describe by Bradford [10]. The activity of alkaline phosphatase (ALP) was assayed using the method of Kochmar and Moss [11] using Randox kit. The concentration of bilirubin was determined using the method of Jendrassic and Grof [12]. Supplied by Randox commercial kits.

2.4 Assay of Prostaglandin Synthase Activity

2.4.1 Principle

Prostaglandins are mediators of the inflammatory response [13], they have been detected in many inflammatory lesions including inflamed joints, [14,15] skin [16]. The synthesis of prostaglandins is catalyzed by the enzyme prostaglandin synthase which is a microsomal enzyme. This enzyme is responsible for the oxidation of the free fatty acid precursor (Arachidonic acid) forming predominantly prostaglandin E2 (PGE_2) and to a less extent, prostaglandin F (PGF_2) and prostaglandin D (PGD_2). Treatment of the synthesized PGE2 with alkali results in the formation of (PGB) which absorbs maximally at 278 nm. Prostaglandin synthase activity was assayed by the method of Yoshimoto et al. [17].

2.5 Isolation of the Enzyme Containing Fraction

Prostaglandin synthase was isolated from beef seminal vesicle by the method of Nugteren et al. [18] as modification by Yoshimoto et al. [17] The frozen beef seminal vesicle obtained from a local slaughter house was partially thawed and freed of fat and connective tissues. A known quantity (1 g) was weighed out, sliced and homogenized in 5 ml of 0.25 M sucrose-EDTA, pH7.8, for 1 min at 4℃. The homogenate was centrifuged at 6,000 xg for 10 mins. The supernatant was decanted and centrifuged again for 10 mins at 15,000 xg. The supernatant was again decanted and centrifuged at 18, 000 xg for 10 mins. The supernatant of this last centrifugation was then used as the crude enzyme preparation since there was no ultra-centrifuge to actually isolate the microsomal fraction.

2.6 Assay for the Enzyme Activity

The above-stated method of Yoshimoto et al. [17] was used in which there is increase in absorption at 278 nm due to the formation of PGB from PGE by alkali treatment. Materials were added in the order shown in the Table 1.

2.7 Effect of Extract on Prostaglandin Synthase Activity

After addition and incubation, each test tube content was extracted with 5 ml ethylacetate and centrifuged for 5 min. The top organic layer (4 ml) of each tube was taken with pipette into a clean test tube and re-extracted with another 5ml of ethylacetate. The top layers were transferred to clean test tubes and the ethyl acetate evaporated under a stream of nitrogen. The residues were dissolved in 2 ml of ethanol and 0.5 ml of 3M KOH added. The absorbance's of the reaction mixtures were read at 278 nm against their respective blanks at 37℃ after 15 mins.

Table 1. Methodology for prostaglandin synthase activities

Materials and order of addition	Test tubes					Blank test tubes				
	1	2	3	4	5	6	7	8	9	10
Enzyme (ml)	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Cofactors (ml) (33 mM) Hydroquinone, 21 mM, Glutathione and 40 nm, haemoglobin. It was incubated for 2 mins at 37℃	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50
Tris-HCl, Buffer, pH, 8.0 ml	1.20	1.20	1.20	0.80	0.30	1.40	1.40	1.40	1.00	0.50
Indomethacin (ml)	-	0.10	-	-	-	-	-	0.10	-	-
(mg/ml)	-	4.00	-	-	-	-	-	4.00	-	-
It was incubated for 2 mins at 37℃										
Extract (ml)	-	-	0.10	0.50	1.00	-	-	0.10	0.50	1.00
(mg/ml)	-	-	1.00	5.00	10.00	-	-	1.00	5.00	10.00
It was incubated for 2 mins at 37℃										
Substrate (Arachidonic acid in Bambara oil) (ml) incubated for 2 mins at 37℃	0.20	0.20	0.20	0.20	0.20	-	-	-	-	-
Citric acid (ml)	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50
Total volume (ml)	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00

2.8 Anti-inflammatory Test

The rats were divided into four groups of three rats each.

Group 1: Rat paw edema treated with normal saline (normal control).
Group 2: Rat paw edema treated with aspirin 100 mg/kg (standard control).
Group 3: Rat paw edema treated with 200 mg/kg dose of the extract.
Group 4: Rat paw edema treated with 400 mg/kg dose of the extract.

2.9 Anti-inflammatory Test

The anti-inflammatory test was carried out using a philogistic agent- induced rat hind paw edema as a model for acute inflammation [19]. The philogistic agent employed in this study was fresh egg albumin. Twenty (20) adult albino rats of either sex (100-150 g) were divided into four (4) experimental groups of five (5) rats each. They were fasted and deprived of water for 18hrs before the experiment. Deprivation of water was to ensure uniform hydration and to minimize variability in edematous response [20]. Various extract doses (200 and 400 mg/kg) dissolved in normal saline were administered orally to group III and IV of the rats respectively. The control group received equivalent amount of normal saline and the reference group was administered 100 mg/kg aspirin. 1 hr post-treatment, inflammation of the hind paw was induced by injecting 0.1 ml of undiluted fresh egg albumin (philogistic agent) into the subplantar surface of the right hind paw. This treatment was found to cause swelling of the paw which retained about the same degree of edema for 3 hr. Thus (inflammation) was assessed as the difference between zero-time paw diameter and that 3 hours after administration of philogistic agent [21]. The right hind paw diameters of the rats were taken immediately before the experiment (zero-time) and at 1 hour intervals after the injection of egg albumin for a period of 7 hr. Average edema at every interval was assessed in terms of difference in diameter after injecting the philogistic agent (D_t) and zero-time diameter (D_0) of the injected paw i.e. (D_t - D_0). The percentage inhibition of edema was also calculated for each dose using the relation:

$$\% \text{ inhibition of oedema} = -\frac{a-x}{b-y} \times 100$$ Perez [22]

Where:

a = Mean paw diameter of treated rats after egg albumin injection.
x = Mean paw diameter of treated rats before egg albumin injection.

b = Mean paw diameter of control rats after egg albumin injection.
y = Mean paw diameter of control rats before egg albumin injection.

2.10 Determination of Membrane Stability (Hypotonicitv-induced Haemolysis)

Membrane stability was determined using the method of Shinde et al. [23].

2.10.1 Principle

During inflammation, lysosomes lyse to release their component enzymes which causes a variety of disorders. Human red blood cell (HRBC) membranes are similar to lysosomal membranes [24], HRBC membrane stability has, therefore, been used as a method to study the mechanism of action of anti-inflammatory drugs [25]. Hypotonicity-induced haemolysis of red blood cells occurs due to osmotically coupled water uptake by the cells, and leads to swelling and lysis. This results in the release of haemoglobins which absorb maximally at 4l8 nm. Hence the optical density at 418 nm, which is an index of haemolysis, is a reflection of the stability of red blood cell membrane. Ox blood was collected in an abattoir in plastic tubes containing 0.1 cm^3 of 3.8% trisodium citrate and was used within 8 hr. Cell suspension was prepared by the method of Murugesh et al. [25], i.e, by centrifuging 5 ml of the blood sample at 3,000 xg for 10 min and discarding the supernant. The pellet was washed twice by re-suspending it in an equal volume of normal saline and centrifuging at 3,000 xg for 10min to. An aliquot (0.1 ml) of the red cell was introduced into 2.5 ml of normal saline and used as HRBCs. Different concentrations of the extract, normal saline, HRBCs and deionized water were prepared and incubated at 37℃ for 1 hr.

After incubation at 37℃ for 1 hr, the incubates were centrifuged at 3,000 xg for 10 min to terminate the reaction. Their absorbances at 418 nm of the supernatant were measured against the blank. Also absorption spectra were scanned to know the extent of the extract conversion of oxyhaemoglobin (540 nm) to deoxyhaemoglobin (570 nm) and methaemoglobin (630 nm). Reaction media containing normal saline and HRBCs served as isotonic (negative control) and the one containing normal saline, deionized water and HRBCs served as hypotonic (positive control). Blank used was 1.2 ml of normal saline in 0.8 ml of distilled water.

2.11 Determination of Anti-platelet Aggregatory Activity

A modification of the method of Born and Cross [26] was used to determine anti-platelet activity. Blood samples were taken from healthy albino rats. Fresh blood samples were drawn by cardiac puncture 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 [27]. Changes in absorption of the platelet rich plasma (PRP) were determined. PRP (0.2 ml), 0.4 ml of 2 M $CaCl_2$, 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.

2.12 Phospholipase A2 Activity Test

The preparation of phospholipase A2 from *B. pulmilus* and assay of the effect of the extract on its activity were performed using the method of Vane [28]. Aliquots (0.5 ml) of re-suspended erythrocytes were mixed with normal saline containing 2 mM calcium chloride and the enzyme preparation and incubated either in the absence or presence of the extract (0.37, 0.74, 1.10 mg/ml) at 37℃ for 1 h. The incubated reaction mixture was centrifuged at 3,000 g for 10 min and the absorption of the supernatant read against the blank at 418 nm. Prednisolone, a known inhibitor of the enzyme was used as control.

2.13 Experimental Design for Hepatoprotective Study

Fifteen (15) Wister rats of either sex weighing between 150 to 180 g were used for this study. Rats were randomly divided into three (3) groups of five (5) rats each. The Group I served as control and was treated with normal saline (1 ml/kg body weight), while Group II and Group III were treated with 200 and 400 mg/kg body weight of the methanol extract of *A. hypogea* orally respectively on daily basis for twenty-eight (28) days, after which rats were sacrificed on the 28th day and blood was collected by cardiac puncture. Serum obtained was used for liver function test.

2.14 Statistical Analysis

The data obtained from the laboratory tests were subjected to one-way analysis of variance (ANOVA). Differences between means at $p \leq 0.05$ were assessed for significance. The results were expressed as mean standard deviation (SD). The analysis was estimated using the computer software: statistical package for social sciences (SPSS), version 21.

3. RESULTS AND DISCUSSION

3.1 Effect of the Methanol Extract of *Arachis hypogea* Leaves on Prostaglandin Synthase Activity

The extract evoked a significant (P<0.05) dose-dependent inhibition of prostaglandin synthase activity when compared with the standard drug indomethacin.

Fig. 1 shows non-significant (P>0.05) decrease in hind-paw oedema (diameter)cm of the rats administered 200 mg/kg b.wt (group 2) and 400 mg/kg b.wt (group 3) when compared with the positive control group administered 10 mg/kg b.wt of aspirin.

3.2 Effect of Methanol Extract of *Arachis hypogea* Leaves on Membrane Stability (Osmotic Fragility)

Fig. 2 treatment with the two doses of methanol leaf extract of *Arachis hypogea* significantly (p< 0.05) lowered percentage haemolysis in the treatment groups when compared with the

Table 2. The extract evoked a significant (P<0.05) concentration related inhibition of prostaglandin synthase activity

	Concentration (mg/ml)	Absorbance	Inhibition of enzyme activity (%)
Control	-	0.40±0.04	0.00
Extract	0.1	0.14±0.005*	65
	0.5	0.12±0.003*	70
	1.0	0.05±0.001*	87.5
	5.0	0.02±0.006*	95
Indomethacin	1.0	0.021±0.004*	94.75
	4.0	0.003±0.003*	99.25

Values of absorbance shown are Mean ± SEM of triplicate determination.
**P<0.05 compared to the control (ANOVA; LSD post hoc).*
Percent inhibition of enzyme activity was calculated relative to the control

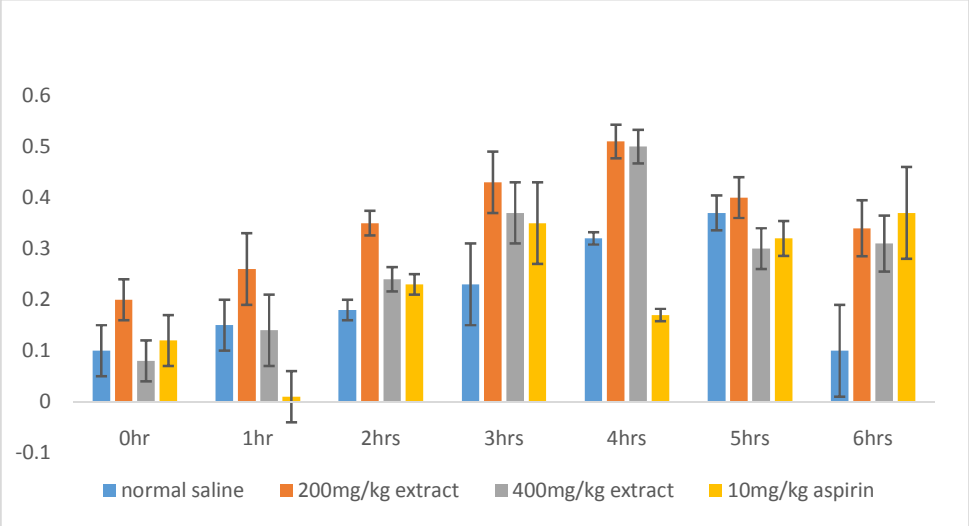

Fig. 1. Effect of methanol extract of *Arachis hypogea* leaves on egg albumin- induced hind paw edema of rats

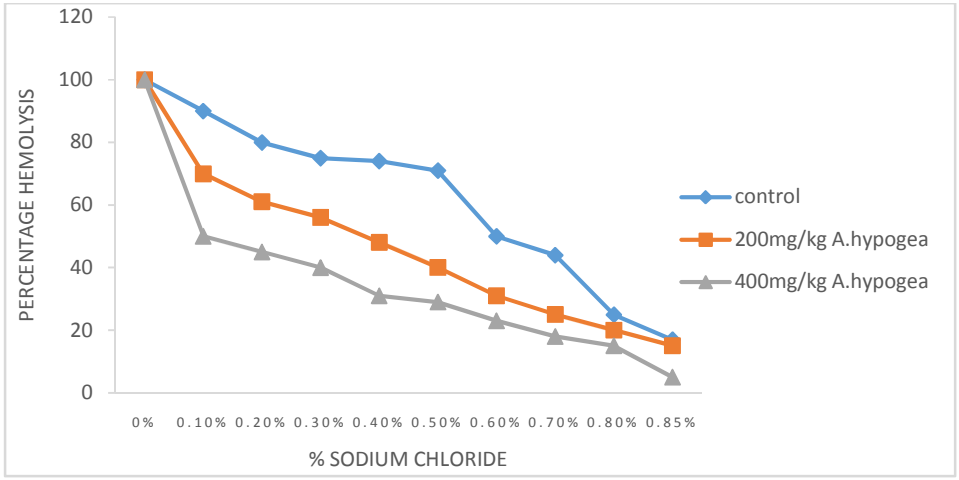

Fig. 2. Effect of methanol leaf extract of *Arachis hypogea* on erythrocyte osmotic fragility

normal control group. However, the animals treated with 400 mg/kg of methanol leaf extract of *Arachis hypogea* had greater RBC membrane stability in NaCl solutions of concentrations 0.1 – 0.5% than those treated with 200 mg/kg of the same extract.

3.3 *In vitro* Effect of Methanol Extract of *Arachis hypogea* Leaves on Platelet Aggregation

Table 3 shows that the different concentrations of the extract inhibited platelet aggregation induced by $CaCl_2$ (2M). The inhibition of platelet by the extract corresponds with that of indomethacin. For example, 0.5 mg/ml and 0.7 mg/ml of the extract gave a percentage inhibition of 53.13 and 68.75 which compare closely to 0.2 mg/ml and 0.4 mg/ml of indomethacin. The result shows that as the concentration of the extract increases, platelet aggregation decreases.

3.4 Effect of the Methanol Extract of *Arachi hypogea* Leaves on Phospholipase A_2 Activity

Table 4 the extract significantly ($P<0.05$) inhibited phospholipase A_2 activity in a concentration-related fashion provoking inhibition comparable to that of prednisolone.

Table 3. *In vitro* effect of methanol extract of *Arachis hypogea* leaves on platelet aggregation

T.T	Pla(ml)	$CaCl_2$ (ml)	N.S	Extract (mg/ml)	Indo	O.D	% Inhibition
1	0.2	0.4	1.9	-	-	0.032±0.001	
2	0.2	0.4	1.8	0.1	-	0.026±0.000	18.75
3	0.2	0.4	1.7	0.2	-	0.022±0.001	31.25
4	0.2	0.4	1.6	0.3	-	0.020±0.001	37.50
5	0.2	0.4	1.5	0.4	-	0.017±0..002	46.86
6	0.2	0.4	1.4	0.5	-	0.015±0.001	53.13
7	0.2	0.4	1.2	0.7	-	0.010±0.002	68.75
8	0.2	0.4	1.7	-	0.2	0.015±0.001	53.13
9	0.2	0.4	1.5	-	0.4	0.011±0.001	65.63

Table 4. Effect of extract on phospholipase A_2 activity

Extract	Concentration (mg/ml)	Absorbance	Inhibition of enzyme activity (%)
Control	-	1.33 ± 0.06	0.00
Methanol extract	0.37	0.64 ± 0.01*	51.88
	0.74	0.14 ± 0.00*	89.47
	1.10	0.02 ± 0.01*	98.49
Prednesolone	1.00	0.11± 0.01*	99.13

Values of absorbance shown are Mean ± SEM of triplicate determination.
**P<0.05 compared to control (ANOVA: LSD post hoc)*

3.5 Effect of the Methanol Extract of *Arachi hypogea* Leaves on Serum Aspartate Aminotransferase Activity of Rats

Fig. 3 shows non-significant ($p>0.05$) increase in serum aspartate aminotransferase activity of the rats administered 200 mg/kg b.wt (group 2) and 400 mg/kg b.wt (group 3) respectively when compared with the control group administered 5 ml/kg b.wt of normal saline.

3.6 Effect of the Methanol Extract of *Arachi hypogea* Leaves on Serum Alanine Aminotransferase Activity of Rats

Fig. 4 shows non-significant ($p>0.05$) reduction in serum alanine aminotransferase activity of the rats administered 200 mg/kg b.wt (group 2) and 400 mg/kg b.wt (group 3) respectively when compared with the control group administered 5 ml/kg b.wt of normal saline.

3.7 Effect of Methanol Extract of *Arachis hypogea* Leaves on Serum Alkaline Phosphatase Activity

Fig. 5 shows non-significant ($p>0.05$) increase in serum alkaline phosphatase activity of the rats administered 200 mg/kg b.wt (group 2) and 400 mg/kg b.wt (group 3) respectively when compared with the control group administered 5 ml/kg b.wt of normal saline.

3.8 Discussion

The use of carrageenan to detect the oral action of anti-inflammatory agents can be said to synonymous to the use of undiluted egg albumin. In rat paw injected with egg albumin, there was a progressive increase oedema which peaked at approximately 3 hours post injection. (Fig. 1) shows that egg albumin is capable of increasing paw volume in rats, this agrees with Ekwueme et al. [29] that 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. The study also show that egg albumin is capable of increasing paw edema 4 hours post induction. The paw width 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 width of the rats that received aspirin was closely similar to the ones that received normal saline. The rats that received 400 mg/kg of the extract had the least paw width. The result showed that 3 hours after oral administration of the extract and egg albumin induction, the paw width of the control (Normal saline) continue to increase while that of the aspirin showed decrease in paw width at 4 hours post induction meanwhile the different doses of the extract showed inconsistency which could be attributed to first-pass hepatic metabolism. During first-pass metabolism, if the extract is rapidly metabolized in the gut wall or liver during this initial passage, then the amount of the unchanged bioactive that gains access to systemic circulation decreases.

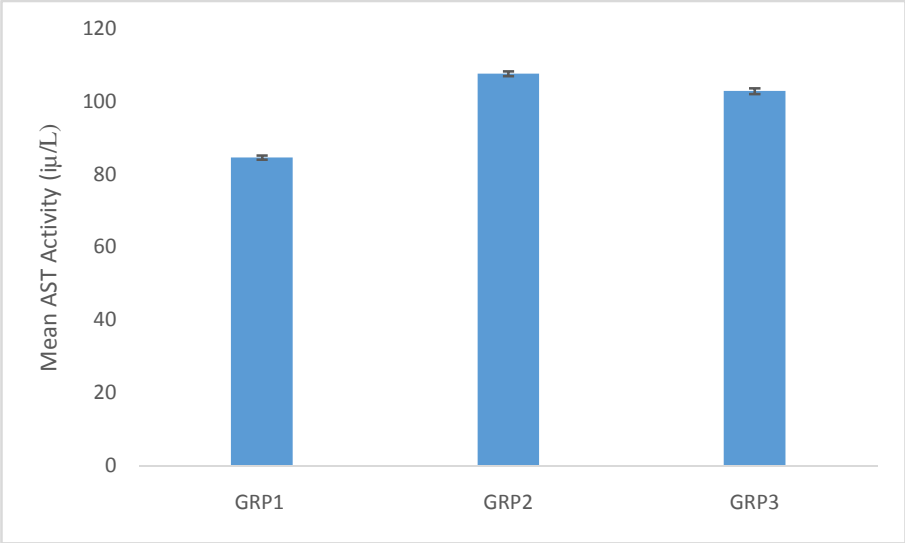

Fig. 3. Effect of methanol leaf extract of *Arachis hypogea* on AST activities

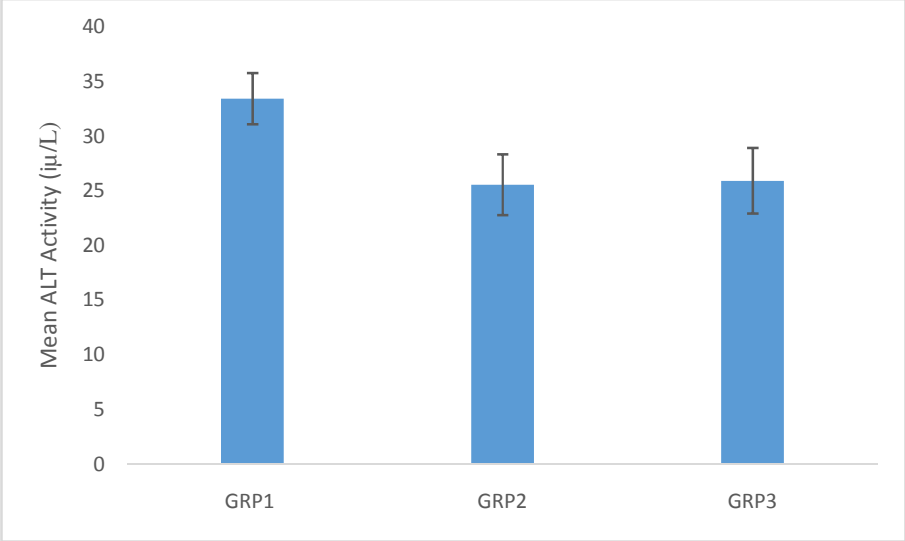

Fig. 4. Effect of methanol leaf extract of *Arachis hypogea* on ALT activities

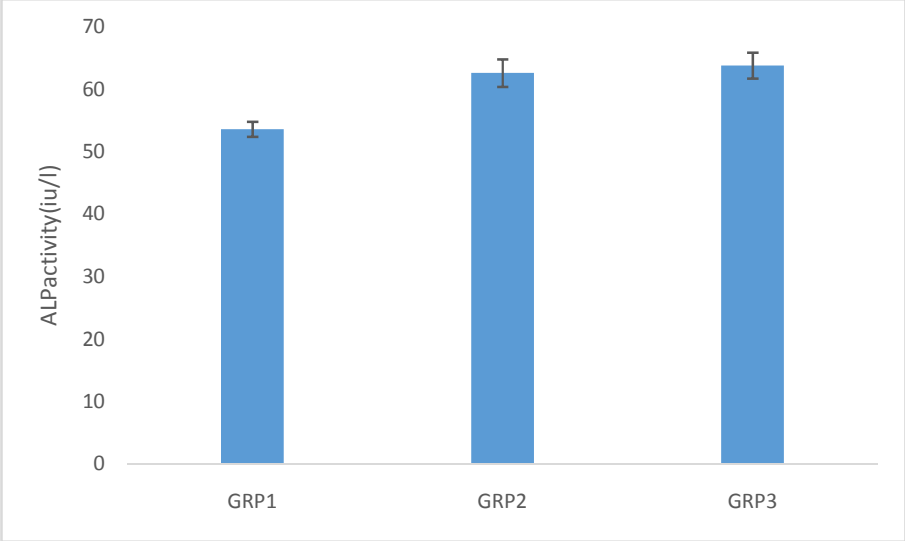

Fig. 5. Effect of methanol leaf extract of *Arachis hypogea* on ALP activities

According to Umukoro and Ashorobi [30], the development of edema in the paw of the rat after the injection of the egg albumin has been described as a biphasic event. The initial phase observed around 1 hr is attributed to the release of histamine, serotonin and kinins while the second phase which is the accelerating phase of swelling is due to the release of prostaglandin-like substances. Williams and Morley [31] reported that Egg albumin induces paw oedema by inducing protein rich exudates containing a large number of neutrophils. These chemical substances produce increased vascular permeability thereby promoting fluid accumulation in tissues that accounts for the oedema [32].

The role of platelets in the inflammatory processes is being increasingly recognized, in addition to their function in haemotasis and thrombosis [33] platelet accumulate in inflammatory sites concomitantly with leukocytes [34] 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 chemotasis, cell survival and proliferation, all of which acceleration inflammatory process [35]. (Table 3) indicates that the extract inhibited significantly ($P<0.05$) $CaCl_2$-induced platelet aggregation in-vitro. The percentage inhibition of platelet aggregation increases with increase in concentration. This shows that the inhibition of platelet aggregation by *Arachis hypogea* is dose dependent implying that as the extract increases, its ability to inhibit platelet aggregation increases too. Maximum platelet aggregation was attained at the 0.7 mg/ml. Moreover, the inhibition of platelet aggregation by the extract might be due to its ability to inhibit PLA_2 (Table 4) which is the first enzyme needed in the synthesis of thromboxanes from Arachidonic acid. 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 ionized 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.

The inhibition of platelet aggregation suggests a probable role of *Arachis hypogea*leaves extract in thrombosis, which in turn prevent aggregation of platelet and thromboembolic disorders that can lead to death.

Consistent with this finding is the inhibitory effect of the extract on prostaglandin synthase isolated from ox seminal vesicles and phospholipase A2 an acyl-hydrolase. These enzymes sequentially mediate the synthesis of prostaglandins. According Foegh and Ramwell, [36], Prostaglandins are synthesized *de novo* by the action of prostaglandin synthase from the free fatty acid precursor, arachidonic acid which is released or mobilized from membrane phospholipids by the action of phospholipase A_2.

Phospholipase A2, cleaves free fatty acids from membrane phospholipids; in this case from erythrocyte phospholipids. The enzyme activity of phospholipase A2 was assayed using its action on erythrocyte membrane on which it creates leakage thus causing hemoglobin to flow out into the medium. The enzyme activity is thus directly related to the amount of hemoglobin in the medium hence the increase in absorbance since hemoglobin absorbs maximally at 418 nm.

Since the extract shows 98% Inhibition of phospholipase A2 at 1.1 mg/ml (Table 4), this suggests that the extract may suppress the synthesis of free fatty acids from membrane phospholipids and consequently deprive prostaglandin synthase of precursors or substrates for the production of prostaglandins. The study agrees with the findings of Iwueke et al. [37].

The sequential inhibition of these two enzymes leads to potent suppression of prostaglandins synthesis and possible amplification of the anti-inflammatory activity of the extract Iwueke et al. [37].

Figs. 4 and 5 shows the result of the effects of methanol extract of *Arachis hypogea* leaves on various aspect of liver functions. The result showed that there were non-significant increase or decrease in the mean levels of alanine aminotransferase (ALT) and Aspartate amino transferase in rats that received 200 mg/kg b.w and 400 mg/kg b.w of the extract respectively, with the control group. Since the ratio of ALT: ALT are indices of hepatocellular integrity. It is indicative that the extract did not damage the liver cells and therefore should not pose a threat to the liver if ingested at the indicated doses. The mean levels of alkaline phosphatase (ALP) and total bilirubin, both of which are indices used to access the excretory function of the hepatocyte, did not also change significantly ($P>0.05$) when compared to the control group.

Results on membrane stability showed that the extract inhibited haemolysis observed in low osmotic medium. This finding is in consonance with the findings of Celine et al. [38] and Agar et al. [39] that an anti-inflammatory drug stabilizes erythrocyte membranes. This then suggest that the leaf extract of *Arachis hypogea* possesses properties which reduces more of metoxhaemoglobin than deoxyhaemoglobin. The stabilization of erythrocyte membrane may be as result of the high content of phenols in the methanolic extract Ibeh et al. [40].

4. CONCLUSION

In conclusion, the results of this study have shown that the leaves of *A. hypogea*

possess anti-inflammatory and hepatoprotective properties mediated by prostaglandin synthesis inhibition and Membrane stabilization. These may contribute to the anti-inflammatory effect. The study also provides empirical evidence for the use of the leaves of *A. hypogea* in folkloric treatment of inflammatory disorders.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All experiments have been examined and approved by the University ethics committee.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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