The Journal of Phytopharmacology 2014; 3(2): 130-138
Online at: www.phytopharmajournal.com

Research Article

ISSN 2230-480X
JPHYTO 2014; 3(2): 130-138
March- April

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Anti-inflammatory and analgesic potentials of *Eleucine indica*

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Abstract

The plant *Eleucine indica* has a long ethnobotanical history because of its use in the treatment painful and inflammatory conditions. This study was aimed at investigating the anti-inflammatory and analgesic activities of ethanolic extract of the whole plant in mice. The anti-inflammatory activity was studied using carrageenin, egg albumin and xylene as phlogistic agents. The analgesic investigation was carried out against acetic acid-induced writhing, formalin-induced pain and hot-plate test. The extract (200 – 600 mg/kg) showed significant ($p< 0.05 - 0.001$) dose-dependent reductions in the mouse paw oedema caused by carrageenin, egg albumin and ear oedema induced by xylene. Mice pretreated with the extract (200 – 600 mg/kg) showed a significant ($p<0.05 – 0.001$) dose – dependent reduction in hind paw-licking caused by formalin, dose-dependent and significant ($p< 0.001$) reduction in acetic acid–induced abdominal constrictions and stretching of the hind limbs and a dose-dependent and significant ($p<0.001$) increase in the latency response in the hot plate test. These results taken together, show that *E. indica* extract has anti-inflammatory and analgesic potentials that can be exploited in the management of pain and inflammatory conditions.

Keywords: *Eleucine indica*, Anti-inflammatory, Analgesic.

Introduction

Current drugs used in the treatment of inflammatory and painful diseases are generally of two classes: opioids and non-opioids. Both classes of drugs have their own perculiar and well known side effects and toxicities. The use of opioid analgesics is limited because of the tendency to cause tolerance and dependence while the non-steroidal anti-inflammatory drugs have the potentials to cause gastric lesions. The development of novel compounds having anti-inflammatory and analgesic and activities with an improved safety profile is still required.[1] The synthetic ones are particularly expensive to develop as they cost millions of dollars. This has prompted a new rush for herbal medicines as the only potential source of newer, safer and better drugs than some of orthodox medicines currently available.[2] Despite enormous the progress made in medical research during the past decades, the effective treatment of many serious diseases remains problematic. Chronic inflammatory diseases remain one of the world's major health problems.[3]

Owing to poor hygiene and malnutrition, children in developing countries often suffer attacks of fever resulting from various infections.[4] These fevers are often accompanied by aches and pains with resultant morbidity and mortality.[5]

Eleucine indica, L. Gaertn (Poaceae) is called nkimenang (Ibibios), and crowsfoot or goose grass (English). It is an annual growing to 0.45m and is considered as an adventitious species that is native in the tropics and subtropical regions.[6] It is one of the medicinal plants used in the treatment of malaria fever among the Ibibios of Southern Nigeria. The whole plant decoction is used to treat joint and malarial pains and to restore menstruation in females suffering from ammenorrhoea. The whole plant especially the root is depurative, diuretic, febrifuge and laxative. It is also used in the treatment of influenza, hypertension, oliguria and urinary retention as well as kidney problems in Trinidad and Tobago.[7,8] The plant has been reported to have phytochemical content of sterol glucoside forms[9] and C-glycosyl-flavone possessing anti-inflammatory activities[10] antibacterial, antioxidant and non-cytotoxic properties[11.] However, there is a lack of scientific literature on its analgesic and anti-inflammatory properties. This present work was therefore conducted in order to ascertain whether the whole plant has the analgesic and anti-inflammatory potentials as claimed in its ethnomedicinal use.

Materials and Methods

Plant material

The plant material (*Eleucine indica*) was collected in April 2009 from Uyo, Akwa Ibom State, Nigeria. The plant was identified and authenticated by Dr. (Mrs.) Margaret Bassey (a plant Taxonomist) in the Department of Botany and Ecological Studies, University of Uyo, where a voucher specimen (UUH1409) was deposited.

Preparation of extracts

The plant material was air-dried and then oven-dried at reduced temperature 35+ 2ºC. It was thereafter ground into powder and cold-macerated in 70% ethanol for 72h, and filtered. The filtrate was dried in vacuo using the rotary evaporator. 30 g of the dried extract was partitioned using various solvents such as n-hexane, chloroform, ethyl acetate, butanol and water to obtain their respective fractions. The crude extract and the fractions were stored in a refrigerator at – 4ºC until required for use.

Preliminary phytochemical investigation

The extract was screened for bioactive ingredients such as saponins, alkaloids, tannins, phlobotannins, flavonoids, anthraquinones, cardiac glycosides and sugar.[12,13]

Animal stock

Adult albino rats and mice were obtained from the Animal House of the University of Jos, Jos, Plateau State and were maintained in the University of Uyo Animal House and fed with growers pellet Feed (Bendel Feeds and Flour mills Ltd, Edo State) with water given ad libitum.

Acute Toxicological Studies

Acute toxicological study was carried out to determine the median lethal dose (LD_{50}) using the modified method of Miller and Tainter[14,15] and was found to be 2090 +0.01 mg/kg.[16]

Evaluation of anti-inflammatory activities

Carrageneen-induced mouse hind- paw oedema

Acute inflammation was induced by sub-plantar injection of a phlogistic agent and measured as increase in the mouse hind- paw linear circumference.[17] Adult albino mice (21-32 g) of either sex were randomized into different groups of six mice per group. They were then used after being fasted for 24h and were allowed free access to water except during the experiment. 0.1ml of freshly prepared carrageenin suspension (1%) in normal saline was injected into the sub-plantar surface of the mouse hind-paw to cause inflammation. The linear circumference of the injected paw was then measured before, 0.5h and hourly for five hours after administration of phlogistic agent. The increase in paw circumference as stated above after administration of phlogistic agent was adopted as the parameter for measuring inflammation.[14,17,18]

The oedema (inflammation) was assessed as the difference in paw circumference between the control and 0.5h, then every hour for 5h after administration of the phlogistic agent. The extract (200-600 mg/kg) was administered intraperitoneally to various groups of mice 1h before inflammation was induced. Control mice were given carrageenin while reference group received acetyl salicyclic acid, ASA (100 mg/kg) intraperitoneally. The average (mean) oedema was assessed by measuring with vernier calipers.

Fresh egg albumin-induced inflammation

Inflammation was induced in mice by the injection of 0.1ml of fresh egg albumin into the subplantar surface of the right hind paw.[19,20] Oedema was assessed as the difference in paw circumference between the control and 1-5h after administration of the phlogistic agent.[21] Hence, the linear circumference of the injected paw was measured before and 0.5 to 5h following the administration of the phlogistic agent. The adult albino mice of either sex were randomized and divided into five groups of six mice per group. Group 1 served as control and received 10ml/kg of distilled water. Groups 2, 3 and 4 were administered with 200, 400 and 600 mg/kg of the extract (i.p) respectively. Group 5 animals were administered with only acetyl salicylic acid 100 mg/kg (i.p). Each mouse was administered with 0.1ml of fresh egg albumin subcutaneously (s.c) 30 min after extract and drug treatment into the right paw. The linear circumference of the paw was measured every 0.5h for 5h using vernier calipers. All the animals were fasted for 24h before the experiment but water was withdrawn during the experiment.

Xylene-induced ear oedema

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 15min was allowed for it to act. Adult albino mice of either sex were randomized and divided into five groups of six mice per group. Group 1 animals received 10ml/kg of distilled water and served as control. Groups 2, 3, and 4 received 200, 400 mg and 600 mg/kg of *E. indica* (i.p) respectively. Group 5 animals were administered with 4mg/kg of dexamethasone orally. All treatments were given to the mice 30 min before the induction of inflammation. The animals were fasted 24h before the experiment started. The animals were thereafter sacrificed under light anaesthesia and both ears were cut off. The difference between the ear weights were recorded as the oedema induced by the xylene.[22]

Evaluation of analgesic activities

Acetic acid-induced writhing

The abdominal constriction following intraperitoneal injection of 0.1 ml of 3% acetic acid consisting of the contraction of abdominal muscle together with a stretching of hind limbs was carried out using standard methods.[23-25] Adult albino mice were randomized and divided into five groups of six mice per group and fasted for 24h but allowed access to water. Group 1 served as negative control and received 10ml/kg of distilled water while groups 2 - 4 were pretreated with 200 – 600 mg/kg of *E. indica* extract intraperitoneally. Group 5 received standard drug Acetyl salicylic acid 100 mg/kg, i.p. After 30min, Acetic acid (0.1 ml) was administered (i. p). The numbers of writhing movements were counted for 30min. Antinociception was expressed as the reduction of the number of abdominal constrictions between control animals (distilled water treated mice) and mice pretreated with the extract.

Formalin-induced paw licking

Mice were used to analyze the first phase of formalin induced licking. 20 ml of 2.5% formalin solution (0.9% formaldehyde) made up in phosphate buffer solution (PBs concentration: NaCl, 137mM; KCl, 2.7mM and phosphate buffer, 10mM) was injected subcutaneously under the surface of the right hind paw of each mouse. The amount of time the mouse spent licking the injected paw was timed, and was used as indication of pain. The first of the nociceptive response normally peaks 5 min after injection and the second phase 15-30 min after formalin injection, which represent the neurogenic and inflammatory pain response, respectively.

Adult albino mice of either sex were randomized and divided into five groups of six animals per group. Group 1 served as control animals and received 10 ml/kg distilled water. Groups 2, 3 and 4 were pretreated with 200, 400 and 600 mg/kg of *E. indica* extract respectively 30 min before being challenged with buffered formalin. Group 5 animals received 0.1 g/kg of acetylsalicylic acid (ASA) intraperitoneally.[26-28]

Hot plate test

The effect of extract on hot plate-induced pain was investigated in adult mice using standard procedure.[13,29] The hot plate test was used to measure the response latencies. The hot plate was kept at 45+1ºC throughout these experiments. The mice were placed into a glass beaker of 50 cm diameter on the heated surface, and the time(s) between placement and shaking or licking of the paws or jumping was recorded as the index of response latency. An automatic 30sec cut-off was used to prevent tissue damage. The mice were divided into five groups of six mice per group. All the animals were fasted 24h before the experiment but allowed water. Group 1 animals served

as the control and were administered with distilled water. Groups 2, 3, and 4 were pretreated with 200, 400 and 600 mg/kg of *E. indica* intraperitoneally (i. p) respectively, 30min prior to the placement on the hot plate. Group 5 received 0.1 g/kg of acetyl salicylic acid intraperitoneally (i. p).

Statistical Analysis

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

Results

Preliminary phytochemical investigation

The phytochemical investigation indicated the presence of alkaloids, tannins, flavonoids, cardiac glycosides and simple sugar.

Anti-infammatory activity

Carrageenin –induced hind-paw oedema in mice

The extract showed good anti-inflammatory effect against acute inflammation by suppressing dose – dependently the increase in the mouse paw oedema caused by carrageenin. This inhibition was statistically significant ($p< 0.05$-0.001) relative to control. The inhibition caused by the extract (600 mg/kg) was comparable to that of the acetyl salicylic acid and was maximal after 5 hours of administration of phlogistic agent (Table 1).

Egg albumin-induced oedema in mice

The results show that the extract caused inhibition of egg albumin – induced oedema in mice over a period of 5h. These effects were dose- and time-dependent and statistically significant ($P<0.01 – 0.001$) relative to control (Table 2).

Xylene –induced ear oedema in mice

The result indicates a dose-dependent inhibition of mice ear oedema by the extract. This inhibition was statistically significant relative to control. The degree of inhibition was favourably comparable with the standard drug (Dexamethasone) as shown in Table 3.

Table 1: Effect of *E. indica* on carrageneen-induced hind-paw inflammation in mice

Treatment /Dose (mg/kg)	Time (hours)						
	0	**0.5**	**1**	**2**	**3**	**4**	**5**
Control	0.24 ± 0.00	0.38 ± 0.01	0.39 ± 0.01	0.42 ± 0.01	0.42 ± 0.01	0.38 ± 0.01	0.36 ± 0.01
200	0.23 ± 0.01	0.36 ± 0.01	0.35 ± 0.01^{c}	0.35 ± 0.00^{c}	0.34 ± 0.00^{c}	0.34 ± 0.00^{c}	0.32 ± 0.00^{c}
400	0.21 ± 0.00^{c}	0.35 ± 0.00^{a}	0.35 ± 0.00^{c}	0.34 ± 0.00^{c}	0.32 ± 0.00^{c}	0.31 ± 0.00^{c}	0.31 ± 0.00^{c}
600	0.20 ± 0.00^{c}	0.34 ± 0.00^{c}	0.33 ± 0.00^{c}	0.31 ± 0.00^{c}	0.30 ± 0.00^{c}	0.28 ± 0.00^{c}	0.27 ± 0.00^{c}
ASA 100	0.23 ± 0.00	0.37 ± 0.00	0.35 ± 0.00^{c}	0.33 ± 0.00^{c}	0.31 ± 0.00^{c}	0.30 ± 0.00^{c}	0.28 ± 0.00^{c}

Values represent Mean± SEM. Significant at $^{a}p<0.05$, $^{b}p<0.01$, $^{c}p<0.001$; (n=6)

Table 2: Effect of *E. indica* on egg-albumin – induced inflamation in mice

Treatment/ dose (mg/kg)		Time (hours)						
		0.0	**0.5**	**1**	**2**	**3**	**4**	**5**
Control		0.24 ± 0.00	0.38 ± 0.01	0.37 ± 0.00	0.37 ± 0.00	0.36 ± 0.00	0.34 ± 0.00	0.34 ± 0.00
Extract	200	0.25 ± 0.00	0.37 ± 0.00	0.34 ± 0.00	0.33 ± 0.00^{b}	0.31 ± 0.00^{b}	0.29 ± 0.00^{b}	0.28 ± 0.00^{b}
	400	0.24 ± 0.00	0.36 ± 0.01	0.33 ± 0.00^{a}	0.32 ± 0.00^{b}	0.30 ± 0.00^{b}	0.28 ± 0.00^{b}	0.28 ± 0.00^{b}
	600	0.27 ± 0.00^{b}	0.36 ± 0.00	0.31 ± 0.00^{b}	0.30 ± 0.00^{b}	0.30 ± 0.00^{b}	0.27 ± 0.00^{b}	0.26 ± 0.00^{b}
ASA	100	0.25 ± 0.01	0.35 ± 0.01	0.35 ± 0.01	0.33 ± 0.00^{b}	0.32 ± 0.01^{b}	0.30 ± 0.00^{b}	0.29 ± 0.01^{b}

Values represent Mean ± SEM. Significant at $^{a}p<0.01$; $^{b}p<0.001$; (n=6)

Table 3: Effect of *E. indica* extract on xylene – induced ear oedma in mice

Treatment/ dose (mg/kg)	WT of RT ear (g)	WT of LT ear (g)	Increase in Ear WT (g)	% Inhibition
Control	0.100 ± 0.00	0.031 ± 0.00	0.069 ± 0.00	
Extract 200	0.057 ± 0.00[a]	0.025 ± 0.00	0.031 ± 0.00[a]	55.07
400	0.056 ± 0.00[a]	0.032 ± 0.00	0.025 ± 0.00[a]	63.77
600	0.051 ± 0.00[a]	0.030 ± 0.00	0.021 ± 0.00[a]	69.56
DEXA 4	0.055 ± 0.00[a]	0.030 ± 0.00	0.025 ± 0.00[a]	63.77

Values represent Mean + SEM. Significant at ap<0.001. (n=6). Key: WT = Weight, RT = Right, LT = Left, DEXA = Dexamethasone.

Analgesic activities of *E. indica*

Acetic acid- induced writhing in mice

The extract (200 – 600 mg/kg) dose-dependently reduced acetic acid–induced abdominal constrictions and stretching of the hind limbs. This reduction was significant (p< 0.001) relative to control as shown in Table 4.

Formalin-induced paw-licking in mice

The animals pretreated with the extract (200 – 600 mg/kg) showed a significant (p<0.05–0.001) dose–related reduction in hind paw licking caused by formalin relative to control (Table 5).

Hot plate test

Animals pretreated with *E. indica* extract (200–600 mg/kg) depicted a dose – dependent increase in the latency response in the hot plate test. These observed increases in latency response (analgesic effect) were statistically significant (p<0.001) relative to control as shown in Table 6.

Table 4: Effect of *E. indica* on acetic acid- induced writhing in mice

Dose (mg/kg)	Time intervals (mins)							
	5	10	15	20	25	30	Total inhibition	% inhibition
Control	15.50 ± 0.76	12.83 ± 1.40	9.50 ± 1.12	9.00 ± 0.73	6.00 ± 0.97	5.83 ± 0.40	58.66 ± 5.38	
Extract 200	9.67 ± 0.42[a]	8.33 ± 0.21[a]	7.83 ± 0.70	6.67 ± 0.22	5.83 ± 0.48	5.17 ± 0.40	43.50 ± 2.43	25.84
400	8.33 ± 0.42[a]	1.83 ± 0.31[a]	1.67 ± 0.33[a]	0.83 ± 0.48[a]	0.83 ± 0.30[a]	0.67 ± 0.33[a]	14.16 ± 2.17	75.86
600	8.00 ± 0.78[a]	1.33 ± 0.42[a]	0.83 ± 0.40[a]	0.50 ± 0.34[a]	0.67 ± 0.33[a]	0.33 ± 0.21[a]	11.66 ± 2.48	80.12
ASA 100	4.83 ± 0.31[a]	4.33 ± 0.21[a]	4.00 ± 0.26[a]	3.50 ± 0.22[a]	1.33 ± 0.21[a]	1.00 ± 0.26[a]	18.99 ± 1.47	67.63

Values represent Mean ± SEM. Significant at [a]p <0.001; (n=6)

Table 5: Effect of *E. indica* on formalin – induced paw-licking in mice

Dose (mg/kg)		Time Interval (mins)						
		5	10	15	20	25	30	
Control		10.67 ± 1.12	8.50 ± 0.56	11.67 ± 0.67	15.17 ± 0.60	17.17 ± 0.40	18.17 ± 0.48	81.35 ± 3.83
Extract	200	12.67 ± 0.21	2.00 ± 0.26[b]	4.83 ± 0.31[b]	4.50 ± 0.34[b]	3.83 ± 0.31[b]	2.50 ± 0.22[b]	30.33 ± 1.65
	400	10.00 ± 0.36	1.00 ± 0.36[b]	4.00 ± 0.26[b]	3.33 ± 0.21[b]	2.33 ± 0.21[b]	1.67 ± 0.21[b]	22.33 ± 1.61
	600	8.33 ± 0.21[a]	0.00 ± 0.00[b]	3.33 ± 0.21[b]	2.50 ± 0.22[b]	1.33 ± 0.21[b]	1.00 ± 0.36[b]	16.49 ± 1.21
ASA	100	4.50 ± 0.22[b]	1.00 ± 0.26[b]	1.33 ± 0.21[b]	1.00 ± 0.26[b]	0.00 ± 0.00[b]	0.00 ± 0.00[b]	7.83 ± 0.95

Data expressed as Mean ± SEM. Significant at [a]p< 0.05; [b]p<0.001; (n=6)

Table 6: Effect of *E. indica* on (hot plate test) - in mice

Groups	Dose (mg/kg)	Reaction Time (sec) (Mean±SEM)	% Inhibition
Control		4.00 ± 0.36	
Extract	200	5.17 ± 0.31	29.25
	400	7.17 ± 0.31[a]	79.25
	600	8.83 ± 0.31[a]	120.75
ASA	100	14.83 ± 0.31[a]	270.75

Values represent Mean ± SEM. Significant at [a]p <0.001; (n=6)

Discussion

Inflammation comprises a complex series of reparative and protective responses to tissue injury irrespective of the cause - infection, auto-immune stimuli or mechanical injury.

Anti-inflammatory, analgesic and antipyretic activities have similar underlying mechanisms but compounds differ in their profile of activity. For instance, the corticosteroids are potent anti-inflammatory drugs but are not analgesics. Paracetamol and the opiod analgesics have an analgesic effect with little anti-inflammatory effect. But many other non-steroidal anti-inflammatory drugs (NSAIDS) have both analgesic and anti-inflammatory activities, e.g. aspirin. Anti-inflammatory drugs are used in the treatment of disorders which lead to inflammation, pyrexia and pain of whatever origin – rheumatoid conditions, gout, dysmenorrhoea, neoplastic diseases and headache.[30]

The anti-inflammatory drugs such as NSAIDS act by inhibiting cyclo-oxygenase and as a result prostaglandin synthesis. These also have antipyretic activity, since prostaglandins are involved in the mediation of pyrexia. Free radical scavenging agents also play a role in inflammation because liberation of free radicals is known to cause tissue damage during the inflammatory process. Flavonoids and phenolics are thought to act by preventing the generation or action of free radicals.[30]

Carrageenin-induced paw oedema is a commonly used primary screening test of new anti-inflammatory agents and is known to have two distinct phases. The first phase (1-2h) is due to the release of histamine or serotonin and the second phase of oedema is due to the release of prostaglandins/protease and lysosome which peak at 3h.[31] Carrageenin paw oedema is known to be sensitive to cyclooxygenase inhibitors and has been used to evaluate the effect of non-steroidal anti-inflammatory agents which mainly inhibit cyclooxygenase involved in prostaglandin synthesis.[32] The effect of *E. indica* on Carrageenin-

induced inflammation in these mice may in part be due to inhibition of the enzyme cyclooxygenase, leading to inhibition of prostaglandin synthesis.

Albumin-induced oedema is a biphasic response characterized by an early phase mediated through the release of histamine, serotonin and kinins while the late phase is associated with the release of prostaglandins and mediated by bradykinin, lencotrienes, poly-morphonuclear cells and prostaglandins produced by tissues macrophages.[33] These results depict that the extract acts on both early and late phases of inflammation.

Xylene is commonly used to investigate the role of Phospholipase A2 (PLA2) in the pathophysiology of inflammation.[34] Dexamethasone was preferred as the standard drug to non-steroidal anti – inflammatory agents because Xylene-induced ear oedema is less sensitive to non-steroidal anti-inflammatory agents.[35] It is suggested that an interaction of the extract with PLA2 may in part be its possible mechanism of anti- inflammatory action.

Formalin produces a clear biphasic response and acts differently in the early and late phases of pain and can be used to ascertain the mechanisms of pains and analgesia.[22] This model is suggested as a suitable test for chronic pain. It produces a biphasic nociceptive response which probably involved two stimuli. The first phase, neurogenic, follows immediately after the injection of formalin and last 3 - 5 min and is a consequence of chemical stimulation of pain nociceptors. It involves Substance P and bradykinin. The second phase is inflammatory pain and starts 15 – 20 min after formalin injection and last 20 – 40 min. This second phase results from sensitization of both peripheral and central neurons involved in nociception and involves histamine and prostaglandins.[36]

Analgesics that act centrally such as narcotics inhibit both phase equally while peripherally-acting drugs such as steroids (dexamethazone, hydrocortisone) and non-steroidal anti-inflammatory drugs like aspirin suppress primarily the late phase.[37] *E. indica* extract, however, did not inhibit both phases equally but it did produce analgesic effect on both phases. The degree of inhibition was more pronounced during the late phase. These taken together, suggest that this extract is a centrally acting analgesic.

Acetic acid is known to cause inflammatory pain acting indirectly by inducing the release of endogenous mediators such as PGE2 and PGE2α in peritoneal fluids as well as lipooxygenase products, which stimulate the nociceptive neurons.[38] The results of the acetic acid – induced writhing suggest strongly that the mechanism of this extract may in part be linked to its inhibition of lypooxygenase and/or cyclooxygenase in pheripheral tissues, reducing PGE2 synthesis and interfering with the mechanism of transduction in primary afferent nociceptor.

The hot plate model indicates involvement of central pain pathways and is selectively used for screening centrally-acting opiate analgesic drugs.[24] Pain is centrally mediated through several complex processes such as opiate, dopaminergic, descending noradrenergic and serotonergic systems. It is well known that thermal nociceptive tests are more sensitive to opioid μ-agonists[31] which sugests this as a possible mechanism of its analgesic effect.

Conflicts of interest

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

Conclusion

The anti-inflammatory and analgesic activities of ethanolic extract (200 – 600 mg/kg) of the whole plant in mice showed significant, dose-dependent responses. The anti-inflammatory activity was studied using carrageenin, egg albumin and xylene as phlogistic agents. The analgesic investigation was carried out against acetic acid-induced writhing, formalin-induced pain and hot-plate test. *E. indica* extract, therefore, has anti-inflammatory and analgesic potentials that can be exploited in the treatment of conditions associated with pain and inflammation.

Acknowlegement

The authors are grateful to Miss Susannah Attah, Sifonobong Akpan and Nsikan Malachy of the Department of Pharmacology and Toxicology, University of Uyo for their technical assistance and for the University of Uyo for the ETF scholarship.

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