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Full Length Research Paper

Anti-inflammatory property of the methanol leaf extract of *Parinari kerstingii* (ENGL) in rats

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The anti-inflammatory effect of the extract of *Parinari kerstingii* leaves was investigated using egg albumin-induced rat paw oedema, leukocyte mobilization, and acetic acid-induced vascular permeability assay methods. Heat and hypotonicity-induced heamolysis of human red blood cell membrane were also used to assess its membrane stabilizing effect and to determine its inhibitory property on phospholipase A_2 activity. Acute toxicity and phytochemical tests were also determined using standard methods. The methanol crude extract (MCE) of *P. kerstingii* reduced ($p<0.05$) the acetic acid-induced vascular permeability and increased agar-induced leukocyte mobilization in rats dose-dependently. Vascular permeability was inhibited by 24.85, 26.04 and 48.52% with 100, 200 and 400 mg/kg of the MCE, respectively. The total leukocyte count of the treated groups increased significantly ($p<0.05$) relative to the control group. The percentage membrane stability exhibited by the MCE was comparable with drug control, indomethacin. The MCE contains principles that protected the erythrocyte membranes effectively. More so, the extract inhibited ($p<0.05$) the activities of phospholipase A_2 and showed no significant difference in the phospholipase A_2 inhibitory effect as compared to the standard drug, prednisolone. The extract showed no toxicity at 5000 mg/kg. Phytochemical screenings revealed the presence of tannins, saponins, reducing sugars, phenols, soluble carbohydrates, alkaloids, terpenoids, steroids, hydrogen cyanide glycosides and flavonoids. This study indicated that the MCE of *P. kerstingii* leaf is relatively safe for consumption and has anti-inflammatory property. Also, it could prevent the haemolysis of human erythrocyte membrane.

Key words: Anti-inflammatory, *Parinari kerstingii,* acute toxicity, phytochemicals, membrane stability.

INTRODUCTION

Inflammation is a complex physiological response of vascular tissues to harmful stimuli and the body's protective mechanism to eradicate noxious stimuli and hence promote tissue repairs (Ferrero-Miliani et al., 2007). Activation of inflammation results in the generation of inflammatory mediators such as histamine, kinin,

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and prostaglandin leading to increased blood flow, leakage of plasma proteins and fluids, and migration of neutrophils from blood vessels into sicken tissues (Chaitanya et al., 2011). Chronic inflammation has become the major challenge in the world today and its implications in virtually all human diseases have motivated a global scientific research (Morales et al., 2014). For instance, a lot of inflammatory processes have been revealed to induce insulin resistance, reduce insulin secretion and dysfunction of β-cells (Akash et al., 2013; Uzayisenga et al., 2014). Several herbal drugs contain certain principles with the ability to stabilize biological membranes when exposed to induced lyses (Oyedapo et al., 1997, 2004).

Parinari kerstingii Engl. is an evergreen plant of the genus *Parinari* and family Rosaceae growing up to the height of 20 m with ovoid shaped fruit and is widely distributed in tropical West Africa (Burkill, 1997). It is variously called aramon (Ivory Coast), kakyiki (Ghana), ningelia (Togo), okpe (*Yoruba-Ife* of Togo), and ƙaiƙayi (Nigeria: Hausa). The plant is sometimes harvested from the wild for local medicinal use. It is used traditionally, for the treatment of bronchopneumonia and feverish pains and also serves as emetic and purgative agent (Burkill, 1997). Presently, there is no scientific report on the folkloric use of *P. kerstingii,* thus this research documented the observed anti-inflammatory effect of the MCE of the leaves of the plant in rats.

MATERIALS AND METHODS

All chemicals used in this study were acquired from Sigma Aldrich, Germany.

Plant

Fresh leaf samples of *P. kerstingii* were obtained from Otuku town of Nsukka Local Government Area, Enugu State, Nigeria. These were authenticated by Mr. A. Ozioko of the Bioresources and Development Centre Programme (BDCP), Nsukka. A voucher specimen (Interceded 0615) was deposited at the same centre. The leaves were air-dried and ground using a mechanical grinder. The resulting powdered crude drug (5000 g) was macerated in methanol for 24 h, filtered with a Whatmann No. 1 filter paper and then concentrated using a rotary evaporator (IKA, Germany) at an optimum temperature of 40 to 50°C.

Animals

Swiss albino mice (18 to 28 g) and Wistar rats (110 to 170 g) of both sexes were gotten from the animal house in the Faculty of Pharmaceutical Sciences, University of Nigeria, Nsukka. They were acclimatized to laboratory condition in a seven day period; water and growers mash (Niger Feeds, Nigeria) was given *ad libitum*. Ethical approval on the use of laboratory animals was obtained from the committee of University of Nigeria on the care and use of laboratory animals, in accordance to the revised National Institute of Health Guide for Care and Use of Laboratory Animal (Pub No.85-23, revised 1985).

Phytochemical analysis

Standard qualitative test (Trease and Evans, 2008) was carried out for the detection of alkaloids, glycosides, steroids, terpenoids, flavonoids, tannins, reducing sugars, soluble carbohydrates, resins and saponins.

Acute toxicity test

The method according to Lorke (1983) was used for the study, which was in two phases. Firstly, twelve mice of were grouped into four: Group 1 was not fed with any drug and used as vehicular control. Groups 2, 3, and 4 received 10, 100, and 1000 mg/kg extracts orally in that order. The mice were then observed under 24 h for irregularity in behavior and/or death. The second phase was similarly designed but group 2 mice were administered 1500 mg/kg of the extract while groups 3 and 4 were given the crude extract at 2900 and 5000 mg/kg, respectively. These were also similarly observed for signs of toxicity, and the lethality dose (LD_{50}) was calculated as the square root of the product of the lowest lethal dose and the highest non-lethal dose or the geometric mean of consecutive doses for which 0 and 100% survival rates were recorded.

Egg albumin-induced paw oedema in rats

The procedure by Niemegeer et al. (1964) was adopted. The Wistar rats were grouped into 5, of 5 rats each. They were respectively administered via oral route, distilled water (5 ml/kg) and the crude extract (at 100, 200 and 400 mg/kg). The last group (5) was given 10 mg/kg indomethacin. After 30 min, 0.1 ml of freshly prepared egg albumin was injected into the sub plantar region of the left hind paw of each rat. The paw diameter was measured with the aid of vernier caliper at 0, 1, 2, 3, and 4 h after the injection of egg albumin.

Assay for membrane stabilization

P. kerstingii crude extract was screened for inhibition of haemolysis of HRBC induced by heat; hypotonic solution (distilled water) was evaluated (Shinde et al., 1999) with some modifications.

Heat induced haemolysis

The MCE of the study plant was dissolved in an isotonic phosphate buffer solution. Five centrifuge tubes were each loaded with 5 ml of the extracts in the respective doses of 100, 200, 400, 800, and 1000 µg/ml and arranged in sets of 4 per dose. Two sets of control tubes contained 5 ml of the vehicle and 5 ml of 200 µg/ml of indomethacin, respectively. A 0.1 ml HRBC suspension was gently mixed with the content of each tube. A regulated water bath at 54°C was used to incubate one pair of the tubes, while a second pair was maintained at -10°C in a freezer for 20 min. Next, the tubes were centrifuged at 1300 g for 3 min, followed by the estimation of the haemoglobin content of the supernatant using a Spectronic Spectrophotometer (21D Milton Roy) at 540 nm. Percent inhibition of haemolysis was calculated, viz.:

$$\% \text{ Inhibition of hemolysis} = 1 - \frac{OD2 - OD1}{OD3 - OD1} \times 100$$

where OD1 = absorbance of test sample unheated, OD2 = absorbance of test sample heated, and OD3 = absorbance of control sample heated.

Hypotonicity induced haemolysis

The MCE was reconstituted in distilled water to form a hypotonic solution. Similarly, 5 ml of the graded doses of the extracts (100, 200, 400, 600 and 800 µg/ml), in centrifuge tubes were grouped in pairs per dose. An isotonic solution containing 5 ml of the MCE in the dose of 100 to 1000 µg/ml was also similarly paired (per dose). A 5 ml of the distilled water and another 5 ml of indomethacin (200 µg/ml) were loaded into separate capillary tubes as vehicle and drug control, respectively. A 0.1 ml of erythrocyte suspension was added to each of the tubes and mixed gently. The tubes and contents were then incubated for 1 h at 37°C, and centrifuged for 3 min at 1300 g. Absorbance (OD) of the haemoglobin content of the supernatant was estimated at 540 nm using the Spectronic spectrophotometer. The percentage heamolysis was thus determined by the mathematical expression:

$$\% \text{ Inhibition of haemolysis} = 1 - \frac{OD2 - OD1}{OD3 - OD1} \text{ X } 100$$

where OD1 = absorbance of isotonic test solution, OD2 = absorbance of hypotonic test samples, and OD3 = absorbance of control hypotonic solution. Heamolysis produced in distilled water was taken to be 100%.

Phospholipase A_2 activity test

The phospholipase A_2 was prepared from *Aspergillus niger* and was used to evaluate the effect of the methanol crude extract (MCE) on its activity (Vane, 1971).

Preparation of enzyme

Nutrient agar plates were prepared by dissolving 28.0 g of nutrient agar in 1000 ml of distilled water. The nutrient agar solution was homogenized in a water bath, 100°C, for 10 min and dispensed into sterilized conical flasks. The agar was autoclaved at 121°C for 15 min and then distributed into sterilized petri dishes. *Aspergillus niger* was then inoculated on the plates and left to grow for 3 days. The nutrient broth was prepared by dissolving 15 g of Sabouraud dextrose agar in 1000 ml of distilled water, homogenized in a water bath for 10 min and dispensed into 250 ml conical flasks. The conical flasks were sealed with cotton wool and foil paper. The broth was then autoclaved at 121°C for 15 min. The broth was allowed to cool to room temperature and then the organism in the Petri dishes were aseptically inoculated into the broth and incubated for 72 h at room temperature. The culture was transferred into test tubes containing 3 ml of phosphate-buffered saline, and centrifuged at 3000 g for 10 min. The supernatant was used as the crude enzyme preparation. 5 ml of blood was drawn out from a healthy volunteer and dispensed into plastic tubes containing 0.01 ml of 1% EDTA as an anticoagulant. They were also similarly centrifuged and the supernatant (plasma) was discarded. The erythrocytes were re-suspended in a volume of normal saline and plasma (1:1) and centrifuged to discard the supernatant so formed. This solution of red cells was reformed to 40% (v/v) suspension with phosphate buffered saline and this served as the substrate for phospholipase. 0.2 ml of $CaCl_2$ (2 mM), 0.2 ml of human red blood cell (HRBC), 0.2 ml of the crude enzyme preparation and varying concentrations of normal saline and the test sample or reference drug were incubated in test-tubes for 1 h. The control contained the human red blood cell suspension, $CaCl_2$ and free enzyme. The blanks were treated with 0.2 ml of boiled enzyme separately. The incubation reaction mixtures were centrifuged at a speed of 3000 g for 10 min. Samples of the supernatant (1.5 ml) were diluted with 10 ml of normal saline and the absorbance of the solutions read at 418 nm. Prednisolone was used as the reference drug. The percentage of maximum enzyme activity and percentage inhibition was determined as follows:

$$\% \text{ Maximum enzyme activity} = \frac{OD \text{ of test}}{OD \text{ of control}} \times 100$$

$$\% \text{ Inhibition} = 100 - \% \text{ maximum activity of the enzyme}$$

Leukocyte mobilization test in rats

The method outlined by Ribeiro et al. (1991) was used to check the effect of the MCE *P. kerstingii* on *in vivo* leukocyte mobilization induced by an inflammatory stimulus. Twenty five adult Wistar rats of both sexes (110 to 150 g) divided into five groups of five rats each were used for the test. Groups 3, 4 and 5 were administered varied doses of the extract (100, 200 and 400 mg/kg), while groups 1 (vehicle control) and 2 (treatment control) received distilled water and indomethacin (10 mg/kg), respectively. Three hours after oral administration of the extracts, distilled water or reference drug, each animal in the respective groups received intraperitoneal injection (i.p) of 0.5 ml of 3% w/v agar suspension in normal saline. Four hours later, the animals were sacrificed and the peritoneal cavities washed with 5 ml of a 5% solution of EDTA in phosphate buffered saline (PBS). The peritoneal fluid was recovered and both total and differential leukocyte counts (TLC and DLC) were performed on the perfusates using a manual cell counter after staining with Wright's stain. The percent inhibition of leukoctye migration was calculated using the formula:

$$\% \textit{ Leukocyte inhibition} = 1 - \frac{T}{C} \textit{ X } 100$$

where T represents the leukocyte count of the treated groups.

Vascular permeability test in rats

The effect of the extract on acetic acid induced vascular permeability was assessed by a modification of the method of Whittles (1964). Twenty five adult Wistar rats of both sexes (120 to 170 g) divided into five groups of five rats each were used. The animals were fasted for 10 h prior to the experiment and were then administered with varied doses of the extract and drug as stated earlier. Three hours later, each animal received 0.5 ml intravenous injection of 1% Evans blue solution. Vascular permeability was induced 30 min afterwards, by (i.p) injection of 1 ml of 0.6% acetic acid. The animals were sacrificed 20 min later, and their peritoneum washed with 10 ml of normal saline. The recovered peritoneal fluid was centrifuged and the absorbance of the supernatant measured at 610 nm using a spectrophotometer.

Statistical analysis

The data was expressed as Mean ± standard error of mean (SEM). Analysis of variance (ANOVA) followed by post hoc and Dunnet-t-test was used to statistically analyze the data. P values less than 0.05 ($P<0.05$) were considered as significant.

RESULTS

Acute toxicity

The LD50 > 5000 mg/kg body weight.

Qualitative phytochemical analysis

The paw volume of all the treated groups were significantly (P≤0.05) reduced from the first hour after oedema induction as compared to the control group (Table 2). The extent of oedema inhibition for the treated groups increased with time. *P. kerstingii* extracts at test doses of 100, 200 and 400 mg/kg reduced the egg albumin induced-oedema by 53, 46.7 and 47%, respectively at the 4th hour as compared to the standard drug (51%). In the control group, egg albumin-induced paw oedema was sustained for 2 h after which it reduces significantly (P≤0.05) when compared with the 4th hour.

Table 5 shows the effect of *P. kerstingii* extract on phospholipase activity. The result shows that phospholipase activity of the extract at different doses was non-significant (P≤0.05) as compared to the standard drug, prednisolone.

The extract of *P. kerstingii* leaves produced a significant increase (p≤0.05) in agar induced leukocyte mobilization into the peritoneal cavity. The proportion of neutrophils in the perfusate was higher than lymphocytes and other cells in all the groups. The total leukocyte count of the treated groups increased significantly (p≤0.05) when compared with that of the control.

Intraperitoneal injection of 0.6% acetic acid evoked an increased vasodilation and permeability of the blood vessel of the animals as indicated by the leakage of Evans blue across the epithelial walls of the blood vessel into the peritoneal cavity. Table 7 shows that the *P. kerstingii* extract elicited a significant and dose dependent reduction (p≤0.05) in vascular permeability.

DISCUSSION

Bioactive compounds present in plants exhibit varied biochemical and pharmacological actions in animals when ingested (Nwogu et al., 2008). The extract of *P. kerstingii* leaves revealed the presence of glycosides, reducing sugars, saponins, triterpenes, flavonoids, tannin, soluble carbohydrates, cyanide, steroids, phenols and alkaloids (Table 1). Some of the constituents of the extract have been documented to possess analgesic and anti-inflammatory activities (Park et al., 2001; Okoli et al, 2007).The presence of flavonoids in the leaf of *P. kerstingii* could account for its use as an antinflammatory agent as reported by Ekwueme et al. (2011), prevention of damage caused by free radicals in the body (Dweck and Mitchell, 2002), treatment of diarrhoea (Schuier et al., 2005), *P. kerstingii* leaves, due to its flavonoid content could also be used as antipyretic, analgesic and spasmolytic agents. Flavonoids exhibit dramatic effects on immune and inflammatory cells; these can be either immunosuppressant or immunostimulatory (Huang et al., 2010). The phytochemical analysis of the extract also revealed the presence of alkaloids and this indicates that the leaves could be used in hypertension treatment (Olaleye, 2007). Kumar and Subrahmanyam, (2013) reported that tannins possess immune-stimulating activities. Various plants that contain tannins are used for wound healing (Okwu and Josiah, 2006; Nayak et al., 2007), treatment of dysentery, diarrhoea and urinary tract infection (Okwu and Josiah, 2006; Fahey, 2005). This suggests the possible potential of *P. kerstingii* leaves in the treatment of dysentery, diarrhoea, urinary tract infection and in wound healing. Ekwueme et al. (2011), reported that the saponin content of *senna* might be responsible for its anti-inflammatory properties and for its immunomodulating effect and could as well be used to treat hyperglycaemia, to cleanse and purify blood, treat hypertension (Fahey, 2005), and might also have cholesterol binding properties, and haemolytic activities (Okwu, 2004). This also suggests the possible potential of *P. kerstingii* leaves in the treatment of inflammatory, hyperglycaemia, hypertension, cleansing and purification of blood and as an immunomodulating agent.

The egg albumin-induced inflammatory reactions have been shown to be due to the release of inflammatory mediators (Heller et al., 1998; Nunez-Guillen et al., 1997; Ndebia et al., 2007). Egg albumin causes inflammation of the rat paw similar to carrageenan and the extract inhibited the development of paw edema in the treated animals at 0.5 and 4.0 h post injection of irritant corresponding to the two phases of the inflammatory response. The extract of *P. kerstingii* produced significant acute anti-inflammatory effects on egg albumin-induced paw oedema, which is a model of acute inflammation used in the study of non-steroidal anti-inflammatory agents (Di Rosa et al., 1971). The effects of the extract was most pronounced at 3 h after induction of oedema, an action which was similar to that of the standard drug (indomethacin), suggesting its usefulness in the management of acute inflammation.

The inhibition of haemolysis was not dependent on the doses, increasing with decreased concentration of the extract in the medium and was comparable with that obtained for indomethacin. Membrane stabilization leads to the prevention of leakage of serum protein and fluids into the tissues during a period of increased permeability caused by inflammatory mediators (Chaitanya et al., 2011). It is well known that the vitality of cells depends on the integrity of their membranes (Ferrali et al., 1992). Exposure of red blood cell to injurious substances such as hypotonic medium, phenylhydrazine and heat results in lysis of its membrane and this is accompanied by haemolysis and oxidation of haemoglobin (Augusto et al., 1982; Ferrali et al., 1992). The haemolytic effect of hypotonic solution is related to excessive accumulation of fluid within the cell resulting in the rupturing of its membrane. Such injury to RBC membrane will further render the cell more susceptible to secondary damage through free radical-induced lipid peroxidation (Augusto et al., 1982; Ferrali et al., 1992). This conception is

Table 1. Phytochemical content of methanol extract of *Parinari kerstingii* leaves.

Phytochemical	Bioavailability
Alkaloids	++
Saponins	++
Steroids	+
Terpenoids	++
Glycosides	++
Tannins	++
Flavonoids	+++
Reducing sugar	++
Phenols	+
Hydrogen cyanide	+
Soluble carbohydrate	+

+ Mild; ++ Moderate; +++ Abundance.

Table 2. Effect of extract on egg albumin-induced rat paw oedema.

Treatment	Dose (mg/kg)	0 h	½ h	1 h	2 h	3 h	4 h
Control	-	0.27±0.03	0.68±0.06	0.69±0.05	0.74±0.10	0.69±0.05	0.66±0.035
Indo	10	0.27±0.06	0.66±0.11 ⌂ 82.9 ₳ 17	0.57±0.10 ⌂ 62.5 ₳ 37.5	0.55±0.11 ⌂ 52.8 ₳ 47	0.49±0.06 ⌂ 41.7 ₳ 58	0.49±0.07 ⌂ 48.9 ₳ 51
Extract	100	0.30±0.02	0.61±0.07 ⌂ 65.9 ₳ 34	0.57±0.04 ⌂ 56.2 ₳ 43.7	0.56±0.06 ⌂ 49 ₳ 50.9	0.51±0.07 ⌂ 43.8 ₳ 56	0.51±0.05 ⌂ 47.6 ₳ 53
Extract	200	0.24±0.03	0.57±0.03 ⌂ 78 ₳ 28	0.57±0.02 ⌂ 68.75 ₳ 31	0.53±0.03 ⌂ 54.7 ₳ 45	0.55±0.057 ⌂ 62.5 ₳ 37.5	0.47±0.04 ⌂ 53 ₳ 46.7
Extract	400	0.25±0.04	0.62±0.08 ⌂ 78.7 ₳ 21	0.61±0.02 ⌂ 75 ₳ 25	0.58±0.05 ⌂ 62 ₳ 37.7	0.49±0.05 ⌂ 50 ₳ 50	0.49±0.04 ⌂ 53 ₳ 47

⌂ = % Inflammation; ₳ = % Inhibition of inflammation; Indo = indomethacin.

consistent with the observation that the breakdown of bio-membranes leads to the formation of free radicals which in turn enhance cellular damage (Halliwell et al., 1988; Maxwell, 1995). The human erythrocyte membrane was protected by *P. kerstingii* extract against lysis induced by hypotonic solution and heat. Lyses of lysosomes occur during inflammation, thus releasing their component enzymes that result to various disorders. Non-steroidal anti-inflammatory drugs (NSAIDs) act by inhibiting the release of lysosomal enzymes or by stabilizing the lysosomal membranes (Mounnissamy et al., 2008). Since human red blood cell (HRBC) membranes are like lysosomal membrane components (Mounnissamy et al., 2008), the inhibition of hypotonicity and heat induced red blood cell membrane lysis was employed as a measure of the mechanism of anti-inflammatory activity of *P. kerstingii* extract (tables 3 and 4). Compounds with membrane-stabilizing properties are well known for their ability to interfere with the early phase of inflammatory reactions, namely the prevention of the release of phospholipases that trigger the formation of inflammatory mediators (Aitadafoun et al., 1996) and according to Anosike et al. (2012), anti-inflammatory drugs usually offer protection of erythrocyte membrane against lysis induced by heat and water. These results indicate that the observed anti-inflammatory activities of this plant are related to their membrane stabilization activity and contained principles that protected the erythrocyte

Table 3. Effect of *P. kerstingii* extract on heat induced haemolysis of HRBCs.

Treatment	Conc. (µg/ml)	Mean absorbance±SD at 540 nm		% Inhibition of analysis
		Heated solution	Unheated solution	
Control	-	0.707±0.002		-
Extract	100	0.034±0.060^{b}	0.705±0.013^{ef}	0.3
	200	0.094±0.050^{d}	0.701±0.090^{def}	1.0
	400	0.084±0.408^{c}	0.575±0.002^{b}	21.1
	600	0.076±0.003^{a}	0.591±0.008^{bc}	18.4
	800	0.091±0.001^{a}	0.472±0.052^{a}	38
	1000	0.107±0.005^{a}	0.445±0.044^{a}	44
Indomethacin	200	0.017±0.004^{a}	0.621±0.0014^{bcde}	13.1

Values represent mean ± standard deviation of triplicate sample. Percent inhibition of of haemolysis was calculated relative to control. Mean values, down the column, with different letters as superscripts are considered significant at $p < 0.05$.

Table 4. Effect of *P. kerstingii* extract on hypotonicity induced haemolysis of HRBCs.

Treatment	Conc. (µg/ml)	Mean absorbance±SD at 540 nm		% Inhibition of analysis
		Isotonic solution	Hypotonic solution	
Control	-	0.390±0.04		-
Extract	100	0.236±0.13^{ab}	0.150±0.02^{abc}	69.22
	200	0.147±0.01^{a}	0.167±0.02^{ab}	62.79
	400	0.279±0.07^{b}	0.146±0.01^{ab}	99.30
	800	0.243±0.06^{ab}	0.216±0.02^{c}	69.28
	1000	0.238±0.03^{ab}	0.178±0.03^{abc}	63.04
Indomethacin	1000	0.195± 0.01^{ab}	0.160± 0.02^{ab}	84.48

Values represent mean ± standard deviation of triplicate sample. Percent inhibition of of haemolysis was calculated relative to control. Mean values, down the column, with different letters as superscripts are considered significant at $p < 0.05$.

Table 5. Effect of *P. kerstingii* extract on phospholipase A_2 activity.

Treatment	Conc. (µg/ml)	Mean absorbance ± SD	% Inhibition
Control	-	1.6±0.31^{b}	-
Extract	100	0.81±0.05^{a}	49.1
	200	0.78±0.03^{a}	50.9
	400	0.80±0.04^{a}	49.6
	600	0.86±0.02^{a}	46
	800	0.85±0.06^{a}	46.5
Prednisolone	200	0.76±0.01^{a}	51.8

membranes effectively. The mode of action of the extract and standard anti-inflammatory drug could relate to binding to the erythrocyte membranes followed by alteration of the surface charges of the cells. This might have prevented physical interaction with aggregating agents or promote dispersal by mutual repulsion of like charges which are involved in the haemolysis of red blood cells. It has been reported that flavonoids,

Table 6. Effect of methanol extract of *P.kerstingii* on leukocyte mobilization.

Treatment (%)	Dose (mg/kg)	TLC (×10^4)	Differential leukocyte mobilization (%)				
			Neutrophils	Lymphocytes	Monocytes	Basophils	Eosinophils
Control		7800±25.4^a	59.8±2.6^b	35.6±2.6^a	2.4±0.5^a	-	1.4±0.5^a
Indomethacin	10	12400±3.8^b	61.2±3.1^b	35.2±2.3^a	1.8±0.8^b	-	1.6±0.5^a
	100	11680±9.6^d	62.6±3.7^b	33.6±2.5^a	1.8±0.5^b	-	1.8±0.4^a
Extract	200	15880±1.3^c	64±3.1^{ab}	33.2±2.2^{ab}	1.4±0.5^b	-	1.0±0.0^a
	400	12400±7.9^b	66.8±3.8^a	30.6±2.6^b	1.4±0.5^b	-	1.2±0.8^a

Table 7. Effect of methanol extract of *P. kerstingii* on acetic acid-induced vascular permeability test in rats.

Treatment (%)	Dose (mg/kg)	Absorbance	% Inhibition
Control	-	0.033±0.002	-
Indomethacin	10	0.027±0.001	18.34
	100	0.025±0.003	24.85
Extract	200	0.025±0.001	26.04
	400	0.017±0.001	48.52

triterpenoids and other phenolic compounds exerted profound stabilizing effect on lysosomal membrane both *in vivo* and *in vitro* and possess ability to bind cations, thereby stabilizing erythrocyte membranes and other biological macromolecules (Middleton, 1996; El-Shabrany et al., 1997; Awe et al., 2009; Oyedapo et al., 2004).

Inflammation, pain and fever are all associated with enhanced production of prostaglandins (Rang et al., 2003). Phospholipase A_2 cleaves free fatty acids from membrane phospholipids, for instance, from erythrocyte phospholipids. The phospholipase A_2 enzyme activity was carried out using its action on erythrocyte membrane which created a leakage that resulted in extravasation of hemoglobin 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. Inhibition of phospholipase A_2 suggests that the extract may suppress the synthesis of free fatty acids from membrane phospholipids and hence prostaglandin synthase is depriving of substrates for the production of prostaglandins (Iwueke et al., 2006). Anti-inflammatory and immunosuppressive steroids inhibit arachidonic acid and its metabolites (prostaglandins) by induction of lipocortin which inhibits phospholipase A_2. 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.

Lymphocyte and neutrophils were mobilized than the other leukocytes. Neutrophil mobilization of the groups treated with different concentrations of extract increased in a concentration dependent manner. The increased neutrophil mobilization in our study is in agreement with the report of Vega and Corbi (2006) and Ekwueme et al. (2015) which state that, the number of phagocytic cell increases in the blood stream during injury and are responsible for increase in white blood cell count during infection. The extract triggered an increase mobilization of neutrophil which then fight and eradicate the harmful agent using myeloperoxidase present in the primary granules, lactoferrin and gelatinase present in the secondary granules, to degrade extracellular matrix, digest phagocytosed material, exert antimicrobial activity and initiate inflammation (Dale et al., 2008). The increase in neutrophil by the extract shows its ability to recruiting dendritic cells and monocyte that complete innate clearance of invading microbes and also initiate more specific adaptive immune responses (Vega and Corbi, 2006).

Again, the mobilization of neutrophil by the extract(table 6) indicates that the extract stimulates the generation of respiratory burst, hence generates ROS used in killing microbes. This is consistent with the report of Puga et al. (2012) which states that the mobilization of neutrophil stimulates the generation of respiratory burst by activating nicotinamide adenine dinucleotide phosphate (NADPH) oxidase which generates ROS used in killing microbes. In addition, the extract can also mop up this ROS when they are produced in excess due to its

antioxidant effect. The mobilization of lymphocyte decreased gradually as the concentration of the extract increased.

The inhibitory effect of *P. kerstingii* extract at various doses was comparable to that of indomethacin. Results demonstrated the effectiveness of the methanol extract of *P. kerstingii* against induced vascular permeability and infiltration of inflammatory cells to an injured area as was carried out using acetic acid induced vascular permeability test and agar induced leukocyte migration. Two major components of the inflammatory response mechanism are vascular changes leading to increased vascular permeability and emigration of leukocytes from the circulation to the site of inflammation (Ezike et al., 2015). Inhibition or suppression of any of these reactions could ultimately alleviate the extent and magnitude of the inflammatory response. In this study, intraperitoneal injection of 0.6% acetic acid resulted in an increased dilation and permeability of the blood vessels of the animals which was indicated by the increased leakage of fluids, including Evans blue across the blood vessel epithelial walls. Administration of the extract of *P. kerstingii* caused a significant and dose dependent reduction in vascular permeability of the extract treated animals.

Conclusion

The study revealed that *P. kerstingii* is rich in flavonoids and other phenolic compounds have been associated with decreased risk of developing inflammatory and other related diseases and also has inhibitory effect of vascular changes that occurs during inflammation. It was reported that stimulation of leukocyte by the extract plays a significant role in immune response and that the anti-inflammatory property of the extract is not at the level of leukocyte mobilization (Ekwueme et al., 2015). This is in line with our result which showed stimulatory effect on leukocyte mobilization. It can therefore be deduced from this study that the extract has anti-inflammatory and immunostimulatory effect and could be used in boosting immune response as revealed in the models studied. This study has membrane stabilizing and phospholipase A_2 inhibitory effects, and may offer some beneficial effects on haemolytic diseases and in the management of inflammatory conditions.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

REFERENCES

Aitadafoun M, Mounieri C, Heyman SF, Binistic C, Bon C , Godhold J (1996). "4-Alkoxybenzamides as a new potent phospholipase A2 inhibitors." Biochemical Pharmacology 51:737-742.

Akash HMS, Rehman K, Chen S (2013). Role of inflammatory mechanisms in pathogenesis of type 2 diabetes mellitus. Journal of Cellular Biochemistry 114:525-531.

Anosike CA, Obidoa O, Ezeanyika LU (2012). Membrane stabilization as a mechanism of the anti-inflammatory activity of methanol extract of garden egg (*Solanum aethiopicum*). DARU Journal of Pharmaceutical Science 20:1-7.

Augusto O, Kunze KL. Montellano PR (1982). N-phenylprotoporphyrin formation in the haemogolobinphenylhydrazine reaction. The Journal of Biological Chemistry 257:6231-6241.

Awe EO, Makinde JM, Adeloye OA, Banjoko SO (2009). Membrane stabilizing activity of Russeliaequisetiformis, Schlecht and Chan. Journal of Natural Products 2:3-9.

Burkill HM (1997). The useful plants of west tropical Africa. Vol. 4. Royal Botanical gardens, Kew England P 180.

Chaitanya R, Sandhya S, David B, Vinod KR, Murali S (2011). HRBC Membrane Stabilizing Property of Root, Stem and Leaf of Glochidionvelutinum. International Journal Research of Pharmaceutical and Biomedical Science 2(1):256-259.

Dale DC, Boxer L, Liles CW (2008). The phagocytes: neutrophils and monocytes. Blood 112(4):935-945.

Di Rosa M, Giroud JP, Willoughby DA (1971). Studies of the mediators of the acute inflammatory response induced in rats in different sites by carrageenan and turpentine. Journal of Pathololgy 104 (1):15-29.

Dweck AC, Mitchell D (2002). Emblicaofficinalis [Syn: *Phyllanthus Emblica*] or Amla: the Ayurvedic wonder. Chesham Chemicals Limited. London.

Ekwueme FN, Nwodo O FC, Joshua P E, Nkwocha C, Eluka PE (2015).Qualitative and quantitative phytochemical screening of the aqueous leaf extract of Senna mimosoides: Its effect in *in vivo* leukocyte mobilization induced by inflammatory stimulus. International Journal of Current Microbiology and Applied Sciences 4(5):1176-1188.

Ekwueme FN, Oje OA, Nwodo OFC, Ozoemena NF (2011). Anti-inflammatory capacity of the aqueous leaf extract of *Senna mimosoides* on inhibition of rat oedema, platelet aggregatory activity and prostaglandin synthase activity. Journal of Medicinal Plants Research 5(14):3028-3036.

El-Shabrany OA, El-Gindi OD, Melek FR, Abdel-Khalk SM, Haggig MM (1997). Biological properties of saponin mixtures of *Fagoniacretica* and *Fagoniamollis*. Fitoterapia 62:219-222.

Ezike AC, Onyeto CA, Nwabunike IA, Mbaoji FN, Attah BE, Amanambu SO, Okoli CO (2015). Anti-inflammatory activity of *Buchholziacoriacea* Engl. (Capparaceae) leaf extract: evaluation of components of the inflammatory response involved. Journal of Complementary and Integrative Medicine 12(2):153-158.

Fahey JW (2005). Moringa oleifera: A review of the medical evidence for its nutritional, therapeutic, and prophylactic properties. Trees of Life Journal 15(1):1-15.

Ferrali M, Signorni C, Ciccoli L, Comporti M (1992). Iron release and membrane damage in erythrocytes exposed to oxidizing agents, phenylhydrazine, divicine and isouramil. Biochemical Journal 285:295-301.

Ferrero-Miliani L, Nielson OH, Andersen PS, Girardin SE (2007). Chronic inflammation: importance of NOD2 and NALP3 in interleukin-1β generation. Clinical Experimental Immunology 147(2):227-235.

Halliwell B, Hoult JRS, Bllake DR (1988). Oxidants, inflammation and anti-inflammatory drugs. The FASEB Journal 2:501-508.

Heller A, Koch T, Schmeck J, Van Ackern K. (1998). Lipid mediators in inflammatory disorders. Drugs 55(4):487-496.

Huang R, Yu Y, Cheng W, Yang CO, Fu E, Chu C (2010). Immunosuppressive effect of quercetin on dendritic cell activation and function. The Journal of Immunology 184(12):6815-6821.

Iwueke AV, Nwodo OFC, Okoli CO (2006). Evaluation of the anti-inflammatory and analgesic activities of Vitex doniana leaves. African Journal of Biotechnology 5(20):1929-1935.

Kumar GVP, Subrahmanyam SN (2013). Phytochemical analysis, in-vitro screening for antimicrobial and anthelmintic activity of combined hydroalcoholic seed extracts of four selected folklore indian medicinal plants. Der Pharmacia Lettre 5(1):168-176.

Lorke D (1983). A new approach to practical acute toxicity testing. Archeology of Toxicology 54:275-287.

Maxwell SRJ (1995). Prospects for the use of anti-oxidant therapies.

Drugs 49:345-361.
Middleton JE (1996). Biological properties of plant flavonoids: An overview. International Journal of Pharmacognosy 34:344-348.
Morales G, Paredes A, Olivares A, Bravo J (2014). Acute oral toxicity and anti-inflammatory activity of hydroalcoholic extract from *Lampayamedicinalis* Phil in rats. Biological Research 47:6.
Mounnissamy VM, kavimani S, Balu V (2008). Evaluation of anti inflammatory and membrane stabilizing properties of ethanol extract of *Canjerarehedi.* Iranian Journal of Pharmacology and Therapeutics 6:235-237.
Nayak BS, Raju SS, Orette FA, Rao AVC (2007). Effects of Hibiscus rosasinensis L (Malvaceae) on wound healing activity: A preclinical study in a spraguedawley rat. International Journal of Low and Extreme Wounds 6(2):76-81.
Ndebia EJ, Kamgang R, Nkeh-ChungagAnye BN (2007). Analgesic and anti-inflammatory properties of aqueous extract from leaves of *Solanum torvum*. (solanaceae). African Journal of Traditional, Complementary and Alternative medicines 4(2):240-244.
Niemeger CJE, Verbiuggen FJ, Janssen PAJ (1964). Effect of various drugs on carrageenan induced oedema in rat hind paw. Journal of Pharmacy and Pharmacology 16:310-816.
Nunez-Guillen ME, JA Silva-Emimi, Souccar C, Limdala AJ (1997). Analgesic and anti -inflammatory activities of the aqueous extract of *Plantugo major* (L). International Journal of Pharmacognosy 6:465-472.
Nwogu LA, Igwe CU, Emejulu AA (2008). Effects of Landolphiaowariensisleaf extract on the liver function profile and haemoglobin concentration of albino rats. African Journal of Biochemistry Research 2(12):240-242.
Okoli CO, Akah PA (2004). Mechanism of the anti-inflammatory activity of the leaf extract of *Culcasia scandens*, P. Baeuv. (Araceae) Pharmacology Biochemistry and Behaviour 79:473-481 https://doi.org/10.1016/j.pbb.2004.08.012 PMID15582018.
Okoli CO, Akah PA, Nwafor SV, Anisiob AI, Ibegbunam IN, Erojikwe O (2007). Anti-inflammatory activity of hexane leaf extract of *Aspiliaafricana* C.D. Adams. Journal of Ethnopharmacology 109(2):219-225.
Okwu DE (2004). Phytochemicals and Vitamin content of indigenous species of southeastern Nigerian. Journal of Sustainable Agriculture and the Environment 6 (1):30-37.
Okwu DE, Josiah C (2006). Evaluation of the chemical composition of two Nigerian medicinal plants. African Journal of Biotechnology 5(4):357-361.
Olaleye MT (2007). Cytotoxicity and antibacterial activity of methanolic extract of *Hibiscus sabdariffa*. Journal of Medicinal Plants Research 1(1):009-013.
Oyedapo OO, Akinpelu BA, Orefuwa SO (2004). Anti-inflammatory effect of *Theobroma cacao*, root extract. Journal of Tropical Medicinal Plants 5:161-166.
Oyedapo OO, Akindele VR, Okunfolami KO (1997). Effects of the extracts of Olaxsubscorpioides and *Aspiliaafricana* on bovine red blood cells. Phytotherapy Research 11:305-306.
Park EH, Kahng JH, Lee SH, Shin KH (2001). Anti-inflammatory principles from *cactus*. Fitoterapia 72:288-290.
Puga I, Cols M, Barra CM, He B, Cassis L, Gentile M, Comerma L, Chorny A, Shan M, Xu W, Magri G, Knowles DM, Tam W, Chiu A, Bussel JB, Serrano S, Lorente JA, Bellosillo B, Lloreta J, Juanpere N, Alameda F, Baró T, de Heredia CD, Torán N, Català A, Torrebadell Rang HP, Dale MM, Ritter JM, Moore PK (2003). Pharmacology Fifth Edition Churchill Livingstone, Edinburgh.
Puga I, Cols M, Barra CM, He B, Cassis L, Gentile M, Comerma L, Chorny A, Shan M, Xu W, Magri G (2012). B cell-helper neutrophils stimulate the diversification and production of immunoglobulin in the marginal zone of the spleen. Natural Immunology 13(2):170.
Ribeiro RA, Flores CA, Cunha FQ, Ferreira SH (1991). IL-8 causes in vivo neutrophil migration by a cell dependent mechanism. Immunology 73:472-477.
Schuier M, Sies H, Billek B, Fischer H (2005). Cocoa-related flavonoids inhibit CFTR-mediated chloride transport across T84 human colon epithelia. Journal of Nutrition 135(10):2320-2325.
Shinde UA, Phadke AS, Nair AM, Mungantiwar AA, Dikshit VJ, Saraf VO (1999). Membrane stabilizing activity–a possible mechanism of action for the anti-inflammatory activity of *Cedrusdeodara* wood oil. Fitoterapia 70:251-257.
Trease E, Evans WC (2008). Pharmacognosy, Williams Charles Evans. 15th edition London: Saunders Publisher.
Uzayisenga R, Ayeka PA, Wang Y (2014). Anti-diabetic potential of Panax notoginseng saponins (PNS): A review. Phytotherapy Research. 28:510-516.
Vane JR (1971). Inhibition of prostaglandins synthesis as a mechanism of action for aspirin-like drugs. Nature 231:232-235.
Vega MA, Corbí AL (2006). Human macrophage activation: Too many functions and phenotypes for a single cell type. Immunologia 25:248-272.
Whittle BA (1964). The use of changes in capillary permeability in mice to distinguish between narcotic and nonnarcotic analgesics. British Journal of Pharmacology 22:246-253.