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ANTI-INFLAMMATORY AND ANALGESIC EFFECTS OF METHANOLIC EXTRACT OF *Afrofritomia sylvestris* LEAF

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Abstract: **Methanolic extract of the leaf of *Afrofritomia sylvestris* was investigated for its anti-inflammatory and analgesic effects. The extract was evaluated using carragenaan-induced paw oedema in rats (anti-inflammatory effect) as well as acetic acid-induced writhing (analgesic effect) in mice, after intra-peritoneal injection of the extract (250mg/kg, 500mg/kg and 1000mg/kg). The negative control animals were given normal saline (10ml/kg) and the effects were compared with that of Acetylsalicylic acid (100mg/kg), as a positive control drug. Each experiment consisted of twenty five animals divided into 5 groups of 5 animals each. Tail immersion reaction time and Naloxone antagonism of the extracts and morphine were further used to investigate the mode of action of the analgesic activity of the leaf. The extract significantly inhibited carageenan-induced hind paw inflammation in rats (P<0.05-0.01) in a dose-dependent manner. The extract also dose dependently (250-1000mg/kg) inhibited acetic acid-induced writhing in mice (P<0.01). The methanolic extract failed to raise the pain threshold of mice towards heat stimulus and Naloxone did not show any significant antagonism (blocking effect) against the extract in the tail immersion experiment, thus ruling out the involvement of opioid receptors in the mechanism of analgesic action of the extract. Phytochemical analyses of the plant show the presence of flavonoids, alkaloids, saponins, tannins, steroids, triterpenes and cyanogenic glycosides. The LD_{50} of the extract was determined to be 3050±223.65mg/kg using the method of Tainter and Miller.In this study, methanolic extract of *Afrofritomia sylvestris* leaf was found to possess significant anti-inflammatory and analgesic effects in the tested models.**

Keywords: ***Afrofritomia sylvestris*****, anti-inflammatory effect, analgesic effect, methanolic extract, carrageenan.**

I. INTRODUCTION

Inflammation is the most common biological response to a variety of stimuli and local injury [1]. The reaction to injury of cells and body tissues occurs through different factors such as infections, chemicals, thermal and mechanical injuries [2]. Inflammation is often exacerbated by the resultant swelling or oedema of tissue, pain (due to increased pressure in tissues during oedema formation or by inflammatory mediators) or even cell damage [3]. Analgesic and anti-inflammatory agents are required for treating inflammatory diseases. Many anti-inflammatory drugs are potential inhibitors of cyclooxygenase (COX) pathway of arachidonic acid metabolism which produces prostaglandins. Prostaglandins are potent vasodilators which also contribute to erythema, oedema and pain [4]. One of the therapeutic classes of drugs used in the treatment of inflammatory diseases such as asthma, arthritis and cardiovascular diseases are the Non-steroidal anti-inflammatory drugs (NSAIDs), due to their efficacy for a wide variety of pain and inflammatory conditions [5]. However, the side effects of most of the clinically available anti-inflammatory agents limits their use. For example, some of the Non-steroidal anti-inflammatory drugs (NSAIDs) may cause gastric ulceration, bleeding and kidney damage due to their nonselective inhibition of both COX-1 and COX-2 isoforms of the cyclooxygenases enzymes [6-10]. Owing to safety concerns associated with the use of synthetic anti-inflammatory and analgesic agents, the public prefer to take natural anti-inflammatory and analgesic treatments from edible materials such as fruits, spices, herbs and vegetables [11]. Also, newer

anti-inflammatory and analgesic drugs devoid of the aforementioned side effects are being searched for all over the world as alternatives to NSAIDs and opiates [12-13]. Therefore, research into medicinal plants such as this, would serve as a lead for newer analgesic and anti-inflammatory drugs.

The nutritive potential of *Afrofritomia sylvestris* leaf has been reported. It is a good source of mineral elements and in the southeastern Nigeria, rural inhabitants supplement their leafy needs with the "hunters weed" which is often added to soup [14]. This study aimed to investigate the anti-inflammatory and analgesic effects of *Afrofritomia sylvestris.*

II. MATERIALS AND METHODS

Plant materials:

Afrofritomia sylvestris leaves were collected from their natural habitat at Eniong Offot village in Uyo metropolis (Akwa Ibom State, Nigeria) and the plant was properly authenticated by Dr. (Mrs.) M.E.Bassey, a plant taxonomist in the department of Botany and Ecological studies, University of Uyo. Voucher specimen was deposited in the herbarium of the department of Pharmacognosy and Natural medicine, Faculty of Pharmacy, University of Uyo, Nigeria (Voucher code: KKA2).

Plant preparation and extraction:

The methanolic extract was prepared by maceration (cold extraction) of 450g of the air-dried, powdered leaves of *Afrofritomia sylvestris* using methanol in an extracting jar. This set up was allowed to stand for 72 hours with occasional shaking. The extracts were filtered, concentrated until constant weights were achieved and stored in a refrigerator at 2-8^{o}C for use in subsequent experiments. This procedure was repeated 3 times for maximal extraction (yield, 45.0$\pm$0.02g).

Phytochemical Screening:

Preliminary phytochemical screening of the methanolic extract was carried out using the standard procedures [15-16) to identify the different phyto-constituents present in the extract.

Animals:

The animals (mice and rats) of both sexes were obtained from the animal house of the department of Pharmacology, University of Calabar. Rats weighing (100-140g), mice weighing (30-35g). They were maintained on standard animal pellets (Pfizer feeds) and water *ad libitum.* Housed in cages to acclimatize to the animal house in the Department of Pharmacology, University of Calabar and maintained under standard conditions (12 light and 12h dark cycle, 25$\pm$ 2^{o}C).

Acute toxicity:

The method of Tainter and Miller [17] was used for the determination of Median Lethal Dose ($LD5_0$). The Methanolic extract of *Afrofritomia sylvestris* was injected intraperitoneally into the mice. Five groups of five (5) mice per group were used for the tests. Groups 1-5 were injected with 2000, 2500, 3000, 3500 and 4000 respectively. Physical signs of toxicity such as drowsiness, stretching, reduced mobility, decreased breathing rate were observed after 30 minutes of sample administration. Mortality in each group within 24 hours was recorded, percentage mortality calculated and the percentage was transformed into probit values by referring to the tables for "transformation of percentage to probits. A graph of percentage death in (probits) was plotted against log-dose and the dose corresponding to probit 5 i.e 50% was read to be the $LD_{50.}$

Anti-inflammatory study:

Carrageenan-induced rat hind paw Oedema:

The anti-inflammatory activity was evaluated according to the method described by Winter et al [18]. A total of 25 albino Wistar rats of both sexes were used in the experiment. They were divided into five groups of five rats each. Carrrageenan was freshly prepared as 1% (w/v) suspension in sterile 0.9% NaCl before the experiment. A volume of 0.1ml of Carrageenan was injected into the plantar tissue of the rat right hind paw. Methanolic extracts (250, 500 and 1000mg/kg) and Acetylsalicylic acid, ASA (100mg/kg) were administered by oral gavage 1 hour before carrageenan injection. Animals in the control group were given normal saline, 10ml/kg.

The increase in paw diameter 1,2,3,4 and 5 hours after the administration of the phlogistic agent between the control rats and those in the test groups was adopted as the parameter for measuring inflammation [18, 19-22]. The average (mean) oedema was assessed by measuring with vernier calipers and the percentage inhibition calculated as follows:

% inflammation = AD_t/AD_0 x100

Where: AD_t= Average diameter of oedema at time, t.

AD_0= Average diameter of oedema of control rats at the same time

Therefore, % inhibition = 100-% inflammation.

Acetic acid induced writhing in mice (Analgesic activity):

The abdominal constrictions resulting from intra peritoneal injection of 3% acetic acid consisting of the contraction of the abdominal muscles together with the stretching of hind limbs were carried out according to the methods described by Santos et al and Correa et al [23-24]. The animals were divided into 5 groups of 5 mice per group. Group 1 served as control (normal saline 10ml/kg), while groups 2,3 and 4 were pretreated with 250mg, 500mg and 1000mg/kg of methanolicextract of *Afrofritomia sylvestris*, intra peritonealy respectively. Group 5 were pretreated with ASA (100mg/kg). After 30 minutes, acetic acid was administered by the same route. The number of writhing movements were counted for 30 minutes. Antinociception (analgesia) expressed as the reduction of the number of abdominal constrictions between control animals and mice pretreated with the drug (extracts for groups 2-4 and ASA for group 5).

Data was calculated according to the following formula:

$$\% \text{ inhibition} = \frac{\text{Mean no. of writhes in control group- Mean no. of writhes in test group}}{\text{Mean number of writhes in control group}} \times 100$$

Tail immersion reaction time and Naloxone antagonism in mice:

Albino mice of either sex weighing between 22-25g were used and divided into 7 groups of 10 animals each. Group 1 (saline 10ml/kg) served as control, while groups 2-4 were given 250, 500 and 1000mg/kg of methanolic extract of *Afrofritomia sylvestris* intra peritonealy. Group 5 animals were given 5mg/kg of Naloxone intra peritonealy, 15 minutes before administration of 1000mg of extract. Group 6 were given 5mg/kg of morphine intra peritonealy. Group 7 animals were given 5mg of Naloxone intra peritonealy 15 minutes before the administration of 5mg/kg of morphine. The tail, (up to 3cm) was then dipped in a beaker of water maintained at 55 ± 0.5^0C. The time in seconds to withdraw the tail clearly out of the water was taken as the reaction time. The first reading (0 minutes) was taken immediately after administration of the test drugs and the readings were taken 30, 60, 90, 120 and 150 minutes later.

Statistical analysis:

The results were described as the mean $\pm$ standard error of mean (SEM), and statistical differences were compared using students' t-test. A probability level of less than 5% was considered statistically significant.

III. RESULTS

Phytochemical screening:

Preliminary Phytochemical screening revealed the presence of alkaloids, saponins, cyanogenic glycosides, triterpenes, flavonoids and tanins.

Acute toxicity:

The LD_{50} was determined to be 3050 ± 223.65mg/kg.

Carrageenan-induced paw oedema in rats:

The effects of methanolic extract of *Afrofritomia sylvestris* leaf on carrageenan-induced oedema in rat hind paw are presented in Table 1. The extract demonstrated a significant anti-inflammatory activity against acute inflammation by suppressing in a dose dependent manner the increase in the rat paw oedema caused by carrageenan (Table 1). This was

comparable to the prototype drug, Acetylsalicylic acid (Aspirin). The suppression was significant (P<0.05-0.01) and was maximal after 5hr of administration of carrageenan. The percentage inhibition of the rat paw oedema was very high at 1000mg/kg dose of the extract; this was so throughout the duration of the test (Table 2) and was greater than that of Acetylsalicylic acid (100mg/kg). The percentage inhibition of the rat paw oedema at the dose of 500mg/kg was comparable to that of Acetysalicylic acid (100mg/kg).

Acetic acid-induced writhing in mice (Analgesic activity):

The effects of methanolic extract of *Afrofritomia sylvestris* leaf on acetic acid-induced writhing in mice are presented in Table 3. The data presented are the mean number of writhings per 30 minutes (± standard errors) of the animal groups of five per group. Differences in the number of writhings compared with control were significant (P<0.01). Data in table 3 show that the extracts (250-1000mg/kg) dose-dependently reduced acetic acid-induced abdominal constrictions and stretching of hind limbs. The percentage inhibition of writhing of 1000mg/kg of the extract is 67.28% and is higher than that of Aspirin (51%) which in turn is slightly higher than that of 500mg/kg of the extract (48.39%).

Tail immersion reaction time and Naloxone antagonism:

The effect of methanolic extract of *Afrofritomia sylvestris* leaf and morphine on tail immersion reaction time and Naloxone antagonism in mice are presented in Table 4. Data in Table 4 show that the reaction times after drug treatment with the extracts (250-1000mg/kg) were not significant (p>0.05) compared to the control at all three dose levels, except for the dose of 1000mg/kg where a significant (p<0.01) action was observed at 120 minutes. However, morphine, a centrally acting analgesic showed significant (p<0.01) increase in reaction time when compared to the control. Pretreatment with Naloxone, a narcotic antagonist caused a significant (p<0.01) reduction in the reaction time when compared with morphine alone. On the other hand, pretreatment with naloxone before administration of 1000mg/kg of the extract did not cause any significant (p>0.05) effect on the reaction time to the heat stimulus.

Table 1: Anti-flammatory activity of methanolic extract of *Afrofritomia sylvestris* leaf (Carrageenan- induced oedema in hind paw) in the rat

Test group	Dos mg/kg	Diameter of oedema (mm) (mean ± SEM)				
		1h	2h	3h	4h	5h
Control		6.18± 0.03	6.54± 0.02	6.78± 0.05	6.56± 0.02	6.48± 0.03
Methanolic extract	250	5.28± .02**	5.24± .02**	4.82± .038*	4.68± 0.02*	4.56± .02*
Methanolic extract	500	4.42± 0.05*	4.12± 0.03*	4.02± 0.03*	3.78± 0.03*	3.50± .04*
Methanolic extract	1000	4.16± 0.04*	3.54± 0.05*	3.32± 0.05*	3.12± 0.02	2.66± .04*
ASA	100	4.80± .06**	4.40± .06**	4.10± .04**	3.86± .05**	3.54± .05*

Significance relative to control *=P<0.01; **=P<0.05

ASA = Acetysalicyclic acid; n=5

Table 2: Comperative anti-inflammatory effect of Aspirin and methanolic extract of Afrofritomia sylvestris on carrageenan-induced inflammation percent inhibition

Test group	Dos mg/kg	Percent inhibition with time (hour)				
		1h	2h	3h	4h	5h
Control		-	-	-	-	-
Methanolic extract	250	14.56	19.88	28.91	28.66	29.63
Methanolic extract	500	28.48	37.00	40.71	42.38	45.99
Methanolic extract	1000	32.69	45.87	51.03	52.44	58.95
ASA	100	22.33	32.72	39.53	41.16	45.37

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Table 3: Analgesic effect of methanolic extract of *Afrofritomia sylvestris* leaf on acetic acid-induced writhing in mice

Test Group	Dose (mg/kg)	No. writhing per 30 minutes (mean ± SEM)	% - Inhibition of writhing
Control		130.2 ± 0.88	
Methanolic extract	250	86.6 ± 2.01*	33.49
Methanolic extract	500	67.2 ± 0.52*	48.39
Methanolic extract	1000	42.6 ± 0.83	67.28
ASA	100	63.8 ± 1.0	51.00

*significantly different ($P<0.01$) from the negative control using students' t-test (n=5)

ASA= Acetylsalicylicacid.

Table 4: Effect of methanolic extract of *Afrofritomia sylvestris* leaf and Morphine on tail immersion reaction time and Naloxone antagonism in mice

Test Group	Dose (mg/kg)	Reaction time after drug treatment (S) (Mean + SEM)						
		Time Interval (mins)	0	30	60	90	120	150
Control			2.28 ± 0.05	2.32 ± 0.04	2.37±0.05	2.39±0.05	2.41±0.04	2.45±0.04
Methanol Extract	250		2.32 ± 0.05	2.28 ± 0.05	2.32 ±0.05	2.39±0.04	2.44±0.05	2.37±0.05
Methanol Extract	500		2.30±0.05	2.37±0.05	2.41±0.04	2.45±0.04	2.53 ±0.05	2.48±0.05
Methanol Extract	1000		2.39±0.06	2.45±0.05	2.50±0.06	2.53±0.05	2.63±0.07	2.50 ±0.06
Naloxone+ Methanolic extract	5:1000		2.41±0.04	2.44±0.05	2.48±0.05	2.56±0.03	2.63±0.04	2.48±0.03
Morphine	5		2.43±0.05	7.98±0.03[b]	8.12±0.04[b]	10.26±0.04[b]	10.71±0.04[b]	10.52±0.02[b]
Naloxine+ Morphine	5:5		2.32±0.04	2.42±0.03[c]	2.48±0.02[c]	2.53±0.03[c]	2.57±0.03[c]	2.50±0.03[c]

Significance related to control: [a]$P<0.05$; [b]$P<0.01$.

Significance of morphine+naloxone related to morphine only: [c]$p<0.01$; n=10

IV. DISCUSSION

The methanolic extract of *Afrofritomia sylvestris* leaf caused marked anti- inflammatory activity. The observed activity was dose- dependent. Carageenan-induced oedema experimental model for acute inflammation is believed to be biphasic. The two phases of inflammatory response are usually due to direct stimulation of nociceptors in the paw which culminates in centrally mediated pain with release of substance P in the neurogenic (early) phase. The late phase on the other hand is observed as a result of release of histamine, serotonin, bradykinin and prostaglandins. Some drugs, especially opioid analgesic agents, inhibit both phases equally while peripherally acting drugs such as nonsteroidal anti-inflammatory drugs (NSAIDs) and steroidal anti-inflammatory drugs only inhibit the late phase [25].

The study has also shown that the methanolic extract of *Afrofritomia sylvestris* leaf caused significant analgesic effect against acetic acid-induced writhing (chemically induced) pain in mice in a dose-dependent manner. Acetic acid induced writhing response is used to evaluate peripherally acting analgesics. The model causes pain sensation by triggering localized inflammatory response leading to the release of free arachidonic acid from the tissue phospholipid [26]. The percentage inhibition of writhing produced by 500mg/kg of extract was closed to that produced by 100mg/kg of aspirin. It seems that the analgesic property is peripherally mediated.

In order to distinguish between central and peripheral analgesic action of *Afrofritomia sylvestris* leaf, the analgesic effects of methanolic extracts of *Afrofritomia sylvestris* leaf and morphine on Tail immersion reaction time and Naloxone antagonism model in mice were examined. From the above, it is observed that all the three doses of methanolic extract (250-1000mg/kg) produced insignificant analgesic action, while morphine, a centrally acting analgesic showed significant analgesic effect throughout the duration of the experiment. Pre-treatment of the animal with Naloxone, a narcotic antagonist was able to block the analgesic effects of morphine while pretreatment with naloxone before administration of

1000mg/kg of the extract did not have any significant effect on the already analgesic action of the extract to the heat stimulus. It is known that centrally acting analgesic drugs elevate the pain threshold of mice towards heat and pressure. Since *Afrofritoma sylvestris* leaf extract failed to raise the pain threshold, therefore, the leaf extract is not centrally acting. This view is further supported by the fact that naloxone did not show any antagonism (blocking effect) against the extract.

Results of the phytochemical analysis have shown that *Afrofritomia sylvestris* contains bioactive substances such as alkaloids, saponins, cyanogenic glycosides, triterpenes, flavonoids and tanins. Flavonoids and saponins are well known for their ability to inhibit pain perception as well as anti-inflammatory properties due to their inhibitory effects on enzymes involved in the production of the chemical mediator of inflammation [27].

V. CONCLUSION

In conclusion, the present study shows that methanolic extracts *of Afrofritomia sylvestris* leaf have significant anti-inflammatory and analgesic effects which may in part be related to its chemical constituents such as flavonoids and saponins and these effects are similar to those of aspirin. On the basis of available data presented, the analgesic effect of *Afrofritomia sylvestris* appears to be peripherally mediated. However, further work is required to fully elucidate the exact mechanism of anti-inflammatory and analgesic actions. The methanolic extract therefore, may serve as a lead in the development of a new anti-inflammatory and analgesic drug.

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