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Full Length Research Paper

Analgesic and anti-inflammatory effects of the ethanol extract of *Elytraria marginata* Vahl (whole plant) in Wistar rats

J.S. Ashidi[1*], O.J. Ojetola[1], P.I. Aziba[2] and E.O. Ajaiyeoba[3]

[1]Department of Plant Science and Applied Zoology, Olabisi Onabanjo University, Ago-Iwoye. Ogun State, Nigeria.
[2]Department of Pharmacology, Obafemi Awolowo College of Health Sciences, Olabisi Onabanjo University, Sagamu, Ogun State, Nigeria.
[3]Department of Pharmacognosy, University of Ibadan, Ibadan, Nigeria.

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The analgesic and anti-inflammatory effects of the ethanol extract of *Elytraria marginata* were studied using hot plate, thermal-induced pain and carrageenan-induced acute inflammation models in adult Wister rats. The preliminary phytochemical constituents of the extract were also ascertained. Inflammation was induced by injecting 0.1 ml of 1% carrageenan into the sub-planter surface of the right hind paw of the rats. Ethanol extract of *E. marginata* with doses of 50, 100 and 150 mg/kg and diclofenac 10 mg/kg were administered orally to separate groups of rats. Control group received 10 ml/kg of distilled water. The results showed that the extract (50, 100 and 150 mg/kg) significantly ($p<0.05$) reduced the carrageenan-induced rat paw oedema in a dose-dependent manner. The oedema reductions at a dose of 150 mg/kg at the 4th h were comparable to that obtained for diclofenac, the standard anti-inflammatory drug at the 3rd hour. The extract also showed a very good analgesic response against hot plate-induced analgesia (thermal stimuli) in the rats. Administration of the extract doses (50, 100 and 150 mg/kg) and ibuprofen 100 mg/kg produced a significant ($p<0.05$) reduction in the pain induced by the hot plate (thermal stimuli) in all the experimental groups when compared with the control. Preliminary phytochemical analysis showed the presence of alkaloids, flavonoids, tannins and saponins. These findings indicate that the ethanol extract of *E. marginata* has both analgesic and anti-inflammatory effects and could be beneficial in alleviating painful inflammatory conditions.

Key words: *Elytraria marginata* plant, inflammation, analgesia, diclofenac sodium, ibuprofen.

INTRODUCTION

Inflammation is a pathophysiological reaction of living organisms to injuries that leads to the local accumulation of plasma fluids and blood cells. Though a defense mechanism, the complex events and mediators involved in the inflammatory reaction can induce, maintain, or intensify numerous diseases (Carey et al., 2009).

In recent times, attention to medicinal plants has increased globally, and a number of evidences have shown the immense potential of medicinal plants traditionally used in various parts of the world (Anosike et

*Corresponding author. E-mail: joseph.ashidi@kcl.ac.uk: ashidisenu@yahoo.com.
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al., 2009). Medicinal plants are considered to be an important source of new chemical substances with potential therapeutic effects (Goji et al., 2010). However, studies on inflammatory diseases are gaining much responsiveness, and the side effects of the currently available anti-inflammatory drugs pose a major problem during their clinical use (Kayaalp, 1998). Hence, the development of newer and more powerful anti-inflammatory drugs with less significant side effects is imperative.

The study of plants which are used traditionally in treating inflammatory conditions should be useful in the search for new drugs with anti-inflammatory and analgesic potentials. In Nigeria, many plant products are employed for their analgesic and anti-inflammatory effects and their efficiency is traditionally acclaimed. One of such plant is *Elytraria.marginata* Vahl. (Acanthaceae).

E. marginata is locally known as "*ewe eso*" in Yoruba in Nigeria. It is an annual or short-lived perennial herb, the stem is up to 9 inches high, with small white or bluish flowers and it is widely found in Tropical Africa. The plant has been reported for its use in the treatment of measles in traditional practices in Nigeria (Sonibare et al., 2009), and has been reported as one of the plants used locally in the treatment of sterility in women in Ivory Coast (Assi, 1980). Djah and Nueba (2011) also reported that *E. marginata* is used traditionally by Anyi-Ndenye women during pregnancy to ensure good development of pregnancy and to facilitate labour. *Elytrariaacaulis*, another species of *Elytraria* has been reported to be an anti-hyperglycemic agent on the alloxan-induced diabetes in rats (Kumudhavalli and Jayakar, 2011).

Though the plant has been used in traditional medicine in treating inflammatory condition, there is no scientific evidence for such activities available in literature. The objective of the present study is therefore to investigate the analgesic and anti-inflammatory effects of the ethanol extract of *E. marginata* on carageenan-induced oedema and hot plate-induced analgesia in experimental models in order to validate its traditional use and determine the probable phytochemical constituents present in the ethanol extract of *E. marginata* as the basis for its pharmacological actions.

MATERIALS AND METHODS

Plant collection and identification

The fresh plant of *E. Marginata* was purchased from Ago-Iwoye local market in August, 2012 and was identified by Mr. Shosanya, O.S. of the Forestry Herbarium at the Forestry Research Institute of Nigeria (FRIN), Ibadan, Oyo State, Nigeria. The voucher number allocated to the herbarium specimen is FHI 109760.

Experimental animals

A total number of 30 adult Wistar rats of both sexes were purchased from the Department of Physiology, College of Veterinary Medicine of the Federal University of Agriculture, Abeokuta, Ogun State, Nigeria. The animals weighed between 150 to 200 g. The animals were put under a well-ventilated condition and were housed in a cage of three animals each. They were allowed to acclimatize with the new environment before theexperiment commenced, and were adequately fed with pelletized feed and water *ad libitum.*

Chemical reagents and drugs

Absolute Ethanol, carrageenan, diclofenac sodium (reference drug), ibuprofen (reference drug), hydrochloric acid (HCl), distilled water, ammonia solution, dragendroff reagents, chloroform, lead acetate solution, concentrated sulphuric acid (H_2SO_4), glacial acetic acid, ferric chloride solution ($FeCl_3$), absolute ethanol (BDH) and Frankfurt or Diclofenac sodium (SWIPHA, London) were used in the study

Preparation of the extract

The dried plant of *E. marginata* was ground with an electric blender. The ground plant, 0.45 kg was macerated in 3 L ethanol for a period of one week. The resulting solution was filtered and the filtrate was concentrated to dryness using a water bath at 50°C. After determination of percentage yield, the extract was stored in the refrigerator (4°C) until needed for analysis.

Preliminary phytochemical screening

Phytochemical screening was carried out on *E. marginata* to test for alkaloids, anthraquinones glycosides, cardiac glycosides, flavonoids, saponins, tannins and cardenolides using standard protocols according to Sofowora (1993) and Ajaiyeoba (2002).

Test for alkaloid

To 0.2 g of the crude extract of *E. marginata,* 10 ml of 10%, HCl was added and placed on a water bath for 5 mins. The extract was then filtered and allowed to cool. The pH was then adjusted to about 6 to 7 by adding 10% ammonia and using litmus paper. Then 5 ml of the filtrate was taken into separate test tubes and small quantity of Wagner's, Mayer's and Dragendorff's reagents were added and observed. The presence of turbidity or precipitation indicates the presence of alkaloid (Sofowora, 1993).

Test for anthraquinone glycoside

To 0.2 g of the crude extract of *E. marginata,* 2 ml of 10% HCl was added and placed on a water bath for 5 mins and filtered while still hot, then allowed to cool. The filtrate was partitioned with equal volume of chloroform and shaken gently. The chloroform layer (lower layer) was transferred to a clean test tube and equal volume of the ammonia solution was added and then shaken gently. The presence of delicate rose-pink layer on the test solution indicated the presence of anthraquinones (Sofowora, 1993).

Test for cardiac glycoside

To 0.2 g of the crude extract was added water and 2 to 3 drops of lead acetate solution then was shaken gently and filtered. To the filtrate, 2 ml of chloroform was added and then 1 ml concentrated

H_2SO_4 was carefully added to form a lower layer. A reddish brown colour at interface was observed for cardiac glycoside (Sofowora, 1993).

Test for flavonoid

To 0.2 g of the crude extract of *E. marginata,* 10 ml of ethanol was added and 3 drops of Ferric chloride ($FeCl_3$) was added. A dark green colour indicated the presence of flavonoid (Ajaiyeoba, 2002).

Test for saponin

Crude extract of *E. marginata* (0.2 g) was placed in a test tube containing 10 ml of distilled water, and then boiled for f5 mins and then filtered. The filtrate was shaken vigorously and observed. The presence of froths indicated the presence of saponin (Sofowora, 1993).

Test for tannin

Crude extract of *E. marginata* (0.2 g) was decocted with 20 ml of distilled water by boiling for 10 mins and filtered while hot and allowed to cool. Ferric chloride reagent (0.1%) was added to the filtrate. A blue-black, green or blue green indicated the presence of tannins (Sofowora, 1993).

Test for cardenolides

Crude extract of *E. marginata* (0.2 g) was added 2 ml of glacial acetic acid containing a drop of $FeCl_3$ solution. Then 1 ml of concentrated H_2SO_4 was gently added to form under layer. A brown ring indicated the presence of cardenolides (Sofowora, 1993).

Anti-inflammatory response

The carrageenan-induced acute inflammation model was employed (Winter et al., 1962). A total number of fifteen rats were fasted for 6 h and divided into 5 groups of 3 rats per group. Deprivation of water was to ensure uniform hydration and to minimize variability in oedematous response (Winter et al., 1963). Various doses of the extract (50, 100 and 150 mg/kg) were administered orally to groups 1 to 3, respectively. Group 4 received oral administration of distilled water (10 ml/kg) as negative control while group 5 received oral administration of diclofenac sodium (10 mg/kg) as positive control. After 1 h, 0.1 ml of freshly prepared 1% carrageenan suspended in distilled water was injected into the subcutaneous region of the right hind paw of the rats. The measurement of the paw was taken before (initial paw volume) and after the injection of the carrageenan at 0.5, 1, 2, 3, 4 and 5 h using a Vernier caliper. The mean paw circumference and percentage inhibition of oedema was determined using the methods of Okpo et al. (2001) and Akah (2009). The percentage inhibition was calculated using the modified formula used by Ahmad et al. (1992) below:

Percentage inhibition = (A–B)/A × 100

Where A is mean paw size of the control group and B is the mean paw size of the treated group.

Analgesic response (thermal stimuli)

Hot plate-induced analgesia was employed according to Ilodigwe and Akah (2009). Fifteen rats were fasted for 6 h and divided into 5 groups of 3 rats per group. The extract (50, 100 and 150 mg/kg) was administered orally to groups 1 to 3, respectively. Group 4 received oral administration of distilled water (10 ml/kg) as negative control while group 5 received oral administration of Ibuprofen (100 mg/kg) as positive control. After 1 h, each rat was gently placed on the hot plate maintained at 55±0.5°C and the time required by the rat to lick the paw or jump was taken as the response. The cut-off time or latency response was 15 seconds to avoid tissue damage. The percentage inhibition was calculated using the modified formula below:

Percentage inhibition = (A–B)/A x 100

Where A is mean increase in latency of the treated group and B is the mean increase in latency of the control group.

Statistical analysis

The results of the experiment were expressed as Mean ± Standard error mean (SEM). Numerical data were analysed for homogeneity of variance using Bartlett's test. The data were then analysed using one-way ANOVA to determine whether results in a particular group is significantly different from those in the corresponding control groups. The analysis of variance was followed by Tukey post hoc for intergroup comparisons. $P<0.05$ were considered as significant.

RESULTS

The preliminary phytochemical screening of the ethanol extract of *E. marginata* (Table 1) showed the presence of alkaloids, cardiac glycoside, flavonoids, saponins and tannins. Other constituents like anthraquinones and cardenolides were absent.

Carrageenan-induced oedema

The effect of *E. marginata* ethanol extract on carrageenan-induced oedema is shown in Table 2. The extract produced a dose-dependent inhibition of oedema induced by carrageenan. The percentage inhibition produced by the extract EM (150 mg/kg) was comparable to that produced by diclofenac sodium, 10 mg/kg (standard drug) at the 3rd h post-carrageenan injection. Highest percentage of the inhibitory effect of the extract (39.56%) was observed at a dose of 150 mg/kg body weight at the 5th h. This inhibitory effect was comparableto that exhibited by diclofenac sodium (40.91%) at 10 mg/kg body weight at the 4th h.

Effect on hot plate analgesia

A significant ($p<0.05$) and dose-dependent elevation of the treatment reaction time to thermal pain was evident in the extract-treated animals. The effect of the extract (150 mg/kg) was comparable to that produced by 100 mg/kg of ibuprofen (standard drug) as displayed in Table 3.

Table 1. Result of phytochemical screening of the *E. marginata* ethanol extract.

Plant constituent	Occurrence
Alkaloid	+
Anthraquinone glycoside	-
Cardiac glycoside	+
Flavonoid	++
Saponin	++
Tannins	+
Cardenolides	-

Key: + Present in moderate concentration; ++ Present in abundance;Absent.

Highest percentage of the inhibitory effect of the extract (72.46%) was observed at a dose of 150 mg/kg. The inhibitory effect was greater compared to that of Ibuprofen at a dose of 100 mg/kg (67.15%).

DISCUSSION AND CONCLUSION

The present study showed some of the pharmacological basis for the ethnomedicinal use of *E. marginata* in the treatment of inflammation. Carrageenan-induced inflammation is one of the most employed models reported for screening of clinically effective anti-inflammatory agents. Oedema formation due to carrageenan in rat is a biphasic event. The initial phase of oedema is attributed to the release of histamine and serotonin, and the second phase of oedema is due to the release of prostaglandins, protease and lysosomal enzymes. However, it has been reported that the second phase is sensitive to the most clinically effective anti-inflammatory agents (Thakare et al., 2009).

The ethanol extract of *E. marginata* showed a good anti-inflammatory activity against acute inflammation induced by carrageenan in a dose-related manner at 3rd and 5th h. Oedema results from the action of inflammatory mediators such as histamine, serotonin and bradykinin at the site of a local inflammatory insult (Harriot et al., 2004). The early phase of oedema, beginning from 1 h after the administration of the irritant (carrageenan), is due to the release of histamine and serotonin, while the later phase, occurring from 3 to 5 h after the administration of the irritant (carrageenan) is induced by bradykinin, protease, prostaglandin and lysosome (Wallace, 2002; Harriot et al., 2004). The reduction in oedema exhibited by ethanol extract of the study plant showed that it contains active constituents which block the release of histamine and serotonin from mast cells and inhibit the activity of other inflammatory mediators.

From this study, *E. marginata* ethanol extract demonstrated significant (*$p<0.05$) and dose dependent anti-inflammatory activity against carrageenan-induced paw oedema. The effect of the extracts was most pronounced, 3 h after the induction of oedema, an action which was similar to that of diclofenac sodium, showing its usefulness in the management of acute inflammation. *E. marginata* (150 mg/kg) caused 39.56% inhibition of carrageenan-induced paw oedema compared to that of the standard drug (diclofenac sodium, 10 mg/kg) which was 40.91% at the 4th h.

Thermal painful stimuli are selective for the evaluation of centrally, but not peripherally acting analgesic drugs (Chau, 1989). *E. marginata* (150 mg/kg) produced 72.46% pain inhibition compared to that of the standard drug (Ibuprofen, 100 mg/kg) which produced 67.15% pain inhibition. These results suggest that the extract possesses non-steroidal anti-inflammatory drugs-like analgesic activities, mediated through both the peripheral and central mechanisms. However, the result of the thermal stimuli (hot plate) experiment showed that the extract is more effective in alleviating central pain. Since pain is an integral part of inflammation, the analgesic activity shall certainly be a beneficial factor during inflammatory condition.

The phytochemical screening of the plant showed that it contains alkaloids, flavonoids, saponins, tannins and phenols. To the best this study knowledge and within the scope of available literature, this is the first report of phytochemical screening on *E. marginata*. In a study on related species, *Elytrariaacaulis*, twopyrazole alkaloids were isolated and characterized to be with asomnine and 4'-hydroxywithasomnine (Ravikanth et al., 2001). In another study, asomnine was shown to have inhibitleukotriene metabolis mLTB4, and well had double inhibitory effect on COX-1 and COX-2 enzymes (Wube et al., 2008). Meanwhile, naturally occurring alkaloids, flavonoids and saponins have been found to elicit analgesic and anti-inflammatory properties (Fernanda et al., 2002; Anaga and Onehi, 2010). According to Manthey (2000), prostaglandins, a group of powerful pro-

Table 2. Effect of ethanol extract of *E. marginata* on the carrageenan-induced rat paw oedema.

Parameters	Mean paw size in centimeter ± standard error mean (SEM)						
	Paw sizes at different time intervals						
Treatment dose	0 min	30 min	60 min	120 min	180 min	240 min	300 min
EM (50 mg/kg)	0.46±0.02	0.71±0.03^{ns} (-4.41)	0.78±0.04^{ns} (-6.85)	0.82±0.03^{ns}(-3.80)	0.75±0.08^{ns} (9.64)	0.70±0.07* (20.45)	0.67±0.06* (26.37)
EM (100 mg/kg)	0.43±0.01	0.67±0.02^{ns} (1.47)	0.62±0.04* (15.07)	0.61±0.03* (22.78)	0.57±0.03* (31.33)	0.58±0.02* (34.09)	0.57±0.02* (37.36)
EM (150 mg/kg)	0.47±0.01	0.62±0.03^{ns} (8.82)	0.62±0.04* (15.07)	0.59±0.03* (33.90)	0.56±0.03* (32.53)	0.57±0.03* (35.23)	0.55±0.01*(39.56)
Distilled water (10 ml/kg)	0.46±0.01	0.68±0.03	0.73±0.03	0.79±0.02	0.83±0.01	0.88±0.02	0.91±0.02
Diclofenac sodium (10 mg/kg)	0.47±0.01	0.58±0.08* (14.71)	0.58±0.03* (20.55)	0.58±0.03*(26.58)	0.53±0.01* (36.14)	0.52±0.01*(40.91)	0.51±0.01* (43.96)

Percentage inhibition is shown in parenthesis, n=3, reduction in oedema of the treated groups (*p<0.05) was significant compared with the control groups. Values with (ns) are not significant when compared with the control groups. All values are expressed as mean paw size in centimetre (cm) ± standard error mean (SEM). The extract showed a dose-dependent inhibition of oedema induced by carrageenan. Percentage inhibition by the extract EM (150 mg/kg) can be compared with that produced by diclofenac (10 mg/kg), standard drug used after the 3rd h. EM= *Elytrariamarginata* ethanol extract. n= number of parameters (rats) in each group.

Table 3. Effect of *E. marginata* on the thermal pain induced by hot plate in rats.

Parameters	Mean increase in latency or time spent on the hot plate in seconds ± Standard error mean (SEM)		
Extract/Drug dose	Before treatment (sec)	After treatment (sec)	Percentage inhibition (%)
EM (50 mg/kg)	1.38±0.20	2.32±0.14*	40.52
EM (100 mg/kg)	1.69±0.20	3.68±0.60*	54.08
EM (150 mg/kg)	1.90±0.32	4.72±0.03*	72.46
Distilled water (10 ml/kg)	1.37±0.21	1.37±0.21^{ns}	-
Ibuprofen (100 mg/kg)	1.38±0.12	4.11±0.10*	67.15

The percentage inhibition for all the treated groups were significant (*p<0.05) compared with that of the control groups. Values with (ns) are not significant, n=3. EM (150 mg/kg) showed highest inhibitory effect and all values are expressed as Mean latency or time spent on the hot plate in seconds (sec) ± Standard error mean (SEM). The extract showed a dose-dependent inhibition of thermal pain by the hot plate.

inflammatory signaling molecules have been shown to be effectively inhibited by flavonoids. Therefore, the analgesic and anti-inflammatory activities of *E. marginata* may be due to the presence of alkaloids, flavonoids and other polyphenols.The ethanol extract of *E. marginata* showed significant analgesic effect, which justified its use in managing pain brought by high body temperature (heat) and pain during labour in pregnant women in traditional practices, the reason why it is called "*ewe eso*" meaning easy leaf". It also showed moderate and acute anti-inflammatory effect, which may be responsible for its use in treating measles and other skin sores. Using both the pharmacological and biochemical parameters in different animal models showed that the study plant, *E. marginata,* is a promising anti-inflammatory and analgesic agent and may be useful for the treatment of inflammatory conditions.

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Conflicts of interest

The authors have none to declare.

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