Nigerian Journal of Biochemistry and Molecular Biology 2016; 31(1&2): 53-64

0189-4757/96 $3.0 + 0.00
Printed in Nigeria

Available online at http://www.nsbmb.com.ng/

NJBMB/036/16

Anti-inflammatory Activity of n-Hexane Stem Bark Extract of *Eucalyptus camaldulensis* (Dehnh)

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Abstract: *Eucalyptus camaldulensis* (Dehnh) is used by the Igala people of Nigeria for the treatment of a wide range of febrile ailments suggesting that the plant could contain antipyretic and/or antibiotic principles. Studies have reported that some antibiotics may possess anti-inflammatory effects. In this study, n-hexane extract of *E. camaldulensis*, reputed for its antibacterial effects, was investigated for anti-inflammatory properties. The stem bark was extracted using n-hexane by cold maceration for 24 hours. Animals used in this study were adult Wistar rats (weighing 140–190 g) of both sexes. The extract was investigated for anti-inflammatory properties using a repertoire of approaches *i.e.* egg albumin- induced rat paw oedema, hypotonicity- induced haemolysis, platelet anti-aggregatory activity, *in vivo* leukocyte mobilization, Phospholipase A_2 activity and prostaglandin synthase activity. The extract significantly ($p<0.05$) inhibited prostaglandin synthase and Phospholipase A_2 activities, prevented hypotonicity- induced haemolysis and rat paw oedema. The effects were comparable to those produced by the standard drug, indomethacin. The extract also significantly ($p<0.05$) aggregated protein rich plasma (PRP) in a concentration dependent manner but the increase in aggregation over time (0 to 8 minutes) was not significant ($p>0.05$). Increasing concentrations of the extract resulted in decreased total leukocyte count (TLC) and increased plasma calcium level. The extract demonstrated significant anti-inflammatory action. The mechanism of action of the anti-inflammatory activity may involve cell membrane stabilization; blocking of prostaglandin synthesis, cell degranulation, histamine, serotonin and bradykinin release; reversal of platelet aggregation and dissociation of platelet aggregates; or lowering of mobilised leukocytes.

KEYWORDS: *Eucalyptus camaldulensis*, Anti-inflammatory, Phospholipase A2, Prostaglandin

1.0 Introduction

Eucalyptus camaldulensis is used by the Igala people of Nigeria for the treatment of a wide range of febrile ailments (Musa *et al.*, 2011), suggesting that the plant could contain antipyretic and/or antibiotic principles. It has been found to be effective against some pathogenic microorganisms involved in wounds, burns and skin infections (Babayi *et al.*, 2004). Mouna and Ladjel (2012) reported that essential oils from *E. camaldulensis* had bactericidal effect against *Pseudomonas aeruginosa* and *Escherichia coli*. Acetone, methanol and water extracts of the leaf, stem and bark of *E. camaldulensis* have been reported to have potent *in vitro* antibacterial activity against *Bacillus megaterium, B. subtilis, Staphylococcus aureus, Micrococcus luteus* and *E.coli* (Pandey and Singh, 2014).

Antipyretic drugs reduce elevated body temperature and are known to act centrally on the temperature regulation center in the brain or peripherally, through vasodilation and heat dissipation (Adesokan *et al.*, 2008). They also inhibit the synthesis of prostaglandin E_2 (Flower and Vane, 1972; Kurokawa *et al.*, 1998).

Over time, it has been noted that certain antibiotics, in addition to their anti-infectious effects have immunomodulatory properties that

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improve the long-term outcome of patients with chronic inflammatory pulmonary diseases (Tauber and Nau, 2008). An ever- increasing number of findings have established that antibiotics, macrolides in particular, may generate anti-inflammatory effects (Buret, 2010). Macrolide antibiotics which are active against Gram-positive bacteria also exhibit a broad spectrum of pharmacological effects including anti-inflammatory activity (Ianaro *et al.*, 1999). Tauber and Nau (2008) also revealed that some other antibiotics like tetracyclines, fluoroquinolones and β-lactam exhibit immunomodulatory and neuroprotective effects accompanied by down-regulation of pro-inflammatory molecules such as nitric oxide (NO), interleukin 1-beta (IL-β) and tumour necrosis factor alpha (TNF-α).

In this study, n-hexane extract of *E. camaldulensis*, which is being studied for its antibacterial effects in our laboratory, is investigated for anti-inflammatory properties. This was to provide pharmacological basis, or otherwise, for its application in ethnomedicine.

2.0 Materials and Methods

2.1 Materials

2.1.1 Plants

The plant materials were collected from Anyigba, North-Central Nigeria. They were identified at the Biological Sciences Department, Kogi State University, Anyigba, Nigeria.

2.1.2 Reagents

Reagents used were of Analytical grade obtained from Aldrich Chemical Co. USA, British Drug House, Poole, England and Lab M Ltd. Lancashire, England.

2.1.3 Animals

Adult Wistar rats (weighing 140 – 190 g) of both sexes were obtained from the Animal House of the Biochemistry Department, Kogi State University, Anyigba, Nigeria. They were housed in metal cages in the laboratory and acclimatised for fourteen days prior to the commencement of the experiments. The animals were fed on standard laboratory food and water *ad libitum*. All animals were handled according to the guidelines for the care and use of animals (The ARRIVE, 2010).

2.2 Methods

2.2.1 Preparation of Extract

The sample was then air-dried at room temperature for three weeks and then pulverised using high speed Creston grinder. The pulverised samples were stored in plastic containers in the open laboratory until they were required for analyses. One thousand grammes of the pulverised plant sample was macerated in five litres of n-hexane in a capped vessel for 24 hours. Thereafter, the macerate was filtered through Whatmann filter paper. The filtrate was then concentrated using a rotary evaporator (HEB RE 52-3) and dried on a water bath at 60°C to obtain the n-hexane crude extract.

2.2.2 Animal Experiments

2.2.2.1 Egg Albumin-induced Rat Paw Oedema Model

The rat paw oedema method of Winter *et al.* (1962) was used. The increase in right hind paw (RHP) volume induced by 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 acclimatized for 7 days before the experiment. The rats were then deprived of food and water for 18 hours before the experiment to ensure uniform hydration and to minimize variability in oedematous response. The first two groups received different doses of the extract (200 and 400mg/kg body weight [b.wt.] in 2 ml of normal saline administered i.p. The control group received equivalent volume of normal saline while the standard group received 100mg/kg b.wt. of indomethacin in 2 ml normal saline. One hour later, acute inflammation was induced by the injection of

0.1 ml of undiluted fresh egg albumin into the sub-plantar of the RHP of the rats.

The volume of the paw oedema was then measured at 0.5, 1, 2, 3, 4, and 5 hours after egg albumin injection. Oedema formation was assessed by water displacement in terms of the difference in the zero time volume of the injected paw and its volume at the different times after egg albumin injection.

For each extract treated animals, the mean inflammation, percentage inflammation and percentage inhibition of inflammation were calculated using the following expressions:

Mean inflammation = $V_t - V_0$
where:

V_0 = volume of oedema at time zero
V_t= vol of oedema at time (0.5....5hrs)

% Inflammation =
$$\frac{\text{Average inflammation of treated group at time t} \times 100}{\text{Average inflammation of control group at time t}}$$

% Inhibition of Inflammation =
$$\frac{(V_t-V_0)\text{Control} - (V_t-V_0)\text{Treated group} \times 100}{(V_t-V_0)\text{Control}}$$

2.2.2.2 Hypotonicity-induced Haemolysis

Fresh venous blood samples (5 ml) were collected from healthy volunteers into plastic tubes containing 0.01ml 1% EDTA to prevent coagulation. The tubes were centrifuged at 3000 rpm for 15 minutes. The supernatants were collected, measured and discarded. Twice the volume of normal saline equivalent to the volume of the supernatant was used to re-dissolve the red blood cell pellet for use. Sixteen test tubes were used, 8 for the main test and the other 8 as the respective blanks. The first tube contained 0.1 ml of blood and 2.4 ml of normal saline. Test tube two contained 0.1 ml of blood, 1.9 ml of normal saline and 0.5 ml of distilled water. Test tube three to eight contained the same volumes of blood and distilled water but varying volumes of extracts and normal saline. The final volume of each test tube was 2.5ml. The blank contained same constituents as the test except the RBC. The mixtures were incubated for 1 hour at 37°C, and afterwards, centrifuged for 3 minutes at 1300 x g. Absorbance of the haemoglobin in the supernatant was read at 540 nm using Spectronic 21D (Milton Roy). The percentage haemolysis was calculated by assuming that the haemolysis produced in the presence of distilled water was 100%. The percentage inhibition of haemolysis was calculated using the following expression:

$$\% \text{ Inhibition} = \frac{Abs_2 - Abs_1}{Abs_3 - Abs_1}$$

Where :
Abs_1 = absorbance of isotonic solution,
Abs_2 = absorbance of test samples,
Abs_3 = absorbance of hypotonic solution,

2.2.2.3 Platelet Anti-Aggregatory Activity

The method of Born and Cross (1963) was used. Blood samples were collected from healthy volunteers. Five millilitres of fresh blood samples were drawn intravenously using 5 ml plastic syringe into plastic tubes containing 0.01 microliter of 1% EDTA. The tubes were centrifuged at 3000 rpm for 10 minutes and the supernatant was collected, diluted twice with normal saline and then used as the platelet rich plasma. Varying concentrations of extracts, 0.2 ml of platelet rich plasma (PRP), 0.4 ml of 2M $CaCl_2$ and normal saline were incubated. The absorbance of the solutions was read at 520 nm at intervals of 2 minutes for 8 minutes.

2.2.2.4 In vivo Leukocyte Mobilisation

The method of Ribiero *et al.* (1991) was used to investigate the effect of the extract on *in vivo* leukocyte migration induced by inflammatory stimulus. One hour after oral administration of the extracts (100, 250 and 500 mg/kg body weight), each rat in the groups (n=5) received intraperitoneal injections of 1 ml of 3% (w/v) agar suspension in normal saline. Four hours later, the animals were sacrificed and the peritoneum washed with 5 ml of a 5% solution of EDTA in phosphate buffered saline. The peritoneal fluid recovered and total and differential leukocyte counts (TLC and DLC) were performed on the perfusates. The perfusates in the tubes were drawn up to the 0.02 ml calibration on the white blood cell capillary and then mixed with 0.38 ml of leukocytes

diluting fluid (3% acetic acid) and left for 10 minutes for all the erythrocyte to lyse. Thereafter, the Neubauer counting chamber was filled with the diluted sample and the set-up examined microscopically in oil-immersion under x100 magnification. The total number of white cells in the 4 large squares was counted. The total leukocyte count (TLC) was calculated from the expression:

$TLC = n \times d \times v$

Where n = number of cells counted
d = the dilution factor, (20)
v = volume factor for counting chamber = 1/0.4 (i.e. 2.5)

Therefore: $TLC = n \times 20 \times 2.5$

The Leishman staining technique was used. Thereafter, distilled water, two times the quantity of stain used to flood the film was added; the set-up was rocked gently for 2 minutes and then left for 15 minutes before rinsing off the stain. The slide was left to dry and then examined on the microscope using the oil immersion objective lens of 100 x magnification. The cells were counted and differentiated on morphological basis using tally counter.

2.2.2.5 Phospholipase A_2 Activity

The method described by Nwodo (1989) was used to obtain enzyme preparation from enterobacter. Fresh blood samples (5 ml) were collected from healthy volunteers into plastic tubes containing 0.01 ml of EDTA to prevent coagulation. These tubes were centrifuged at 3000 rpm for 15 minutes. The supernatant was collected and discarded. Twice the volume of normal saline equivalent to the volume of the supernatant was used to suspend the red cell pellet to obtain the human red blood cell (HRBC). Varying concentrations of the extract (0.33 – 2.67 mg/ml), 0.2 ml of 2 mM $CaCl_2$, 0.1 ml of HRBC and 0.2ml of enzyme preparation of enterobacter were incubated for 1 hour. Then, the incubates were centrifuged at 3000 rpm for 10 minutes. Samples of the supernatant were diluted with 10 ml of normal saline and the absorbance of the solutions read at 418 nm. The control contained same constituents as the test except the extract. The blanks also contained same constituents except the enzyme. The percentage maximum enzyme activity and percentage inhibition was calculated using the following expression:

$$\% \text{ maximum activity} = \frac{\text{Absorbance of Test}}{\text{Absorbance of Control}} \times 100$$

% Inhibition = 100 - % maximum activity

2.2.2.6 Prostaglandin Synthase Activity

Prostaglandin synthase was isolated from beef seminal vesicle using the method of Nugteren *et al.*, (1966). The enzyme activity was assayed using a modification of Yoshimoto *et al.*, (1970) and Flower and Vane (1972). A cofactor solution was prepared by mixing 33 mM hydroquinone, 21 mM glutathione and 40 µM haemoglobin in the ratio 1:1:8. A 7 mg of prostaglandin was placed out into each set of test tubes. 1.5 ml of cofactor solution was added and the mixture was allowed to pre-incubate for 2 minutes at 37°C. The reaction was then started by the addition of 0.2 ml of substrate (melon seed oil was used as a source of arachidonic acid) and allowed to proceed at 37°C for 2 minutes. Varying concentrations of extract and buffer were also added to give a total volume of 2.5 ml. The reaction mixture was incubated at 37°C for 2 minutes and then terminated by the addition of 0.5 ml citric acid (0.2M). The reaction mixture was extracted twice with 5 ml of ethyl acetate and centrifuged at 2500 rpm for 10 minutes. Each time 10ml aliquot of the top organic layer was pipetted out into a clean test tube. The combined ethyl acetate extract was evaporated to dryness with sand bath. The residue was dissolved in 2 ml of methanol. Then 0.5 ml of 3M KOH solution was added to the solution and left for 15minutes. The absorbance of the test against blanks at 37°C was read at 278 nm on a Thermospectronic 4001/4 Spectrophotometer. The blanks contained exactly the same constituents as 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 in place of the extracts. Enzyme activity was calculated using the following expression:

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

2.2.2.7 Effect of Extract on Plasma Calcium Concentration

Twenty animals (weighing 164 ± 23 g) were randomized into five groups of four animals each. Group 1 animals each received a single daily dose of 100 mg/kg b. wt. of the extract p.o for 7 days. Groups 2, 3 and 4 similarly received 400, 800 and 1,200 mg/kg b. wt. of the extract for the same duration. The four animals that served as control received 5 ml of normal saline in lieu of the extract for the same period. On the eighth day, blood samples were collected from the animals by cardiac puncture and transferred into EDTA bottles. The samples were centrifuged (1000 rpm for five minutes) to obtain the plasma as the supernatant. The plasma were then analysed for their calcium level using Jenway Flame Photometer.

2.2.8 Data Analysis

The data obtained were analysed by Statistical Package for the Social Sciences version16 (SPSS Inc. Chicago, USA). Student t-test was used to determine the differences between the means of parameters in the respective test and control groups. Analysis of variance (ANOVA) was used to determine the differences in mean between and within groups. P-values < 0.05 were accepted as significant.

3.0 Results

The crude stem bark n-hexane extract of *E. camaldulensis* significantly ($p<0.05$) inhibited the rat paw oedema of male and female albino rats induced by injection of egg albumin. From Table 1, the inhibition was more apparent from 2 hours. However, increasing the concentrations of the extracts from 200 mg/kg b. wt. to 400 mg/kg b. wt. did not lead to corresponding increase in inhibition of the inflammation in the test animals. The extracts of *E. camaldulensis* produced reduction in rat paw oedema that was comparable to that of the reference drug, indomethacin.

Table 2 shows that the extract of *E. camaldulensis* at the concentrations tested significantly ($p<0.05$) inhibited hypotonicity-induced haemolysis. The significantly ($p<0.05$) increased inhibition was concentration dependent manner.

Table 3 shows that the absorbance of the PRP treated with the extract of *E. camaldulensis* increased over the period of eight minutes, but the increase over time was not significant ($p>0.05$). The absorbance however, increased significantly ($p<0.05$) as the concentration of the extracts increased.

Compared with the control animals, the total leukocyte count (TLC) of the animals treated with 100 mg/kg b. wt. of extract of *E. camaldulensis* increased. Thereafter, it decreased with increasing concentration of the extract. There was gradual decrease in the percentage of neutrophils mobilised with increasing concentration of extracts used in the treatment of the animals. However, there was an increase in the percentage of lymphocytes mobilised as the concentration of extract used for the treatment of the animals increased (Table 4).

The extract of *E. camaldulensis* induced significant ($p<0.05$) concentration dependent inhibition of the activity of Phospholipase A_2 activity when compared with controls (Table 5). Varying the calcium level affects the inhibitory effects of the extract of *E. camaldulensis*. The percentage inhibition of the activity of Phospholipase A_2 activity by a fixed concentration of extract of *E. camaldulensis* was inversely proportional to the concentration of calcium (Figure 1). There was a significant ($p<0.05$) decrease in the inhibitory activities of extract of *E. camaldulensis* on phospholipase A_2 activity with increasing concentrations of calcium.

The activity of the prostaglandin synthase decreased with increasing concentrations of extract of *E. camaldulensis* (Table 6). Compared to the control, the extract of *E. camaldulensis* significantly ($p<0.05$) inhibited

Table 1: Percentage inhibition of egg albumin induced rat paw oedema by n-hexane extract of *E. camaldulensis* stem bark

Treatment	Dose (mg/kg b.wt.)	Sex	t = ½			t = 1			t = 2			t = 3			t = 4			t = 5		
			A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
Indomethacin	**100**	M	1	100	0	2	130	0	1	50	50	0	0	100	0	0	100	0	0	100
		F	1	66	33	1.5	75	25	0.5	20	80	0	0	100	0	0	100	0	0	100
***E. camaldulensis* Extract**	**200**	M	2	200	0	1	66	33	0.5	25	75	0	0	100	0	0	100	0	0	100
		F	1	66	33	1	50	50	1	33	66	0.5	17	83	0	0	100	0	0	100
***E. camaldulensis* Extract**	**400**	M	1.5	150	0	1	66	33	0.5	25	75	0	0	100	0	0	100	0	0	100
		F	1	66	33	1	50	50	0	0	100	0	0	100	0	0	100	0	0	100

t= time in hours; A= average inflammation; B= % inflammation; C= % inhibition of inflammation

Table 2: Percentage inhibition of haemolysis by n-hexane extract of *E. camaldulensis* stem bark

Tube	Concentration (mg/ml)	Inhibition of Haemolysis (%)
3	12.5	22.51
4	25	25.28
5	50	26.61
6	100	36.28
7	200	44.92
8	400	51.12

Table 3: Anti-platelet aggregatory activity of n-hexane extract of *E. camaldulensis* stem bark

Concentration (mg/ml)	Absorbance			
	2 minutes	4 minutes	6 minutes	8 minutes
50	0.767	0.881	0.892	0.901
100	0.831	0.918	0.931	0.94
200	0.918	0.947	0.956	0.963
400	0.938	0.979	0.989	0.997

Table 4: Effect of n-hexane extract of *E. camaldulensis* stem bark on *in vivo* leukocyte mobilisation in rats

Treatment (mg/kg b wt)	Differential Leukocyte Mobilisation (%)			
	TLC	Neutrophils	Lymphocytes	Monocytes
Normal saline	400	57	42	1
100	600	62	38	0
250	400	61	39	0
500	350	54	45	1

Table 5: Inhibition of phospholipase A_2 activity by n-hexane extract of *E. camaldulensis* stem bark

Treatment	Concentration of Extracts (mg/ml)	Absorbance	% Maximum Activity	% Inhibition
Control	-	2.042 ± 0.13	100	0.00
E. camaldulensis	0.33	1.872 ± 0.01	84.32	15.68
E. camaldulensis	0.67	1.851 ± 0.01	83.38	16.62
E. camaldulensis	1.00	1.506 ± 0.02	67.84	32.16
E. camaldulensis	1.33	1.26 ± 0.02	56.76	43.24
E. camaldulensis	2.00	0.763 ± 0.00	34.37	65.63
E. camaldulensis	2.67	0.165 ± 0.01	7.43	92.57

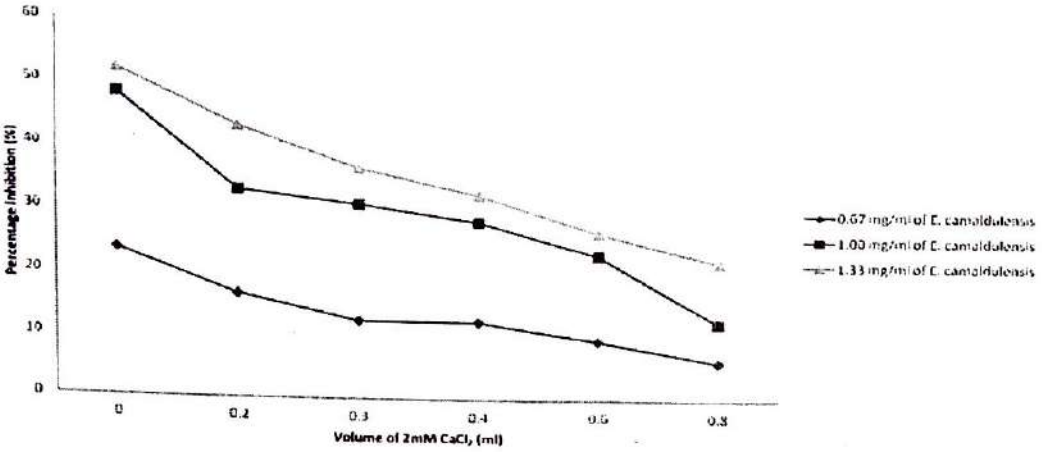

Figure 1: Phospholipase A_2 activity inhibition by n-hexane extract of *E. camaldulensis* stem bark

prostaglandin synthase activity. However, weight for weight, Indomethacin, demonstrated a higher inhibition of the enzyme than the extract of *E. camaldulensis*.

The plasma calcium levels increased with increasing concentration of the extracts (Table 7).

Table 6: Inhibition of prostaglandin synthase activity by n-hexane extract of *E. camaldulensis* stem bark

Treatment	Conc (mg/ ml)	Abs	EA	% I
Control	-	0.173	37.54	0.00
E. camaldulensis	0.1	0.099	21.48	42.78
E. camaldulensis	0.2	0.101	10.96	70.80
E. camaldulensis	1.0	0.296	6.42	82.90
Indomethacin	0.8	0.154	4.18	88.87

Conc = Concnetration; Abs = Absorbance; EA = Enzyme Activity; %I = Percentage Inhibition

Table7: Effect of n-hexane extract of *E. camaldulensis* stem bark on plasma calcium concentrations of rats

Group	Treatment (mg/kg b wt)	Calcium Concentration (ppm)
Control	Normal saline	5.44
E. camaldulensis	100	5.64
	400	6.66
	800	6.56
	1200	5.86

4.0 Discussion

The possession of anti-inflammatory properties by an extract would depend on its ability to affect patho-physiological changes accompanying inflammatory diseases (Ekwueme *et al.*, 2011). 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). Irritant-induced rat paw oedema is associated with three distinct phases. The first phase is mediated by mast cell degranulation and histamine and serotonin release (1 hour), the second phase (60 to 150

minutes) is characterized by bradykinin release and pain, and further eicosanoid production in the late phase (3-4 hours).

At one hour post-injection, the extracts of *E. camaldlensis* exhibited 33 to 50% inhibition of the rat paw, which was higher than that produced by indomethacin, a reference drug. This suggests that the extract may have the capacity to inhibit mast cell degranulation as well as histamine and serotonin release. The extract may also be able to carry out inhibition of bradykinin release and eicosanoid production since the extract markedly inhibited rat paw oedema at 2 hours post injection and beyond. These suggest that the n-hexane extract of *E. camaldulensis* is capable of inhibiting inflammation and hence possesses anti-inflammatory activities. However, increasing the concentrations of the extracts from 200 mg/kg b. wt. to 400 mg/kg b. wt. did not lead to corresponding increase in inhibition of the inflammation in the test animals, but the extract produced rat paw oedema reduction comparable to that of the reference drug, indomethacin.

Since the red blood cell (RBC) membrane is similar to that of lysosomal membrane, inhibition of RBC haemolysis will therefore provide good insights into the inflammatory process (Umukoro and Ashorobi, 2006). Injury to lysosomal membranes usually triggers the release of phospholipase A_2 that mediates the hydrolysis of phospholipids to produce inflammatory mediators (Aitadafoun *et al.*, 1996). Perez *et al* (1995) and Shinde *et al.* (1999) have shown that compounds with membrane-stabilizing properties possess anti-inflammatory activities. The extract inhibited hypotonicity-induced haemolysis, thus have membrane stabilizing properties. This suggests that n-hexane extract of *E. camaldulensis* possesses anti-inflammatory properties. The stabilisation of the membrane may be a probable mechanism for the antibacterial activity of the plant extract.

Platelets are essential for normal haemostasis, and activation of the clotting cascade by trauma results in platelet activation, which is followed by aggregation (Sur *et al.*, 2003). When blood is centrifuged to obtain PRP and an agonist is added, the platelets aggregate and absorb less light so that transmission increases. Reagents such as thrombin, collagen, ADP and $CaCl_2$ bind to specific platelet membrane receptors, activating platelets and triggering series of reactions that culminate in shape change, granule release and aggregation, which are inflammatory actions (Chanarin, 1989). Whether any of these responses occurs, it will still depend on the normal platelet function, the concentration of the agonist used and the levels of certain inhibitory substances. The presence of an inhibitory substance would lead to increased absorbance by PRP, and therefore reversal of the aggregation process. This may contribute to the anti-inflammatory process. Extract of *E. camaldulensis* induced increases in absorbance of PRP. These increases in absorbance of the PRP treated with extracts were concentration dependent. This is an evidence that extracts of *E. camaldulensis* could dissociate aggregates of platelets thereby enhancing easy perfusion of tissues and consequent delivery of nutrients, oxygen and hormones to distant parts.

Cell migration serves several important roles during physiological and pathological processes (Lemen *et al.*, 2012). During an inflammatory reaction, leucocytes are mobilised from the blood to the inflammatory lesion (Wandall, 1985). As part of their defensive roles during inflammation, these cells release their lysosomal contents such as bactericidal enzymes and proteases (Okoli *et al.*, 2008). This is usually a beneficial response, but can also lead to negative consequences (Norman and Kubes, 2005) causing further tissue damage and inflammation. Treatment with the extract induced increased mobilisation of the total leukocyte, the count of which, however, decreased with increasing concentration of the extract. As can be seen from the foregoing, the extracts demonstrated potent anti-inflammatory activity; this could be responsible for the reduction in the total leukocyte mobilised to the site of agar injection. The biphase effect may be explained as follows: at lower concentrations, the extracts exhibit their antibacterial activity by mobilising phagocytic leukocytes to the site of infection. Being a potent anti-inflammatory agent, high concentrations of the extract will lead to lowering of the leukocyte mobilised, as leukocyte mobilisation is an inflammatory process. Partida-Sanchez *et al.*

(2001) demonstrated that the inability of neutrophils to directionally migrate to the site of infection in mice renders the mice susceptible to bacterial infections. In chronic inflammation, the acute process characterised by neutrophil infiltration gives way to predominance of mononuclear phagocyte and lymphocytes (Gallin, 1993). The mobilization of neutrophils in higher percentage than lymphocytes and monocytes to the site of inflammation by the extract of *E. camaldulensis* suggests that the extract is capable of preventing bacterial infection (antibacterial activity) and chronic inflammation (anti-inflammatory activity).

Phospholipids are a major constituent of cell membranes. Cellular phospholipases present in leukocytes and platelets are activated during inflammation and degrade phospholipids to arachidonic acids and other free fatty acids, which can be metabolized to prostaglandins and leukotrienes (Pinckard *et al.*, 1992). Thus, the activity of phospholipase is a pro-inflammatory action. The extract of *E. camaldulensis* inhibited phospholipase A_2 activity significantly in a concentration dependent manner. Phospholipase A_2 (PLA_2) activity is calcium dependent, results of varying calcium levels on extract-induced inhibition of Phospholipase A_2 activity at constant extract concentration revealed that the inhibitory action of the extract on phospholipase A_2 is inversely proportional to the concentration of calcium present in the medium. The inhibition of the PLA_2 by the extracts may not be due to lowering of calcium levels.

Prostaglandins are known inflammatory mediators (Vane, 1976; Ricitto and Fitzgerald, 2011). They are C_{20} fatty-acid derivatives found in almost all tissues in the human body. Prostaglandins also promote inflammatory disorders e.g. drugs that block prostaglandin synthesis are effective against arthritis and similar diseases, and both Vane (1971) and Nwodo (1989) and others believe that inhibition of prostaglandin synthase may account for anti-inflammatory effect of aspirin-like drugs. In addition, it has been shown that antibiotic substances have capacity to inhibit prostaglandin synthesis. The n-hexane extract of *E. camaldulensis* caused inhibition of the activity of prostaglandin synthase in a concentration dependent manner. This suggests that the extract have both anti-inflammatory and antibiotic activities. Another species of Eucalyptus, *E. globulus* has been reported to promote anti-inflammatory actions by inhibiting phospholipase A_2 and prostaglandin synthase activities (Miguel, 2010).

The mechanism of action of the anti-inflammatory activity of the extract may involve cell membrane stabilization; blocking of prostaglandin synthesis, cell degranulation, histamine, serotonin and bradykinin release; reversal of platelet aggregation and dissociation of platelet aggregates; and or lowering of leukocytes mobilised.

In conclsion, crude n-hexane extract of *Eucalyptus camaldulensis* demonstrated significant anti-inflammatory potentials. The mechanism by which the extract brings about the anti-inflammatory action should be investigated.

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