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ANALGESIC AND ANTI-INFLAMMATORY EFFECTS OF ETHANOL EXTRACT OF *ENANTIA CHLORANTHA* STEM-BARK IN RODENTS

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ABSTRACT

Aim of study: To determine the anti-inflammatory, analgesic and anti-oxidant activities of ethanol extract of the stem bark of *Enantia chlorantha* in rodents in Akwa Ibom State Nigeria. **Materials and methods**: A one percent solution of carrageenan, xylene and fresh egg albumin were used in-vivo to test inflammation in Swiss albino mice. Acetic acid (0.1ml of 3% solution), 2.5% formalin solution (0.9% formaldehyde) made up in phosphate buffer solution (PBS concentration: NaCl 137 mM; KCl 2.7 mM and phosphate buffer 10 mM) and an electronic hot plate kept at 45± 1^0C throughout the experiment was used to evaluate the analgesic properties of the extract. The free radical scavenging ability of the extract against 1-Diphenyl-2-Picrylhydrazyl radical (DPPH) in methanol was evaluated. **Results**: The extract showed significant ($p<0.01$-0.001) dose-dependent reduction in inflammation in carrageenan induced edema and fresh egg albumin induced edema relative to control. The standard drug used was acetyl salicylic acid. The xylene induced inflammation experiment showed that the extract exerted significant ($p<0.01$) anti-inflammatory effect at its highest dose. Analgesic effects of the extract were significant ($p<0.05$-0.001 relative to control) and dose dependent in all three (3) models. The extract exhibited radical scavenging activity. The IC_{50} values were: 45.60 µg/ml for the extract and 40.76 µg /ml for ascorbic acid. **Conclusion**: The above results provide support for the traditional use of *Enantia chlorantha* stem bark in treating pain and inflammatory conditions in Akwa Ibom state.

KEYWORDS: *Enantia chlorantha*, stem bark, rodents, ethanol extract.

1. INTRODUCTION

Enantia chlorantha is a fair sized ornamental forest tree that can reach heights of 30m. It grows in dense shade and may be recognized by its bright yellow slash and conspicuous black fruits (Vivien and Faura, 1985). It is located in the West African region and extends from southern Nigeria to Gabon, Zaire and Angola. It is commonly called African white wood, Moambe Jaune and Annikia chlorantha. Locally, the name varies from place to place (Keay, 1998). The Ibios of Akwa Ibom call it Uno eto, the Yoruba's call it Osupupa or dokita Igbo. The Edo people refer to it as Erenbav bogo while Ikale and Boki tribes refer to it as Osumolu and Kakerim respectively. The family is Annonaceae and the specie is chlorantha. There are over 40 different species that grow in Nigeria as is seen by the various names that it is called and the varied uses to which it is applied. Enantia chlorantha is used to treat a wide variety of conditions using its roots, stem bark, fruit and leaves. In Akwa Ibom it is used to treat malaria fever, typhoid fever, jaundice dysentery, wounds, infections high blood pressure and many other related illnesses. It has been used also for anti-viral, anti-candidal and for gastroenteritis (Moody et al, 1995), (Gill and Akinwunmi, 1986). A decoction of the stem bark in illicit gin is usually taken to relieve painful and swollen joints, fever, headache and toothache.

A literature search showed that the plant has been worked on by many investigators who have examined different aspects of the plant. The models they have employed have revealed some significant level of activity in various areas. We aim by this work to use other models in the hope that the mechanism of action of the plant may be inferred.

2.0 MATERIALS AND METHODS

2.1 *Plant material:* The plant *Enantia chlorantha* was collected in January 2012 in Uyo the capital city of Akwa Ibom State, Nigeria. It was identified and authenticated by a Taxonomist in the Department of Botany and Ecological Studies, University of Uyo, Akwa Ibom state Nigeria. A voucher specimen (voucher number UUH 018/13) was deposited with the herbarium of the Department of Pharmacognosy and Natural Medicine, Faculty of Pharmacy, University of Uyo.

2.2 EXTRACTION

The plant stem bark was washed and partially air dried for about 2 weeks at ambient temperature (25^0C ± 1^0C). It was then ground to powder using pestle and mortar. The pulverized sample was divided into two parts. One part was cold macerated in 70% ethanol at room temperature for 72 h and then filtered. The filtrate was dried in a rotary evaporator at 40^0C. This extract is referred to as crude. The other part was successively and gradiently macerated for 72 h at room temperature in the following solvents: n-hexane, chloroform, ethyl acetate, methanol and water to obtain different fractions. The crude extract and the fractions were stored in the freezer at -4^0C until required.

2.3 ACUTE TOXICITY STUDY OF THE EXTRACT

The method of Lorke (1983) was used to determine the LD_{50} of the extract in Swiss albino mice. The extract was administered to three groups of mice containing three (3) mice each at a dose range of 100-1000 mg/kg, (i.p). The animals were observed for physical signs of toxicity and the number deaths in each group within 24 hr were recorded. The animals were fasted for 24 hr prior to the experiment but allowed water *ad libitum*. The LD_{50} was calculated as the geometric mean of the maximum dose producing 0% mortality (A) and the minimum dose producing 100% mortality (B). $LD_{50} = \sqrt{AB}$

2.4 PHYTOCHEMICAL SCREENING

The phytochemical screening of the extract was carried out according to the methods of Odebiyi and Sofowora (1978), Harborne (1984) and Trease and Evans (1996). The following bioactive compounds were screened for their presence: saponins, tannins and alkaloids. Others were flavonoids, antraquinones, cardiac glycosides and reducing sugars.

2.5 ANIMAL STOCK

Mice and rats of the Swiss albino species weighing 18-25 g of both sexes were used for the experiments. They were obtained from the Department Of Pharmacology Animal House In The University Of Uyo, Uyo. The animals were housed in standard plastic cages at room temperature and moisture under naturally illuminated environment of 12:12 h dark/light cycle. They were fed with pelleted feeds (Bendel Feeds) and allowed water *ad libitum*.

2.6 ANALGESIC STUDIES

2.6.1 Acetic acid induced writhing in mice

The method of Santos *et al.*, (1994); Correa, Kyle, Chakrararty and Calixto, (1996) and Besra, Sharma and Gomes, (1996); Nwafor and Okwuasaba (2003) was used to evaluate the analgesic activity of the extract. The abdominal constrictions and stretching of hind limbs were counted following intraperitoneal injection of 0.1 ml of 3% acetic acid. Adult albino mice (18-30 g) were used for this experiment. They were fasted for 24 hr and were allowed access to water *ad libitum* before the experiment. They were then randomized into six (6) groups of six (6) mice per group. Group 1 was given 10 ml/kg, (p.o.) of distilled water. Groups 2-4 were administered with 32.40-96.20 mg/kg, (i.p.) of the extract. Group 6 received acetylsalicylic acid (ASA, 100 mg/kg, i.p.). Group 5 animals received the middle dose of extract (64.80 mg/kg, i.p.) and ASA (100 mg/kg, i.p.). After 30 min, acetic acid (100 mg/kg, i.p.) was administered. The number of writhing movements was counted at 30 min intervals for 5 h. Anti-nociception was expressed as the reduction in the number of writhing or abdominal constrictions between control animals and mice pre-treated with the extract.

2.6.2 Formalin- induced hind paw licking in rats

The method of Correa and Calixto (1993) and Gorski, Correa, Filhe, Yunes and Calixto, (1993); Nwafor and Okwuasaba (2003) was used in this experiment. 20 µl of 2.5% formalin solution (0.9% formaldehyde) made up in phosphate buffer solution (PBS concentration: NaCl 137 mM; KCl 2.7 mM and phosphate buffer 10 mM) was injected into the subplantar space of the right hind paw of each mouse. The amount of time spent licking the injected paw was noted and used as an indication of pain. The first phase of the nociceptive response normally peaks 5 min after injection and the second phase 15-30 min after formalin injection which represents the neurogenic and inflammatory pain response. Adult albino mice (18-30 g) of either sex were randomized into six (6) groups of six (6) mice each. Group 1 received 10 ml/kg, (p.o.) of distilled water. Groups 2-4 were pre-treated with 32.40-96.20 mg/kg, (i.p.) of the extract 30 min before being given the buffered formalin. Group 5 animals received the middle dose of extract (64.80 mg/kg, i.p.) and ASA (100 mg/kg, i.p.). While group 6 was administered with ASA (100 mg/kg, i.p.).

2.6.3 Effect of extract on thermally-induced pain in mice

The effect of the extract on hot plate induced pain was investigated in adult mice according to the methods of Vaz, Cechinel, Yunes and Calixto, (1996) and Nwafor and Okwuasaba,

(2003). The interval between heat application and response was measured. The hot plate is kept at 45±1 0C throughout the experiment. The mice were placed in a glass beaker of 50 cm diameter which was placed on the hot plate. The time (s) between placement and shaking or licking of the paws or jumping was recorded as the index of response latency. An automatic 30 s cut-off in the hot plate was set to prevent tissue damage. Adult albino mice (18-25 g) of either sex were used for this experiment. The mice were randomized into six (6) groups of six (6) mice per group. All animals were fasted for 24 hr prior to the experiment and allowed access to water ad libitum. Group 1 animals received distilled water (10 ml/kg, p.o.). Groups 2-4 were pre-treated with the extract at doses of 32.40- 96.20 mg/kg, (i.p.) respectively 30 min before being placed on the hot plate. Group 5 received the middle dose (64.80 mg/kg, i.p.) of the extract along with ASA (100 mg/kg, i.p.). While group 6 received ASA 100 mg/kg, (i.p.).

2.7 ANTI- INFLAMMATORY ACTIVITIES OF THE EXTRACT

2.7.1 Effect of extract on carrageenin–induced mouse hind paw oedema

The method of Winter, Risley and Nuss, (1962) was used to determine the hind paw linear circumference by subplantar injection of a phlogistic agent. Adult albino mice weighing from 22-30 g of either sex were used for this experiment. They were randomly divided into different groups of six mice per group. They were fasted for 24 hr prior to the experiment and deprived of water only during the experiment. A freshly prepared 1% solution of carrageenin (0.1 ml in normal saline) was injected into the sub-plantar surface of the mouse hind paw. Measurements of the linear circumference were taken at intervals of 30 min for 5 hr after the administration of the phlogistic agent. The increase in paw circumference was taken as the parameter for inflammation (Nwafor and Okwuasaba, 2003; Nwafor and Hamza, 2007). The extract was administered at doses of 32.40, 64.80 and 96.20 mg/kg, (i.p.) 1hr before induction of inflammation. Control mice received distilled water (10 ml/kg, (p.o.) while the reference group received acetyl salicylic acid (100 mg/kg, i.p.). A vernier calliper was used to measure the oedema.

2.7.2 Determination of effect of extract on xylene–induced ear oedema in mice

Inflammation was induced in mice using the topical route of administration of 2 drops of xylene at the inner surface of the right ear and a period of 15 min was allowed to elapse for it to take effect. Adult albino mice (22-25 g) of either sex were randomized into different groups of six mice each. Group 1 received 10 ml/kg, (p.o.) of distilled water. Groups 2, 3 and

4 received 32.40, 64.80 and 96.20 mg/kg, i.p. of extract respectively. Group 5 received the 64.80 mg/kg, (i.p.) of the extract in addition to the standard drug of dexamethasone 5 mg/kg, (p.o.). Dexamethasone (5 mg/kg, .p.o.) was administered to group six (6). All animals were fasted for 24 hr prior to the experiment but allowed water ad libitum. The extract and dexamethasone were administered 30 min before induction of inflammation. The animals were sacrificed under light anaesthesia and both ears were severed. The difference between the ear weights was taken as the oedema induced by xylene (Tjolsen, Berge, Hunskar, Roland, and Hole, 1992; Mbagwu, Anene, and Adeyemi, 2007).

2.7.3 Effect of extract on egg albumin-induced inflammation in albino mice

Increase in the rat hind paw linear circumference induced by subplanter injection of a phlogistic agent was used as the measure of acute inflammation (Nwafor, Jacks and Ekanem, 2007). The phlogistic agent employed in this study is fresh egg albumin (Akah and Nwambie., 1994). Adult albino mice (22-28 g) of either sex were used after 24 hr fast and deprived of water only during the experiment. Inflammation of the hind paw was induced by injecting 0.1 ml of fresh egg albumin into the sub plantar surface of the hind paw. Oedema was assessed as the difference in paw circumference between the control and at 30 min, 90, 120, 150 and 180 min after the administration of the phlogistic agent (Hess and Milonig, 1972). The animals were randomized into different groups of six (6) mice per group. The extract was administered at the dose range of 32.40-96.20 mg/kg, i.p. to groups 2-4. Group six (6) received acetylsalicylic acid (100 mg/kg, p.o.) 1hr before induction of inflammation. Group 1 received distilled water 10 ml/kg, (p.o.). Group 5 was pretreated with extract (64.80 mg/kg, i.p.) 10 min before the administration of ASA at 100 mg/kg, (p.o.). Then 1 hr later, inflammation was induced using egg albumin while group 1 received distilled water (10 ml/kg, p.o.) only.

The linear circumference of the paw was measured every 30 min using a vernier caliper. The average oedema (Ct -Co), percentage induced inflammation and percentage inhibition of oedema was calculated for each dose;

Percentage (%) inflammation= average inflammation at time t/average inflammation of control at same time x100.

Oriowo, (1982); Akah and Njike, (1990).

2.8 SUBCHRONIC STUDY

2.8.1 Effect of extract on cotton pellet granuloma in rats

The method of Mossa, Rafatullahi, and Gulal, (1995) was used for this experiment. Five (5) groups of five (5) rats each were randomly selected, shaved and anaesthetized with chloroform. Thirty (30 g) of sterilized cotton pellet was inserted into the groin region of the rat with a small subcutaneous incision. The incision was sutured with sterile catgut and methylated spirit was applied at the site of incision (Crunkhon and Meacock, 1971). Groups 2-4 were then treated with varying doses of the extract (32.40, 64.80 and 96.20 mg/kg) respectively for 7 days. Group 5 received 10 ml/kg, (p.o.) of distilled water while group one (1) was administered with 100 mg/kg, (i.p.) of ASA and was taken as the positive control. On day 8, the animals were sacrificed and the pellets were removed and debrided of extraneous tissue. The pellets were dried at 650C overnight and then weighed. The average weights of the pellets of the control group as well as the others were calculated. Increase in weight of the pellet was taken as a measure of granuloma formation.

The percentage inhibition will be calculated as: % inhibition = Wc –Wd/Wc x100. Where Wd = difference of pellet weight of the drug treated group. Wc =difference in pellet weight of the control group (Vogel, Scholken, Sandoe, Muller and Vogel, 2002).

3.0 STATISTICAL ANALYSIS

Results were expressed as multiple comparisons of mean ± SEM. Significance was determined using One-way Analysis of Variance (ANOVA) followed by Turkey-Kramer multiple comparison post test. A probability level of less than 5 % was considered significant.

4.0 RESULTS

The intraperitoneal LD50 of the extract in mice was calculated according to the method of Lorke to be 324.0 mg/kg. The LD50 was calculated as geometric mean of the maximum dose producing 0 % mortality (A) and the minimum dose producing 100 % mortality (B). LD50 = √AB. Phytochemical screening of the extract revealed the presence of the following secondary metabolites: saponins, alkaloids, terpenes, cardiac glycosides, anthraquinones and flavonoids. The effect of extract on acetic acid-induced writhing in mice is shown in Table 1. The extract (32.20–96.20 mg/kg) dose-dependently reduced acetic acid-induced abdominal constrictions and stretching of hind limbs. Maximum analgesic effect was observed at 25 min for doses of 64.40 and 96.20 mg/kg. The reduction was statistically significant ($p<0.05$–0.001) when compared to control. The effect of the extract was comparable to that of the

standard drug, ASA (100 mg/kg). The extract inhibited formalin-induced hind paw licking in mice. This inhibition was dose-dependent and statistically significant ($p<0.05–0.001$) relative to control as shown in table 2. Table 3 shows Rats pre-treated with the extract (32.20–96.20 mg/kg, i.p) demonstrated a dose–dependent increase in latency of response in the hot plate test. The increases in the latency of response (analgesic effect) were statistically significant ($p<0.05–0.001$) relative to control and were incomparable to that of the standard drug, ASA (100 mg/kg). In table 4, we see that the extract showed significant ($p<0.01–0.001$) anti-inflammatory effect against carrageenin-induced hind paw oedema relative to control. The extract exerted significant ($p<0.01–0.001$) anti-inflammatory effect at all dose levels tested against oedema induced by egg albumin as seen in table 5. Anti-inflammatory effect of extract against xylene-induced ear oedema in mice is as shown in Table 6. The extract exerted a weak anti-inflammatory effect which was only significant ($p<0.01$) at the highest dose and this was comparable to that of the standard drug, dexamethasone (4.0 mg/kg). The effect of extract on cotton pellet granuloma is shown in Table 7. The extract caused a significant ($p<0.001$) dose dependent effect against cotton pellet granuloma. These effects were not comparable to that of the standard drug, ASA (100 mg/kg).

Table 1: Effect of extract on acetic acid-induced writhing in mice

Treatment/ Dose (mg/kg)	Time Intervals (Min)						
	5	10	15	20	25	30	Total
Control	8.00 ± 0.57	21.33 ± 1.45	17.00 ± 0.57	12.33 ± 0.88	10.66 ± 1.45	12.00 ± 0.88	81.32 ± 5.80
Extract 32.40	2.66 ± 0.33 ^{c}	8.33 ± 0.88^{c}	10.33 ± 1.20 ^{c}	4.00 ± 0.57 ^{c}	1.66 ± 0.66 ^{c}	10.66 ± 1.82	37.64 ± 5.46^{c}
64.80	1.66 ± 0.66 ^{c}	4.00 ± 2.08^{c}	2.33 ± 0.88 ^{c}	1.33± 0.33 ^{c}	1.00± 0.30^{c}	2.66 ± 0.88 ^{c}	11.98 ± 4.83^{c}
96.20	0.66 ± 0.66 ^{c}	2.00 ± 0.57^{c}	1.33 ± 0.66 ^{c}	1.33 ± 0.33 ^{c}	1.00± 0.03^{c}	0.66 ± 0.33 ^{c}	5.98 ± 2.55^{c}
ASA 100	1.00± 0.03^{c}	2.66 ± 1.20 ^{c}	3.00 ± 0.57^{c}	2.00 ± 0.57 ^{c}	1.66 ± 0.88 ^{a}	2.00 ± 0.88^{c}	11.32 ± 4.10^{c}
ASA + Extract 100 + 64.80	1.00± 0.03^{c}	1.00 ± 0.57 ^{c}	2.00 ± 0.86^{c}	4.20 ± 0.57^{c}	0.66 ± 0.33^{c}	0.33 ± 0.33 ^{c}	8.19 ± 2.66^{c}

Data are expressed as mean ± SEM.

Significant at $^{a}p< 0.05$, $^{b}p< 0.01$, $^{c}p<0.001$ when compared to control (n = 6).

ASA = Acetyl salicylic Acid

Table 2: Effect of extract on formalin–induced hind paw licking in mice

Treatment/ Dose (mg/kg)	Time Intervals (Min)						
	5	10	15	20	25	30	Total
Control	30.50 ± 0.30	15.20 ± 1.60	16.50 ± 0.70	10.50 ± 0.35	7.60 ± 0.23	4.56 ± 0.42	54.36 ± 4.10
Extract 32.40	27.30 ± 0.95	7.16 ± 0.28^{c}	6.30 ± 0.30 ^{c}	5.00 ± 0.35 ^{a}	4.00 ± 0.42 ^{a}	3.78 ± 0.40^{a}	53.50 ± 2.75^{c}
64.80	22.25± 0.50^{a}	6.33 ± 0.20^{c}	5.83 ± 0.21 ^{c}	4.25± 0.36 ^{c}	4.16 ± 0.42 ^{a}	3.25 ± 0.21^{a}	46.07 ± 1.73^{b}
96.20	17.15 ± 3.25^{a}	4.16 ± 0.16^{c}	2.30 ± 0.45 ^{c}	1.23 ± 0.16 ^{c}	2.16 ± 0.21 ^{b}	2.30 ± 0.21^{a}	29.50 ± 4.44^{b}
ASA 100	9.45 ± 0.50 ^{c}	2.51 ± 0.22 ^{c}	1.45 ± 0.16^{c}	2.15 ± 0.21 ^{c}	1.66 ± 0.21 ^{c}	0.00± 0.00	17.21 ± 1.30^{c}
ASA + Extract 100 + 64.80	10.15 ± 1.25 ^{c}	1.15 ± 0.30 ^{c}	2.15 ± 0.16^{c}	1.20 ± 0.60^{c}	0.00± 0.00	0.00 ± 0.00	14.66 ± 1.87^{c}

Data are expressed as mean ± SEM.

Significant at $^{a}p< 0.05$, $^{b}p< 0.01$, $^{c}p<0.001$ when compared to control (n = 6)

ASA = Acetyl salicylic Acid.

Table 3: Effect of extract on hot plate test

Group	Dose mg/kg	Reaction Time (sec)	% Inhibition
Control	-	4.03 ± 0.23	
Extract	32.40	6.42 ± 0.87	59.30
Extract	64.80	11.13 ± 0.63^{c}	176.17
Extract	96.20	16.22 ± 0.64^{c}	302.48
ASA	100	23.29 ± 0.44^{c}	477.91
ASA+extract	100+64.80	25.89 ± 0.77^{c}	542.43

Data are expressed as mean ± SEM.

Significant at $^{a}p< 0.05$, $^{b}p<0.01$, $^{c}p<0.001$ when compared to control (n=6).

ASA = Acetyl salicylic Acid.

Table 4 Effect of extract on carrageenin-induced oedema in rats

Treatment/ Dose (mg/kg)	Time Intervals (hr)						
	0	0.5	1	2	3	4	5
Control	0.25 ± 0.01	0.33± 0.01	0.33 ± 0.01	0.32 ± 0.01	0.32 ± 0.01	0.31 ± 0.01	0.31 ± 0.01
Extract 32.40	0.24 ± 0.07a	0.35 ± 0.01^{ns}	0.34 ± 0.02^{ns}	0.33 ± 0.01^{ns}	0.32 ± 0.01^{ns}	0.32 ± 0.01^{ns}	0.32 ± 0.01^{ns}
64.80	0.24 ± 0.02^{a}	0.34 ± 0.01^{a}	0.34 ± 0.02^{a}	0.34 ± 0.01^{a}	0.31 ± 0.01^{a}	0.30 ± 0.01^{c}	0.30 ± 0.01^{c}
96.20	0.25 ± 0.01^{c}	0.34 ± 0.01^{c}	0.34± 0.01^{b}	0.32 ± 0.01^{b}	0.30 ± 0.01^{c}	0.28 ± 0.01^{c}	0.28 ± 0.01^{c}
ASA 100	0.25 ± 0.01^{c}	0.34±0.01c	0.33 ± 0.01^{b}	0.30 ± 0.01^{a}	0.27 ± 0.01 ^{a}	0.26 ± 0.01^{a}	0.25 ± 0.01 ^{b}

Data are expressed as mean ± SEM.

Significant at $^{a}p<0.05$, $^{b}p< 0.01$, $^{c}p< 0.001$ ns=not significant when compared to control (n = 6).

ASA = Acetyl salicylic Acid.

Table 5 Effect of extract on fresh egg albumin-induced oedema in rats

Treatment/ Dose (mg/kg)	Time Intervals (hr)						
	0	0.5	1	2	3	4	5
Control	0.24 ± 0.01	0.33± 0.01	0.32 ± 0.01	0.30 ± 0.01	0.29 ± 0.01	0.27 ± 0.01	0.27 ± 0.01
Extract 32.40	0.25 ± 0.07^{ns}	0.34 ± 0.01^{b}	0.34 ± 0.02^{b}	0.32 ± 0.01a	0.31 ± 0.01a	0.29 ± 0.01^{ns}	0.29 ± 0.01^{ns}
64.80	0.24 ± 0.02^{a}	0.33 ± 0.01^{a}	0.32 ± 0.02^{a}	0.32 ± 0.01^{b}	0.29 ± 0.01^{b}	0.27 ± 0.01^{a}	0.26 ± 0.01^{a}
96.20	0.23 ± 0.01^{a}	0.34 ± 0.01^{b}	0.31± 0.01^{b}	0.29 ± 0.01^{c}	0.26 ± 0.01^{b}	0.26 ± 0.01^{a}	0.25 ± 0.01 ^{a}
ASA 100	0.23 ± 0.01^{b}	0.34±0.01^{a}	0.31 ± 0.01^{b}	0.29 ± 0.01^{c}	0.26 ± 0.01 ^{a}	0.26 ± 0.01^{a}	0.25 ± 0.01 ^{a}
ASA+Extract 64.80 mg/kg	0.24 ± 0.01^{b}	0.32±0.01^{b}	0.29 ± 0.01^{b}	0.28 ± 0.01^{c}	0.25 ± 0.01 ^{a}	0.25 ± 0.01^{a}	0.24 ± 0.01 ^{b}

Data are expressed as mean ± SEM.

Significant at $^{a}p<0.05$; $^{b}p< 0.01$, $^{c}p< 0.001$ ns= not significant when compared to control (n = 6).

ASA = Acetyl salicylic Acid.

Table 6 Effect of extract on xylene-induced ear oedema in mice

Treatment/ Dose (mg/kg)	Weight of Right Ear (g)	Weight of Left Ear (g)	Increase in Ear Weight (g)	% Inhibition
control (normal saline) 0.2ml	0.09 ± 0.02	0.05 ± 0.00	0.04 ± 0.01	-
Extract 32.40	0.08 ± 0.01^{ns}	0.04 ± 0.00^{ns}	0.04 ± 0.01^{a}	20.80
64.80	0.08 ± 0.01^{a}	0.05 ± 0.01^{a}	0.03 ± 0.01a	28.57
96.20	0.06± 0.01^{a}	0.04 ± 0.01^{a}	0.02 ± 0.01^{a}	42.85
Dexamethasone 4.00	0.05 ± 0.01^{b}	0.04 ± 0.01^{b}	0.01 ± 0.00^{b}	50.00
Dexamethasone 4.0+ 64.80 mg/kg	0.05 ± 0.01^{b}	0.04 ± 0.01^{b}	0.01 ± 0.00^{b}	50.00

Data are expressed as mean ± SEM.

Significant at $^{a}p< 0.01$, $^{b}p<0.001$ ns=not significant when compared with control (n = 6).

Table 7 Effect of extract on cotton pellet induced-granuloma

Treatment/ Dose (mg/kg)	Weight of Dry Cotton Pellet Granuloma(g)	% Inhibition of Granuloma Formation
Control (normal saline) 0.2ml	81.71 ± 0.43	-
Extract 32.40	69.40 ± 0.87 ^{a}	15.06
64.80	59.40 ± 0.91^{a}	27.30
96.20	51.72 ± 2.01 ^{a}	36.70
ASA 100	47.25 ± 1.88 ^{b}	42.17

Data are expressed as mean ± SEM.

Significant at $^{a}p< 0.01$, $^{b}p< 0.001$ when compared with control (n = 6).

ASA = Acetyl salicylic Acid.

5.0 DISCUSSION

The extract exerted pronounced anti-inflammatory effect on carrageenin, egg albumin-induced oedema and cotton pellet granuloma at all dose levels tested. Two mechanisms contribute to the development of oedema caused by increased vascular permeability that results from a primary stimulus. One induced by local release or formation of various autacoids and another induced neurogenically by stimulation of primary sensory neurons and subsequent mediator (substance P) release from peripheral ending of fibres (Gamse, Holzer, and Lembeck, 1980); (Amico-Roxas, Caruso, Trombadore, Scifo, and Scapagnime, 1984). According to Lembeck and Holzer (1979), the neurogenic component plays an important role in maintaining the non-neurogenic plasma extravasation since the stimulation of peripheral neurons and subsequent release of substance P from peripheral sensory ending causes further release of histamine from mast cells Nwafor *et al.* (2007). Carrageenan is a sulphated

polysaccharide obtained from seaweed (Rhodophyceae) which is commonly used to induce inflammation and believed to be biphasic (Dirosa, 1972). The first phase is due to release of histamine and serotonin. The second phase is caused by release of bradykinin, protease, prostaglandin and lysosome (Chawla, Singh, Murthy, Gupta, and Sing, 1987). It has been reported that the second phase of the edema is sensitive to most clinically effective anti-inflammatory drugs, which has been linked to the anti-edematous effect of natural products (Lino, Taveria, Viana and Motos, 1997) Prostaglandin plays a major role in the development of the second phase of the reaction, which is measured at around 3 hr time (Al-Rahaily, El-Tahir, Mossa and Rafatullah, 2001). The carrageenin-induced paw edema model in rats is known to be sensitive to cyclo-oxygenase (COX) inhibitors and has been used to evaluate the effects of non-steroidal anti-inflammatory agents which primarily inhibit the enzyme COX -2 involved in prostaglandin synthesis. Based on the results, it can be inferred that the inhibitory effect of ethanol extract on carrageenin–induced inflammation in rats may be due to the inhibition of enzyme cylco-oxygenase. But lipo-oxygenase inhibitors also possess significant anti-inflammatory action against carrageenan induced paw edema. (Lino *et al.*, 1997). The extract also inhibited egg albumin-induced edema demonstrating that it can inhibit inflammation by blocking the release of histamine and 5-HT, two mediators that are released by egg albumin (Nwafor *et al.*, 2007). However, acetyl salicy lic acid (ASA), a cyclo-oxygenase inhibitor reduced significantly edema produced by egg albumin. It was also observed that the extract significantly reduced the granuloma formation in rats. Multiplications of small blood vessels as well as proliferation of fibroblast are the characteristics features of the repair phase of inflammation. The extract effectively reduced the cotton pellet-induced granuloma, suggesting its activity in the proliferative phase of inflammation.

It also exhibited significant anti-inflammatory activity on the cotton pellet test. The cotton pellet granuloma is widely used to evaluate the transudative and proliferative components of chronic inflammation (Parvataneni, Rao, Archana and Rao, 2005). The moist weight of pellets correlates with transudates while the dry weight of the pellet correlates with the amount of granulomatous tissues (Swingle and Shideman, 1972). Chronic inflammation occurs by means of the development of proliferating cells. These cells can either spread or be in granuloma form. Non-steroidal anti-inflammatory drugs decrease the size of granuloma, which results from cellular reaction by inhibiting granulocyte infiltration resulting in inflammation, preventing generation of collagen fibers and suppressing

mucopolysaccharides. The extract showed significant anti-inflammatory activity in cotton-pellet induced granuloma and thus found to be effective in chronic inflammatory conditions, which reflected its efficacy in inhibiting the increase in the number of fibroblasts and synthesis of collagen and mucopolysaccharides during granuloma tissue formation (Swingle and Shideman, 1972).

The extract significantly reduced acetic acid-induced writhing, formalin-induced hind paw licking as well as delayed the reaction time of animals (mice) to thermally-induced pain. Acetic acid causes inflammatory pain by inducing capillary permeability (Amico-Roxas et al., (1984); (Nwafor *et al.*, (2007) and in part through local peritoneal receptors from peritoneal fluid concentration of PGE_2 and PGF_2 α (Deraedt, Jougney, and Falhout, 1980); (Bentley, Newton, and Star, 1983). The acetic acid-induced abdominal writhing is a visceral pain model in which the process releases arachidonic acid via cyclo-oxygenase and prostaglandin biosynthesis plays a role in the nociceptive mechanism (Franzotti *et al.*, 2002). It is used to distinguish between central and peripheral pain. These result, suggested that the extract may be exerting its action partly through the lipoxygenase and/or cyclooxygenase system.

The organic acid has also been suggested to induce the release of endogenous mediators indirectly, which stimulates the nociceptive neurons that are sensitive to NSAIDs and narcotics. The inhibition of acetic acid-induced writhing by the extract at all the doses suggests anti-nociceptive effect which might have resulted from the inhibition of the synthesis of arachidonic acid metabolites (Adzu, Amos, Kapu and Gamaniel, 2003).

Formalin-induced pain involves two different types of pain which are in phases; neurogenic and inflammatory pains (Vaz *et al.*, 1996, 1997) and measures both centrally and peripherally mediated activities that are characteristic of biphasic pain response. The first phase (0 to 5 min), named neurogenic phase resulted from chemical stimulation that provoked the release of bradykinin and substance P while the second and late phase initiated after 15 to 30 min of formalin injection resulted in the release of inflammatory mediators such as histamine and prostaglandin (Wibool, Sae, Reanmongkol, and Wongnawae, 2008); (Yi-Yl *et al.*, 2008). The injection of formalin has been reported to cause an immediate and intense increase in the spontaneous activity of C fiber afferent and evokes a distinct quantifiable behavior indicative of pain demonstrated in paw licking by the animals (Heapy, Jamieson and Russell, 1987). The first phase of formalin-induced hind paw licking is selective for centrally acting

analgesics such as morphine (Berken, Ostunes, Lermioglu, and Ozer, 1991), while the late phase of formalin-induced hind paw licking is peripherally mediated. Algesic (nociceptive) receptors mediate both the neurogenic and non-neurogenic pains. The extracts ability to inhibit both phases of formalin-induced paw licking suggests its central and peripheral activities as well as its ability to inhibit bradykinins, substance P, histamine and prostaglandins which are mediators in the pain (Lembeck and Holzer, 1979).

CONCLUSION

From this study, it can be concluded that the stem bark of *Enantia chlorantha* has observable analgesic and anti-inflammatory effects. Evaluation of sub-chronic toxicological properties showed that in low doses, the extract has proven relatively safe for use in animals employed for these experiments. This is demonstrated in the biochemical parameters and post mortem histology of vital organs. Observable pharmacologically active phytochemical constituents of *Enantia chlorantha* may be responsible for the effects noted in the study. Therefore, this plant may be a good candidate for developing new treatment for pain and inflammatory conditions.

CONFLICT OF INTEREST STATEMENT

The authors hereby declare that we have no conflict of interest whatsoever.

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