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Antinociceptive, Anti-inflammatory and Antioxidant activities of Eleagnine: An alkaloid Isolated from *Chrysophyllum albidum* Seed Cotyledons

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Abstract: Eleagnine, an alkaloid isolated from *Chrysophyllum albidum* seed cotyledon was investigated to evaluate its potential antinociceptive, anti-inflammatory and antioxidant activities. The antinociceptive effects were carried out using flick, hot-plate tests and acetic-acid induced writhings in mice. The anti-inflammatory activity and the pain threshold were assessed on the oedematous right hind paws of rats using plethysmometer and analgesiometer respectively. The antioxidant effect was carried out *in vitro* using DPPH (1,1-diphenyl-2-picrylhydrazyl) assay. The compound demonstrated potent analgesic (in all the four models used both peripheral and central sites), anti-inflammatory and a very weak antioxidant properties. Naloxone (an opioid antagonist) showed that the analgesic effect was mediated through opioid transmission in both central and peripheral sites while its anti-inflammatory effect involve the inhibition of histamine, 5-HT, prostaglandins in carrageenan induced oedema. Antioxidant activity of the eleagnine ranges between 46.1 and 15.1% for the tested concentrations. This was far less than what observed for ascorbic acid. At much lower concentration. These pharmacological effects of this alkaloidal compound described here may suggest that eleagnine is one of the constituents of *Chrysophyllum albidum* responsible for the ethnomedical uses as antimicrobial agent through anti-inflammatory property.

Key words: *Chrysophyllum albidum*, eleagnine, an isolated alkaloid, antinociceptive, anti-inflammatory, antioxidant effects

INTRODUCTION

The *Chrysophyllum albidum* G. Don-Holl. (Sapotaceae) tree is common throughout the tropical Central, East and West Africa regions for its sweet edible fruits and various ethnomedical uses (Dalziel, 1955; Amusa *et al.*, 2003). *Chrysophyllum albidum* fruits (known as African star apple) are widely eaten in southern Nigeria. The fleshy fruit pulp is suitable for jams and is eaten especially as snack by both young and old (Amusa *et al.*, 2003). The African star apple fruit has been found to have highest content of ascorbic acid with 1000 to 3,330 mg of ascorbic acid per 100 g of edible fruit or about 100 times that of oranges and 10 times of that of guava or cashew (Amusa *et al.*, 2003). It is reported as an excellent source of vitamins, irons, flavours to diets (Nwadinigwe, 1982; Adisa, 2000). In addition, its seeds are a source of oil, which is used for diverse purposes. The fruits also contain 90% anacardic acid, which is used industrially in protecting wood and as source of resin, while several other components of the tree including the roots and leaves are used for medicinal purposes

(Adewusi 1997). The bark is used as a remedy for yellow fever and malaria, while the leaves are used as emollients and for the treatment of skin eruptions, diarrhoea and stomachache, which are as a result of infections and inflammatory reactions (Adisa, 2000).

Natural antioxidants have been established to promote health by acting against oxidative stress related diseases such as infections, diabetics, cancer and coronary heart diseases (Burits and Bucar, 2002). Studies have shown a diminished risk of chronic diseases in populations consuming diets high in fruits and vegetables (Pryor *et al.*, 2000). It has been suggested that antioxidants found in large quantities in fruits and vegetables may be responsible for this protective effect (Halliwell, 1994). Generally, food antioxidants act as reducing agents, reversing oxidation by donating electrons and hydrogen ions (Groff and Gropper, 2000). Much attention has been focused on natural antioxidants and some antioxidants isolated from natural sources with high activity have been reported (Okamura *et al.*, 1993; Parasakthy *et al.*, 1996). Most of these natural antioxidants constituents are derived from plant sources.

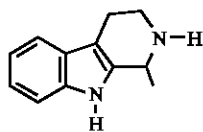


Fig. 1: Eleagnine

In continuation of our studies on antioxidant and anti-inflammatory agents in Nigerian medicinal plants (Iwalewa *et al.*, 2003, 2005; Omisore *et al.*, 2004, 2005) and in our quest for novel antioxidant principle, from natural sources, due to toxicological effects observed from the synthetic ones, we report here the antinociceptive, anti-inflammatory and the antioxidant effects of eleagnine, an alkaloid isolated from *Chrysophyllum albidum* (Fig. 1).

MATERIALS AND METHODS

Chemicals: All chemicals used were of analytical grade obtained from BDH Chemicals Ltd., Poole England and Fluka chemika.

Plant material

Collection, extraction and isolation: The fruits of *C. albidum* bought in Ile-Ife market was identified by Mr. A.T. Oladele of the Herbarium Section, Department of Pharmacognosy, Faculty of Pharmacy, Obafemi Awolowo University, Ile-Ife and voucher specimen (FPH/S/001) was deposited. Isolation of the eleagnine was as previously described (Idowu *et al.*, 2003).

Animals: Swiss albino mice of either sex weighing 20-26 g and Wistar albino rats (150-255 g) were used. The animals were maintained under normal laboratory conditions of humidity, temperature (25±1°C) and light (12 h day: 12 h night), in the Department of Pharmacology Animal House and allowed free access to food and water *ad libitum* for at least 5 days, before the commencement of our experiments. The principle of laboratory animal care (NIH publication No 85-23) guidelines and procedures were followed in this study (NIH publication revised 1985).

Drugs: The following drugs were used: [Disprin® (acetylsalicylic acid-ASA)]-(Reckiti Benckiser); Carrageenin (Sigma) and Indomethacin (KGN Pharmaceuticals). Glacial acetic acid (BDH.), DPPH-1,1-diphenyl-2-picrylhydrazyl (Sigma), Naloxone (Sigma), Histamine (Sigma), Serotonin (Sigma), Cyproheptadine Hydrochloride (Shalina Laboratories Pvt. Ltd., Mumbai-India).

Analgesic activity evaluation: Evaluation of the analgesic properties of eleagnine was carried out by using three

different models of noxious stimuli; namely, chemical, mechanical and thermal stimuli.

Acetic acid-induced writhing method: Control group of mice (n = 5) received normal saline (0.3 mL kg⁻¹ i.p.). Mice in the test groups received eleagnine (5, 10 and 20 mg kg⁻¹ i.p) or acetylsalicylic acid (ASA, 100 mg kg⁻¹ i.p) respectively. ASA was used as the reference analgesic drug for comparison in this study. One hour following eleagnine-, ASA-or normal saline administration, 0.1 mL of a 3% acetic acid solution was injected to each of the test mice intraperitoneally (Koster *et al.*, 1959). The number of abdominal contractions that occurred within the next 20 min following acetic acid administration were counted and recorded. A significant reduction in the number of acetic acid-induced abdominal contractions of the treated mice, compared to the contractions in the untreated control mice, was taken as an indication of analgesic activity.

Hot Plate test method: Control group of mice (n = 6) received normal saline (0.3 mL kg⁻¹ i.p.) only. The control mean reaction time (in sec) was determined and recorded. The test group mice (5 mice per dose or reference drug-dose) were treated with different doses of eleagnine (5, 10 and 20 mg kg⁻¹ i.p) or ASA (100 mg kg⁻¹ i.p.), respectively. One hour following the test agent or ASA-administration, the mice were separately placed in a hot plate (Thermajust, Model 475, TechniLab Instruments, N.J, 07440) maintained at 55±1°C. For both the control and test animals, the reaction time (in sec) was taken as the time when each of the mice jumped out of the beaker on the hot plate. The test mean reaction time (in sec) was also determined for each plant extract dose and ASA.

Tail immersion test method: Control group of mice (n = 6) received normal saline (0.3 mL kg⁻¹ i.p.) only and the mean reaction time (in seconds) was determined. Test groups of mice (6 mice per extract-or reference drug-dose) were treated with eleagnine (5, 10 and 20 mg kg⁻¹ i.p) or ASA (100 mg kg⁻¹ i.p.), respectively. One hour following the drug or reference drug (ASA)-administration, the tail (up to 5 cm) of each mouse was immersed in hot water maintained at 50±1°C (in a 1 L water bath). For both the control and test animals, the reaction time (in sec) was taken as the time when the animals withdrew their tails completely from the hot water in the bath (Parimaladevi *et al.*, 2003). The test mean reaction time (in sec) was calculated for each dose, ASA and the control.

Investigation for opioid receptors mediation in inducing analgesia: Ten mice were fasted overnight (but allowed free access to water) and were divided into 2 groups. The two groups received 5 mg kg⁻¹ Naloxone subcutaneously

(sc). After 45 min a group received 0.3 mL normal saline while the second group got 20 mg kg⁻¹ eleagnine intraperitoneally (i.p.). These mice were then subjected to flick test, hot-plate test and acetic acid induced abdominal contractions for analgesic activities (Dearniyagala *et al.*, 2003)

Analgesimeter method: pain threshold in rats: The oedematous right hind paws induced by 0.1 mL of 1% carrageenin were subjected to an increasing force (pressure) according to the method of Randall and Sellito (1957). One hour following the administration of eleagnine (5, 10 and 20 mg kg⁻¹ i.p.) or IND (10 mg kg⁻¹ i.p.) into the test animals and 0.3 mL kg⁻¹ i.p normal saline into the control animals, the pain threshold was measured mechanically (using Ugo Basile Analgesimeter-Model 09380, Milan, Italy). at 0, 0.5, 1, 2, 3 and 4 h after drug administration. Squealing of the animals as a consequence of application of continuous pressure to their paw was taken as the reaction time of the animals. Thereafter, the pressure (force) stimulus was terminated and the pain threshold was read off from the scale.

Anti-inflammatory activity determination: Inflammation in the hind paw of albino rat was induced as described by Winter *et al.* (1962). 0.1 mL of 1% carrageenan suspension was injected into sub-plantar surface of the right hind limb of each rat. The control group received 0.3 mL normal saline, the treated and positive control groups received eleagnine (5, 10 and 20 mg kg⁻¹ i.p) and 10 mg kg⁻¹ of Indomethacin (IND) respectively by intraperitoneal route, 1 h before carrageenan injection. The volume of each paw was measured by plethysmometer (Cat No. 7150 Ugo Basile 21025 Comerio-Varese Italy) to determine the oedema formation size at 0, 0.5, 1, 2, 3 and 4 h. The difference in the left and the right hind paws was taken as a measure of oedema.

Hind paw oedema induced by chemical mediators in mice: 20 mice were divided into four groups containing five mice each. Ten of the mice received 20 mg kg⁻¹ eleagnine i.p. 30 min after the administration of eleagnine they were divided into two groups. The first group was injected with histamine (1 mg kg⁻¹) while the second group received serotonin (1 mg kg⁻¹) into the subplantar tissue of the right hind paw. The third and the fourth groups serve as the negative and positive controls; they received 0.1 mL of normal saline and 10 mg kg⁻¹ cyproheptadine, respectively. Oedema formation was measured in plethysmometer every 6 min for histamine and serotonin. The difference between the left and right hind paws indicated the oedema formation (Kasahara *et al.*, 2002; Gupta *et al.*, 2003).

Evaluation of antioxidant activity: The determination of the radical scavenging activity of the alkaloid was carried using the DPPH (1,1-diphenyl-2-picrylhydrazyl) assay as described by Mensor *et al.* (2001), with a slight modification. The test sample (2.0 mL) of the varying concentrations (1000, 500 and 250 µg mL⁻¹) was added to 1.0 mL of DPPH (0.25 mM) in methanol. The mixture was incubated in a dark chamber for 30 min and absorbance was taken at 515 nm on a spectrophotometer (Pharmacie Biotech, Novaspec II). The mean absorbance from three replicate assays was then converted into the percentage antioxidant activity (AA%) using the formula:

AA% = 100-{(Abs sample-Abs blank)×100}/Abs control.}, where Abs sample was the absorbance of sample (eleagnine) = sample solution (2.0 mL) plus DPPH solution (1.0 mL, 0.25 mM), Abs blank was the absorbance of blank = Methanol (1.0 mL) plus sample solution (2.0 mL), without DPPH. Abs control was the absorbance of negative control = DPPH solution (1.0 mL, 0.25 mM) plus methanol (2.0 mL), without eleagnine.

Statistical analysis: The data obtained were expressed as mean±SEM and were subjected to statistical analysis using student's t-test. Values of p<0.05 were taken to be statistically significant.

RESULTS

Anti-inflammatory activity and inhibitory: The effect of eleagnine on the acute inflammatory oedema induced by carrageenan on rat hind paw showed a significant (p<0.05) anti-inflammatory activity (Fig. 2). The percentage of inhibition of oedema were 45.2, 61.4 and 55.6% using 5, 10 and 20 mg kg⁻¹ of eleagnine respectively. Indomethacin IND (10 mg kg⁻¹) inhibited the oedema volume by 73.2%. These effects are not dose dependent and not significantly different from each other. However, the effects of different doses of eleagnine were significantly different from the control. From Fig. 2 and 3, it was clearly shown that eleagnine (though not dose dependent at various doses used) suppressed oedema formation mediated by the chemical mediators of inflammation. Eleagnine effectively inhibited the inflammation induced through histamine and serotonin, which could suggest that the eleagnine anti-inflammatory action is through inhibiting the mediators involved in inflammation. The effect of eleagnine (Fig. 2 and 3).

Analgesic effect: Analgesic effect of eleagnine was clearly shown in all the four models used. In the flick test, eleagnine was significant at 20 mg kg⁻¹ only, (Table 1). In both hot plate and acetic-acid induced writhing models, eleagnine showed a reduction in pain dose dependently

Table 1: The antinociceptive activity of Eleagnine (ELG) and the involvement of naloxone (an opioid antagonist) in three models of induced pain in mice

Doses (mg kg ⁻¹)	Flick test (s)	Hot-plate test (s)	Acetic-acid induced writhing (no/20 min)
Control (0.3 mL normal saline)	3.6±0.6	7.4±0.7	63.5±2.4
Control±Naloxone (5 mg kg ⁻¹)	2.5±0.4	11.3±0.9**	57.2±1.6
Eleagnine (5 mg kg ⁻¹)	5.2±2.0	10.4±1.4*	61.0±7.8
Eleagnine (10 mg kg ⁻¹)	4.8±1.6	13.9±1.5**	24.6±7.3**
Eleagnine (20 mg kg ⁻¹)	23.8±9.1**	29.7±7.7**	12.8±5.7**
Eleagnine (20 mg kg ⁻¹) + Naloxone (5 mg kg ⁻¹)	3.12±0.4	12.9±0.8**	52.2±2.4
ASA (100 mg kg ⁻¹)	19.8±1.9**	16.5±2.0**	18.2±2.9**

Values are Mean±SEM of 5 mice. *p<0.05, **p<0.01 was taken as statistically significant value compared to the control

Table 2: Percentage antioxidant activity of eleagnine and ascorbic acid on DPPH scavenging capacity. Ascorbic acid percentage antioxidant activity at 6.0 µg mL⁻¹ was 60.1%

Concentration µg mL ⁻¹	Activity (%)
1000	46.1
500	22.4
250	15.1

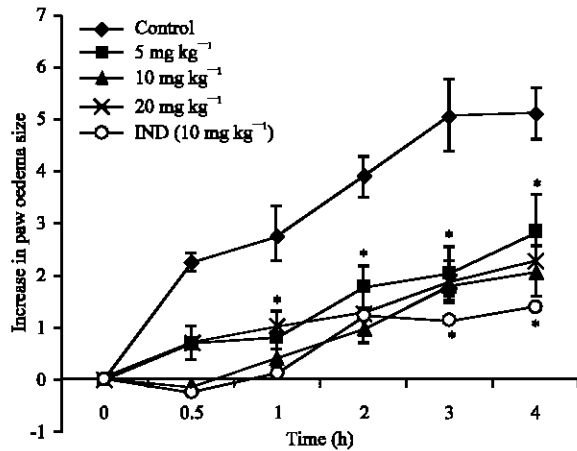


Fig. 2: Anti-inflammatory activity of Eleagnine (ELG) and IND in carrageenan induced oedema of the rat hind paw. Values are Mean±SEM of 5 mice. * p<0.05, was taken as statistically significant value compared to the control

and significantly (p<0.05) at 10 and 20 mg kg⁻¹ dose levels. These activities were however greater when compared to acetylsalicylic acid as the standard drug. Using analgesiometer, a mechanical induced pain on oedematous paw, there was a dose dependent increase in pain threshold with eleagnine (Fig. 4).

Antioxidant activity: In the DPPH assay, ability of eleagnine to scavenge free radicals was measured spectrophotometrically to estimate the percentage antioxidant activity. The results indicated a consistent increase in percentage antioxidant activity with increase concentrations as shown in Table 2. Ascorbic acid exhibited 63.1% antioxidant activity at the concentration of 6.0 µg mL⁻¹

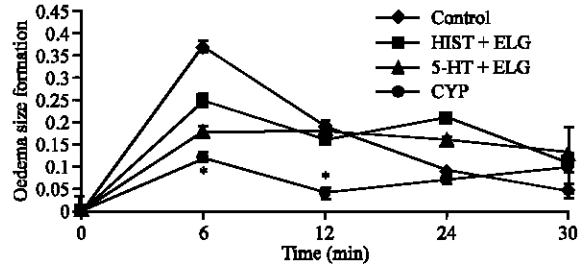


Fig. 3: Inhibitory activity of eleagnine (ELG) on histamine and serotonin induced oedema paw in mice. Values are Mean±SEM of 5 mice. * p<0.05, was taken as statistically significant value compared to the control

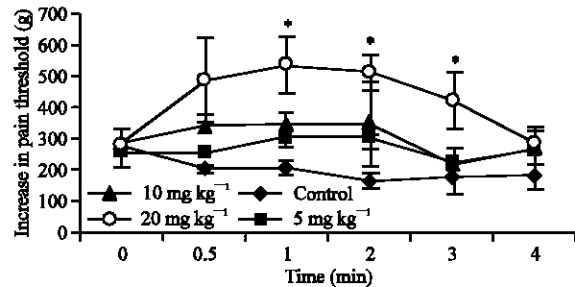


Fig. 4: Analgesic activity of Eleagnine on the mechanically induced pain in rats hind oedematous paw as measured by analgesiometer. Values are Mean±SEM of 5 mice

DISCUSSION

The present study examined the anti-inflammatory, analgesic, antioxidant and biochemical effects of eleagnine, an alkaloid isolated from the seed cotyledon of *Chrysophyllum albidum*. The results showed that the doses used in both mice and rats exhibited antinociceptive and anti-inflammatory activities, however, the compound has a weak antioxidant property in DPPH.

Antinociceptive action of eleagnine had a rapid onset and produce maximum response within 1-2 h. This action was found in all the models used, which indicates that eleagnine is effective against acute peripheral and centrally mediated pains. This observed analgesic effect

could be linked to the involvement of opioids transmission through which naloxone an opioid receptor antagonist blocked the effect of eleagnine in all the models used. It is a well-established fact that NSAID's and some flavonoidal constituents from plant sources exert their anti-inflammatory and analgesic effect through inhibition of cyclo oxygenase pathways (Vane, 1971; Hosseinzadeh and Younesi, 2002). Recent finding also indicate that there are other pathways like induce nitric oxide synthetase (iNOS), cytokines, TNF alpha (Feghali and Wright, 1997). However, in the time-course of oedematous inflammation formation induced by carrageenan, it has been shown that three main mediators are responsible for the acute and chronic inflammatory reactions (Kasahara *et al.*, 2002; Di Rosa *et al.*, 1971).

The first early phase is induced by the presence of histamine, serotonin/bradykinins, while the second late phase is produced mainly by prostaglandins (Kasahara *et al.*, 2002). From Fig. 1 and 2, it was clearly shown that eleagnine (though not dose dependent at various doses used) suppressed oedema formation mediated by the chemical mediators of inflammation. Eleagnine effectively inhibited the inflammation induced through histamine and serotonin, which could suggest that the eleagnine anti-inflammatory action is through inhibiting the mediators involved in inflammation. The effect of eleagnine therefore throws some light into possible constituents of *C. albidum* extract reported by Onabanjo *et al.* (1979) to possess anti-inflammatory activity.

From our quantitative determination, eleagnine appeared as a weak natural antioxidant compare to ascorbic acid activity at a much lower concentration (Table 2). This could be due to the absence of phenolic hydrogen. Phenolic compounds especially the flavonoids have been the secondary metabolites with promising antioxidant activity. Quercetrin is a flavonoid with a better antioxidant activity than ascorbic acid and has been employed, as positive control is some antioxidant experiments (Thabrew *et al.*, 1998). Their activities as been linked to the presence of catechol group in ring B, the 3-OH group in combination with a C2C3 double bond and keto group in position 4 (Trouillas *et al.*, 2004; Saskia *et al.*, 1996). These features are absent in the investigated eleagnine alkaloid. The seed cotyledon of *C. albidum* is so important in Western Nigeria ethnomedicine for various treatments of infections. For example extract of the seed cotyledon is used for vaginal and skin infections (Dalziel, 1955; Idowu *et al.*, 2003; Onabanjo *et al.*, 1979). It is widely known that infection is the condition of multiplication of parasitic organisms or microorganisms like bacteria, fungi, malaria, virus and worms within the body. This can be recognized by fever,

pain, redness, swelling, heat (or warmth), general malaise, increased white blood cell count in the blood. All these are symptoms of inflammatory reactions (www.answers.com/topic/inflammation). There is a link between infections, oxidation from free radicals and inflammation (Omisore *et al.*, 2005). Infection and oxidants ultimately result into inflammatory responses through the release of various autocoids at the site of infection (Onabanjo and Maegraith 1970; Iwalewa and Agbani, 2004; Omisore *et al.*, 2005). Therefore the antimicrobial effect of eleagnine in our previous study (Idowu *et al.*, 2003) could be linked up to the anti-inflammatory, analgesic and antioxidant properties exhibited by this alkaloid in this investigation. These activities of eleagnine (as one of the constituents found in *C. albidum*) could therefore support the ethnomedicinal and ethnobotanical uses of *Chrysophyllum albidum* seed cotyledon.

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