ORIGINAL RESEARCH

Effects of *Dacryodes edulis* Leaf Extract on Pain and Inflammation

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ABSTRACT

Background: *Dacryodes edulis* leaf extract is used in traditional settings in management of many disease conditions including pain and inflammation. However, these empirical claims have not been evaluated scientifically.
Objective: The objective of this study was to determine the effect of ethanol leaf extract of *Dacryodes edulis* on pain and inflammation using rodents.
Materials and Methods: Writhing test using acetic acid for induction of pain and tail-immersion in temperature controlled water were used to study the effect on pain sensation while xylene-induced ear oedema and carrageenan-induced paw oedema were used for the effect of *Dacryodes edulis* on inflammation. Male mice divided into seven groups and three different doses of extract viz,100mg/kg, 200mg/kg and 400mg/kg based on the results of the acute toxicity study and the mean effective dose study, ED_{50} were used for the study. Ethical approval was obtained from the Faculty of Basic Medical Sciences, University of Calabar Animal Research Ethics Committee with number: 012PA31116.
Results: 400mg/kg extract showed significant analgesic activity having 75% inhibition of pain in the writhing test while aspirin showed 80% inhibition of pain. The extract also significantly ($p<0.05$) prolonged flick latency period comparable to morphine in tail immersion test. The extract caused 72% inhibition of xylene induced ear oedema compared to 91% of dexamethasone. Similarly it showed comparable activity ($p<0.05$) with aspirin in the carrageenan-induced paw oedema.
Conclusion: *Dacryodes edulis* leaf extract has activity against pain and inflammation. This validates its application in management of inflammation and pain in local settings.

Keywords: *Dacryodes edulis*, Analgesic, Pain, Anti-inflammatory, Male mice

INTRODUCTION

Many herbs have been used in managing various ailments in man especially in traditional settings. In recent years, there is increasing awareness and general acceptability of use of herbal drugs in medical practice[1].The rise in use of herbal preparations in healthcare delivery has given rise to abuse and adulteration of some of the preparations. The major problems however most of the times is lack of standardization and scientific evaluation of their efficacy, safety and potency. A typical example is the *Dacryodes edulis* Lam (Burseraceae) commonly called African pear. The tree

usually attains a height of up to 40 m in a wild forest but does not exceed 12m in farm plantations. It has a short trunk with a deep, but dense crown. The bark is usually rough with droplets of resin. It usually has compound leaves with about 8 leaflets in pairs. The leaves are usually glossy especially the upper surface. The flowers which are usually yellow and measure about 5-6 mm across[2]. The fruit length varies between four to twelve centimeters and the colour is usually violet or dark blue. However, the flesh is usually light green to pale. The season for fruit bearing is usually around the month of July and the fruits matures at about five months after flowering. *Dacryodes edulis* has two varieties; viz, *edulis* and *parvicarpa*. The fruits of *D. edulis* are larger and the tree branches are ascending and stout. The fruits of D. *parvicarpa* are smaller and the branches are drooping and slender[3]. The leaves concoctions of African pear are applied directly for earaches while the leaf sap is dropped into the ear to relieve ear pains[4]. The leaf decoctions or softened fruit of African pear are taken to relieve constipation. The leaf concoctions are also used to treat digestive tract infections and discomfort[4]. *D. edulis* is also reported to have anti-malarial activity, anti-oxidant activity and anti-bacterial activities[5,6,7] . Iron and copper are found in significant amounts in the fruits of African pear. These minerals are reported to be effective in preventing fatigue, cognitive malfunction, muscle weakness and organ system malfunction[8]. This study was undertaken to evaluate the efficacy and potency of the ethanol leaf extract of *Dacryodes edulis* on pain and inflammation.

MATERIALS AND METHODS

Preparation of plant extract

The leaves of *Dacryodes edulis var edulis* were collected from the African peer tree along MCC road Calabar, Cross River State. The leaves were identified by a taxonomist at the herbarium unit of the Department of Botany at the University of Calabar, Cross River State.

After rinsing the leaves of foreign matter, they were immediately air dried for 48hours and thereafter pulverized to fine powder. A quantity (1kg) of the grounded leaves of the plant was then macerated in about 5litres of 98% ethanol and left for 24 hours. After filtering the extract, the filtrate was evaporated to get a near solid mass using rotary evaporator (Buchi, Germany) and subsequently dried using a water bath set at 55ºC to give a dark green resinous mass.

Pharmacological studies

Ethical approval for the study was given by the Faculty of Basic Medical Sciences, University of Calabar, Nigeria, Animal Research Ethics Committee (FAREC-FBMS) with number; 012PA31116.

Acute toxicity test.

Acute toxicity test was done using Miller and Tainter (1944) method [9]. Thirty five male mice weighing between 19-24g were divided into seven groups of five mice per group. The test was carried out by single oral- administration of the extract at doses of 100, 500,1500, 3000, 5000 and 6000mg/kg to groups 1 to 6 respectively. Mortality and general behaviour were observed continuously for 1, 3 and intermittently for next 6 hours, 24 hours and 48 hours. The parameters watched out for were, gross behavioural changes, grooming, alertness, sedation, loss of righting reflex, tremors and convulsion. From a graph of percentage mortality in probit versus log dose, LD_{50} was calculated

Three doses viz, 100mg/kg, 200mg/kg and 400mg/kg which represented halve, whole and double of the ED_{50} against *Plasmodium berghei berghei* were used in this study. The same doses were used to evaluate the effect of the extract in treating malaria (being processed for publication elsewhere) as well as effect against pain and inflammation since these conditions sometimes present together.

Analgesic activity test

Acetic-acid induced writhing test.

The writhing test method as described by Akuodor *et al*[11] was adopted. A total of 30

albino mice were used in the study. The animals were shared into 5 groups of 6 mice per group. Group one which served as control group, received only 0.2ml of normal saline. Groups two, three and four received extract doses of 100,200 and 400 mg/kg body weight respectively. Group five was given aspirin-150mg/kg body weight. After thirty minutes of administering the drug, an intraperitoneal injection of 0.7% acetic acid was given to cause pain sensation. Afterwards, each mouse was put in a transparent box for observation. The count of number of abdominal constrictions in each mouse was taken for thirty minutes starting from 5 minutes after injecting acetic acid formulation[11]

Tail immersion test

A total of 30 albino mice were used. They were shared into five groups of six mice in each group. The negative control which was group one received 0.2ml of normal saline each. Groups two, three and four were given 100, 200 and 400 mg/kg body weight of the extract, respectively. Group five was given 10mg/kg subcutaneous morphine injection. After thirty minutes of administering the drug, each mouse was restrained in a cylinder, horizontally such that the tail would be hanging freely. A mark was made at the 4cm length of the mice tail and the marked part of the tail dipped in a water bath thermostatically stabilized at 50ºC and the length of time it took for the rat to remove its tail from the water was noted. The prior reading was taken soon before injecting the test and standard medications. The tail flick latency was measured and recorded at 30, 60, 90 and 120 mins. All medications were given by oral route[12].

Anti-inflammatory activity test

Xylene- induced ear oedema

A total of 30 albino mice were used for the study. They were shared into five groups of six mice per group. Negative control which is group one received distilled water only. Group two, three and four received 100,200 and 400 mg/kg of *Dacryodes edulis* leaf extract respectively. Group five was given 4mg/kg of dexamethasone orally. After one hour of administering the drug, oedema was induced in each mouse by putting one drop of xylene at the inside surface of the mouse right ear. After three hours, the animals were euthanized under light ether anaesthesia and the two ears were cut off to equal size and weighed. The mean weight differences between the left and the right ears were evaluated in each group and documented as an index of inflammation[13].

Carrageenan-induced inflammation test.

A total of 30 rats were used for this test. They were shared into five groups of six rats per group. The negative control which is group one was given 1ml of normal saline each. Groups two, three and four were given one hundred, two hundred and four hundred mg/kg body weight of the extracts respectively. Group five was given 150mg/kg body weight of aspirin. One hour later, inflammation was induced in the hind paw with injection of 0.1ml of 1% carrageenan suspension by injecting it into the sub plantar tissues of the right paw. This was done to all the groups. The circumference of the right hind paw of each rat was measured using thread and ruler after 0.5, 1, 2, 3,4, and 5 hours respectively. The mean inflammation of paws in the groups treated with extracts were evaluated with respect to those in the control group and standard. All drugs were given orally[14].

Statistical analysis

Results were expressed as mean±SEM and analyzed with statistical package for social sciences (SPSS version 20) by using one-way analysis of variance (ANOVA) followed by Dunnett's post hoc test. Difference in the mean $p<0.05$ was considered statistically significant.

RESULTS

Results of acute toxicity study

LD_{50} as calculated from the graph of mortality in probit versus log dose is 6741.43mg/kg.

Results of analgesic activity study

The writhing frequency as induced using acetic acid and percentage inhibition by the test extract and standard drug is shown in Table 1.

The tail flick latency period obtained in different groups in the immersion of tail test is shown below in Table 2.

Table 1. Result of the effect of DE on writhing induced by acetic acid

Dose given	No of Writhes	Percentage inhibition
Normal saline 0.2ml	20.00±1.05	Nil
Aspirin 150mg/kg	4.65±0.15	80
100mg/kg DE	9.50±1.25	55
200mg/kg	8.00±1.50	60
400mg/kg	5.50±1.58	75

Values represent mean±SEM; n=6 ($p<0.05$)

Table 2: Effect of DE on thermal stimuli response in tail immersion test

Dose	Pre-treatment(sec)	30 mins(sec)	60 mins(sec)	90 mins(sec)	100 mins(sec)	120 mins(sec)
Normal saline-0.2ml	7.15±0.80	8.20±0.13	9.17±0.25	11.21±0.85	12.02±1.40	14.17±0.09
Morphine - 10mg/kg	7.75±1.00	16.17±1.10	18.67±1.70	21.50±1.30	23.20±0.70	23.95±0.25
DE - 100mg/kg	7.29±0.85	10.15±0.85*	12.05±0.20*	13.67±0.37*	14.00±0.96*	15.82±1.05*
DE - 200mg/kg	7.47±1.05	12.18±0.60*	13.92±0.80*	14.65±0.80*	15.35±0.25*	17.33±1.00*
DE - 400mg/kg	7.65±0.87	15.21±0.76	17.05±0.25	18.35±0.87	18.33±1.70	20.65±1.09

Values represent mean±SEM; n=6; *= Significant difference from the values in morphine group ($p<0.05$)

Result of ear oedema induced by xylene

The activities of inhibiting inflammation by test extracts and standard drug as induced using xylene is shown in table 3.

Result of carrageenan-induced paw oedema.

The result of effect of *Dacryodes edulis* leaf extract on carrageenan induced paw oedema test is shown in Table 4.

Table 3: Effect of *Dacryodes edulis* on ear oedema induced by xylene

Dose	Wt. of right ear(mg)	Wt. of left ear(mg)	Wt. diff. (mg)	% Inhibition of inflammation
Normal saline (1ml)	97±2.00	64±1.00	33±1.00	Nil
Dexamethasone(4mg/kg)	65±4.00	62±1.00	3±2.00	91
100mg/kg DE	87±1.00	62±3.00	25±2.00	24
200mg/kg DE	71±2.00	62±2.00	09±2.00	72
400mg/kg DE	71±6.00	64±2.00	07±4.00	78

Values are expressed as mean ±SEM; n=6; wt=weight; DE = *Dacryodes edulis* ethanol leaf extract

Table 4: Effect of DE on Carrageenan- induced paw oedema (Paw circumference vs time)

Dose	0hr (mm)	0.5hr (mm)	1hr (mm)	2hrs (mm)	3hr (mm)	4hrs (mm)	5hrs (mm)
Normal saline - 1 ml	2.2±0.2	2.9±0.1	3.0±0.2	3.0±0.2	3.2±0.3	3.3±0.2	3.5±0.2
100 mg/kg	2.2±0.2	2.6±0.1*	2.6±0.2*	2.5±0.2*	2.4±0.1*	2.4±0.2*	2.3±0.2
200 mg/kg	2.2±0.1	2.5±0.2	2.5±0.1	2.4±0.2	2.3±0.1	2.3±0.1	2.2±0.1
400 mg/kg	2.1±0.2	2.5±0.1	2.5±0.2	2.4±0.1	2.2±0.2	2.2±0.1	2.2±0.1
Aspirin - 150 mg/kg	2.2±0.1	2.4±0.1	2.4±0.2	2.4±0.1	2.2±0.2	2.2±0.1	2.2±0.1

Values represent mean±SEM, n=6 ($p<0.05$); *= Significant difference from the values in Aspirin group

DISCUSSION

The present study was carried out on *Dacryodes edulis* leaf extract to validate its use in traditional medicine as an analgesic and anti-inflammatory agent. The leaf extract was shown to be largely safe for consumption as shown from the LD_{50} which was above 6000mg/kg. It demonstrated a significant analgesic property both in the writhing test induced by acetic acid and tail-immersion test. DE at 400mg/kg caused 75 per cent inhibition of pain (abdominal contraction) induced by acetic acid as compared to 80 per cent by aspirin taken as a standard drug used in management of pain. Again in the tail immersion test, the flick latency period recorded by 400mg/kg of DE is not significantly different from that recorded for morphine which is a standard narcotic analgesic signifying that at that dose, DE is as effective as morphine in managing pains. These results validated the use of DE decoctions in treatment of pains of various types in the traditional settings in Nigeria[3].

Dacryodes edulis leaf extracts showed comparable capacity (78per cent) in preventing inflammation induced by xylene as compared with the standard drug, dexamethasone which is a corticosteroid. 400mg/kg of DE showed 72 percent inhibition while dexamethasone showed 91 per cent inhibition. Again, in carrageenan induced paw oedema, the extract showed similar capacity as an anti-inflammatory agent as compared with aspirin, taken as a standard drug for management of inflammation, as there is no significant difference in the paw circumference of animals in the 400mg/kg DE group and aspirin group. Although the exact mechanism through which DE elicited its analgesic and anti-inflammatory effects is not clear, however it may be similar to the pathways through which the standard drugs used elicited similar effects since the effects of the extract was comparable to those of the standard drugs.

Aspirin inhibit the activity of the enzyme, cyclooxygenase which leads to the formation of prostaglandins that cause pain, inflammation fever, Morphine binds to opiod receptors and block transmission of nociceptive signals, pain modulating neurons in the spinal cord and inhibit primary afferent nociceptors to dorsal horn sensory projection cell to inhibit pain sensation. Dexamethasone acts primarily via inhibition of inflammatory cells and suppression of expression of inflammatory mediators[15]

The Pharmacological activity of medicinal plants is often determined by the combination of their active constituents. Different flavonoids isolated from different medicinal plants have shown remarkable analgesic and anti-inflammatory activities[16]. The observed analgesic and anti-inflammatory effects seen with *Dacryodes edulis* leaf extract could be attributed to its flavonoids constituents which were detected during Phytochemical analysis of the extract.

CONCLUSION

Dacryodes edulis leaf extract possess a dose dependent analgesic and anti-inflammatory activity in animal models. Further study however may be required to verify these activities in humans.

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