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SOME PHARMACOLOGICAL ACTIVITIES OF HEXADEC-12-ENOIC ACID ISOLATED FROM *CHENOPODIUM AMBROSIOIDES* LINN

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ABSTRACT: *The extractive (labelled Sc), from Chenopodium ambrosioides (Linn), which had been characterised as hexadec-12-enoic acid in an earlier work, was tested for analgesic, antipyretic and anti-inflammatory activities. The result for the acetic acid induced writhing test for Sc revealed very high activity, 96.77%, being significantly higher than that of the standard, aspirin i.e. acetyl salicylic acid, (64.68 %). The hot plate analgesic test showed that the activity was not significant and was short-lived compared to that of morphine. The test for antipyretic activity revealed that it possessed not only antipyretic but also hypothermic activity 3 hours after administration. The anti-inflammatory activity of hexadec-12-enoic acid may be involved prostaglandin synthesis inhibition*.

Keywords: Pharmacological, Analgesic, Anti-inflammatory and Antipyretic

INTRODUCTION

No doubt, pain is always with the human race (as well as other animals), cutting across all races and ages. As a result of this, analgesic, antipyretic and anti-inflammatory agents are important to us. Even though a number of such drugs have been synthesised, there is still need to explore plants that have been reported to possess these activities. *Chenopodium ambrosioides* (Linn) is one of such plants [Lohdip and Oyewale, 2009; Lohdip et al, 2008, 2009; Burkill, 1985; Internet Source[1,2,3]]. This led to the isolation and characterisation of compounds with possible pharmacological activities from the plant [Lohdip, 2012].

Even though the compound of interest here, hexadec-12-enoic acid ($C_{15}H_{29}COOH$), is a fatty acid [Robert and Caserio, 1964; Morrison and Boyd, 2000], it was tested for pharmacological activities. This is due to the fact that in spite of the advantage which fat gained from its early discovery and the recognition of its structure, few chemists specialized in the study of fats [Internet Source[4]]. This work was encouraged by the fact that some fatty acids or oils have been associated with a number of pharmacological activities [Rosenberg, 1979; Internet Source[1,5,6,7]].

This paper reports on studies carried out on an extractive isolated from *C. ambrosioides* L., characterised as hexadec-12-enoic acid [Lohdip, 2012], for analgesic, antipyretic and anti-inflammatory activities.

LITERATURE

Fatty Acids

Hexadecenoic acid also known as palmitoleic acid because it is the analogue of palmitic acid, is a fatty acid, implying that it is also a lipid. It is found in marine animal oils, amphibians, reptiles, mammals and vegetables [Internet Source[4]].

Fatty acids include all fatty acids obtained from natural products which are soluble in fat solvents (diethyl ether, chloroform, benzene and petroleum ether) and insoluble in water. Most fatty acids are soluble in hot absolute or 95 % ethanol [Internet Source[4]]. They most frequently occur as components of natural fats and can be classified into several series: Saturated fats series which have no unsaturation linkages; oleic acid series (or monoethenoid acids), characterized by one double bond; linoleic acid (or diethenoid acids), characterized by two unsaturation linkages;polyethenoid acids series consists of tri- and tetra- ethenoid acids; and ethynoic acids series consisting of at least one triple bond [Internet Source[4]]..

Fats have been recognized as a separate category of foodstuff since prehistoric times. The application of fats and waxes as illuminants, in cosmetics, medicinal and as lubricants dates back before our earliest record of civilized man. As phospholipids, fats are important in managing snake bites [Internet Source[4]]. Lavender Essential Oil successfully treat burns [Internet Source[5]]. Linden Blossom Essential Oil also known as Lime Blossom is a tonic for liver, has anti-inflammatory and antiseptic properties, is good for healing wounds and rejuvenating the body [Internet Source[6]].; Sandal Essential Oil relieves body itching and inflammation of the skin, is effective in relieving dehydrated skin, prevents the skin from forming ugly scars and fights against dry eczema [Internet Source[7]]; Chenopodium Oil known as Ascaridole from *Chenopodium ambrosioides* (Linn) is known to posses antihelminthic property [Internet Source[1]].

CHENOPODIUM ambrosioides (LINN)

Chenopodium ambrosioides (Linn) is a small polymorphic annual or perennial herb, depending on the condition or the place it is grown. It has grooved much branched stem, oval and toothed leaves that are up to 4 cm long and 1 cm wide. The flowers are small and green and grow in long spikes/clusters along its stems. The seeds are very small and green when fresh but black when dry. The plant can grow up to a height of 1m.It is believed to have originated from Mexico and tropical regions of Central and South America, though it has been distributed to most of the world. The plant has a very strong odour [Internet Source[1] ; Burkill, 1985]. The species name ambrosioides, i.e ambrosia-like, and one of the English names, Epazote, refers to the strong odour [Internet Source[1,7]]. The Yorubas in Southwest Nigeria call it ‘ewe imi’ (meaning: leaf of excreta), [Burkill, 1985]. In the Middle Belt region of Nigeria, the Ikulus in Kaduna State call it *‘anyinyung’* (=smelly).Hausa= *kafikashiwari*(meaning: your ordour is worse than excreta) [Lohdip and Oyewale, 2009; Lohdip et al, 2008].

Chemical compounds in *Chenopodium ambosioides*includeascaridole, (a volatile oil, 60-80%), isoascaridole, p-cymene, limonene and α-terpinene [Internet Source[2]]. α-pinene, artiasone, butyric acid, d-camphor, ferulic-acid, geraniol, saponins, stigmasterol, etc. Of the compounds mentioned above the most active ingredient responsible for most documented pharmacological activities of the plant is ascaridole (1,4-peroxido-p-menth-2-ene) [Internet Source[1,3]].

Even though a decoction and infusion of the plant has been reported to be toxic, in the 1970s, the World Health Organization reported that a decoction of the leaves (20 g) rapidly expelled intestinal parasites without any apparent side effects in human. The leaf extract was found to be 100 % effective against the common intestinal parasites, *Ancilostoma* and *Trichuris,* and 50 % effective against Ascacris. In 2001 a study showed that the extract of epazote expelled intestinal roundworms and the common human tapeworm (*Hymenolepsis nana*). *In vitro* studies showed that the oil of Chenopodium and Chenopodium extracts inhibits egg development and maturation of larvae parasites [Burkill, 1985].

In some other research [[Internet Source[1]].] it has been documented to be toxic against snails and against drug-resistant strains of *Mycobactrriumtuberclosis*. I has also bee used for the treatment of peptic ulcers and has been reported to haveantitumorous activity (including actions against several multi-drug resistant tumor cell lines) [Internet Source[1]].

METHODOLOGY

The extractive, characterised as hexadec-12-enoic acid [Lohdip, 2012] and labelled Sc, was tested foranalgesic, antipyretic and anti-inflammatory activities.

Test for Analgesic Activities

The writhing and hot plate tests were carried outusing Albino mice of both sexes (15-30g body weights).

The Writhing Test

The test was carried out as described by Okpo *et al*.,(2001) and Koster *et al*.,(1959), The dose used for the extractive was based on the amount of the fraction obtainable from 100g of the stem powder. This was200 $mgkg^{-1}$, prepared by first mixing with a drop of 'TWEEN 80' before dissolving in distilled water, because it was insoluble in water. It was then administered orally, with the help of apolyethenecanula. Test animals were groups of five mice each (Albino mice of either sexes and 20-30 g body weights; obtained from Veterinary Research Institute Vom, Nigeria). One group was administered 10 cm^3kg^{-1} of distilled water and another was given acetylsalicylic acid i.e. asprin, (100 $mgkg^{-1}$) as controls. A dose of 10 $cm^3kg^{-1}$of acetic acid (0.6 % v/v in normal saline) was administered to all groups of animals, and number of writhes for each animal was counted for 15 minutes.

The Hot Plate Test

The hot plate method employed here was according to standard method [Bisignano,1994]. The same dose of the extractive was employed as above and five mice were used. The extractive was administrated orally and morphine (1 $mgkg^{-1}$) was administered subcutaneously as the standard drug. Temperature of hot plate was raised to 55 ^oC. One animal at a time in a big beaker covered at the top was placed on the hot plate. The time of reaction to stimulus of the mouse was recorded at the 1st, 2nd and 3rd hour after administration.

Test for Antipyretic Activities

The method described by Koster *et al*.,(1959) was employed. The pyrexia was induced by subcutaneous injection of 20 % aqueous suspension of yeast in distilled water (20cm^3kg^{-1}).Same dose of the extractive was employed as above and was administered intraperitoneally

(ip) to five Albino mice (20-30g body weight) each. Distilled water ($10cm^3kg^{-1}$) was administered to another group of five mice, as control.

The rectal temperatures were recorded using a digital thermometer, about 1 hour before the beginning of the experiment (taken as reference point) and then 1,2 and 3 hours, respectively, after administration of the drugs. The results were expressed as mean ± standard error of the data obtained from different animals. The differences between groups were statistically analysed by SPSS student's test for paired data and differences with $P < 0.05$ were considered significant.

Test for Anti-Inflammatory Activity

The experiment was carried out as described by Bamgbose and Naomese, (1981). Rats (5 per group) were allotted to different treatment groups. Oedema was induced in the rats by injecting egg albumin into the sub-plantar tissue of the right hind paw. The linear paw circumference was measured using the cotton thread method [Santos *et al*., 1994)]. The paw swelling at each time was calculated as the difference between the linear circumstance at time t (C_1) and that at 0 hour (C_o).

Distilled water (0.1 cm^3/100 g) and indomethacin (10 $mgkg^{-1}$) were used as control and standard, respectively, while same dose of the extractive was employed as in the antipyretic activities.They were all administered orally 1 hour before injection of thephlogistic agent.

Measurements were again made immediately after injection of the phlogistic agent and at hourly intervals for 3 hours. This was to check forthe effect of the water, extractive and drug after the induction of albumin-induced oedema on the mice.

RESULTS AND FINDINGS

Analgesic Activities

The results for the analgesic activities of hexadeca-12-enoic acid (Sc) from *Chenopodium ambrosioides* (Linn) against acetic acid and hot plate induced pains on mice are summarized on Tables 1and 2, respectively.

Table1:**Result of test for analgesic activity of hexadeca-12-enoic acid (Sc) from Chenopodium ambrosioides (Linn) - acetic acid (AA) -induced writhing test on mice**

Extract/drug	Dose (mg/kg)	No. of Writhes in 15 Minutes After Administering AA	% Inhibition
Water	10	51.67 ± 2.36	-
Sc	200	1.33 ± 0.42	96.77
Acetyl salicylic acid (10mg/kg) (standard)	10	18.25 ± 2.17	64.68

Table 2: Result of hot plate analgesic test for hexadeca-12-enoic acid (Sc) from Chenopodium ambrosioides (Linn)

Extract/ drug	Dose (mg/kg)	Time Of Reaction To Stimulus After Administration (in sec.)					
		1hr	% Inhibition	2hr	% Inhibition	3hr	% Inhibition
Water	10	11.25 ± 1.31	-	11.00 ± 2.00	-	10.40 ± 0.80	-
Sc	200	15.00 ± 4.08	33.33	14.00 ± 1.41	38.46	10.67 ± 0.94	-0.05
Morphine (1mg/kg) (Standard)	1	16.00 ± 0.82	42.22	15.00 ± 0.00	36.36	15.00 ± 0.00	48.08

Antipyretic Activity
The result of the test for antipyretic activity of hexadec-12-enoic acid on Albino mice is summarised on table 3.

Table 3:Result of antipyretic activities of test for hexadeca-12-enoic acid (Sc) from Chenopodium ambrosioides (Linn)

EXTRACT/DRUG	DOSE (mg/kg)	RECTAL TEMPERATURE (^{O}C)							
		After Administration Of Drug/Extracts							
		1hr before expt (control)	16 hr after yeast	1hr after admi	Temp. Drop 1hr	2hr	Temp. Drop 2hr	3hr	Temp. Drop 3hr
Water	10	36.13 ± 0.47	37.97 ±0.37	38.13 ± 1.14	-0.16	38.30 ± 0.27	-0.33	38.00 ± 0.57	0.13
Sc	200	36.23 ± 0.21	37.37 ± 0.52	37.23 ± 0.19	0.14	35.80 ± 1.50	1.57	35.83 ± 1.18	1.54

Anti-Inflammatory Activity

The result for the anti-inflammatory activities of the extractive against albumin-induced oedema on rats paws is summarized in table 4.

Table 4: Result of anti-inflammatory activity test for hexadeca-12-enoic acid (Sc) from Chenopodium ambrosioides (Linn)

EXTRACT/ DRUG (mg/kg)	TIME OF REACTION TO STIMULUS AFTER ADMINISTRATION (IN SEC.)									
	1 hr before albumin	1 hr after albumin			2hr after albumin			3hr after albumin		
	C_i	Ct	Paw swelling	% Inh	Ct	Paw swelling	% Inh	Ct	Paw swelling	% Inh
Indomethacin (5mg/kg)	2.33 ± 0.20	2.90 ± 0.88	0.57	24.47	2.40 ± 0.8	0.07	89.85	2.13 ± 0.9	-0.20	129.41
Sc (200)	2.13 ± 0.09	3.03 ± 0.12	0.90	-16.88	2.73 ± 0.36	0.60	13.04	2.60 ± 0.08	0.47	30.88
Water (10 ml/kg)	2.30 ± 0.08	3.07 ± 0.25	0.77	-	2.99 ± 0.05	0.69	-	2.98 ± 0.05	0.68	-

NOTE: C_i = Initial paw circumference; Ct = Paw circumference at the given time; Inh = Inhibition

DISCUSSION

Analgesic Activities

Acetic Acid Test

The result for the acetic acid induced writhing test for Sc revealed very high activity,96.77%. Statistically determined by SPSS paired sample t-test, compared to the control, the extractive significantly ($P<0.05$) inhibited the pain induced by acetic acid. Also compared to the standard, aspirin, the activity of Sc was significant ($P<< 0.05$).

The ability of the extractive to inhibit acetic acid-induced writhing in mice suggests that the analgesic effect of the extractive could be peripherally mediated [Okpo *et al.,* 2001].The percentage inhibition being higher than that of aspirin (64.68 %) is an indication that the extractive, hexadec-12-enoic acid could be a good analgesic.

The Hot Pate Test

Comparing the time taken for perception of pain by animals administered the extractive to the time taken for animals in the control group (Table 2), there were 33.33 % and 38.46 % inhibitions one (1) and two (2) hours after administration, respectively, while there was no inhibition three (3) hours after administration. This implies that the activity was not significant and was short-lived compared to that for morphine.

The inhibitions as compared to the control were not significant, ($P > 0.05$). Also compared to the standard, Morphine, the activities of the extractive was not significant ($P > 0.05$).

The results of the analgesic tests in this work suggest that the extractive, hexadec-12-enoic acid, possesses analgesic activity which could be peripherally mediated, since it inhibited acetic acid-induced writhing in mice. The hot plate test revealed that any analgesic activity centrally mediated, exhibited by this fraction, is not significant and is short-lived.

The analgesic activity exhibited by hexadec-12-enoic acid here corroborated with the analgesic activity for the crude extract of *C. ambrosioides* (Linn) reported [Lohdip and Oyewale, 2009], as well as confirms the assertion in literature that *C. ambrosiodes* Linn possesses analgesic activity [Internet Source[1]].

Antipyretic Activity

Comparing the temperature drops in animals administered the extractive to the temperature drop in control group (Table 3), the extractive showed antipyretic activity. Infact, it possessed not only antipyretic but also hypothermic activity as 3 hours after administration, the temperatures even dropped lower than that measured before administration of yeast [Koster *et al*.,1959]. It could be said that the extractive possesses antipyretic activity which expanded to at least 3 hours after administration.

Ant-Inflammatory Activity

The percentage inhibition on paw swelling was determined by comparing the linear paw circumference of animals treated with the extractive and indomethacin (the standard drug) to that obtained in the control group administered only water. Low swelling implies that the extracts were active.

The result revealed that Sc gave 30.88 % inhibition lower than for the standard drug, indomethacin.The effects of the extractive was more pronounced at the later stages of the inflammation suggesting it may correspond to the phase of prostaglandin release, which is the third and final phase in carrageen on oedema; other phases are the initial phase which involves the release of histamine and 5HT, the second phase is mediated by Kinins [Di Rosa *et al.*,1971]. Most anti-inflammatory agents inhibit cyclooxygenase enzyme which is involved in prostaglandin synthesis at the site of inflammation [Wannang, 2005]. The anti-inflammatory activity of hexadec-12-enoic acid isolated from *C. ambrosioides,* therefore, may involve prostaglandin synthesis inhibition.

IMPLICATION TO RESEARCH AND PRACTICE

This work has justified some claims that Chenopodium ambrosioides L. is used against pain, headache, fever [Lohdip and Oyewale,2009] and that it possesses anyi-inflammatory activity [Lohdip *et al.*, 2008; Internet Source[1]]. The findings in this work could also be used as a lead to synthesis and development of new analgesic, antipyretic and anti-inflammatory drugs.

CONCLUSION

The acetic acid-induced writhing test in mice revealed that hexadec-12-enoic acid possessed analgesic activity that could be peripherally mediated, but did not possess a significant centrally mediated analgesic activity. The antipyretic test showed that the extractive does not only possesses antipyretic activity, but also hypothermic. It also possesses anti-inflammatory activity which may involve prostaglandin synthesis inhibition. These findings suggest that the analgesic and antipyretic activities reported to be present in the crude aqueous extract of Chenopodium ambrosioides Linn (1) could be solely attributed to hexadec-12-enoic acid.

The findings reported in this paper have also succeeded in enlisting hexadec-12-enoic acid as an analgesic, antipyretic, as well as anti-inflammatory agent. The discovery of new analgesic, antipyretic and anti-inflammatory agents of local plants origin, such as mentioned above, could replace some already existing drugs which are mostly expensive and unaffordable by the poor of the society. This could help in meeting tbeMilleniumDevelopement Goals (MDGs) which include improvement of health and reduction of mortality for all, especially women and children.

FURTHER RESEARCH

Further research will involve isolation and characterization of more compounds from Chenopodium ambrosioides L. These will also be tested for pharmacological activities.

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