IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Human Journals
Research Article
November 2022 Vol.:25, Issue:4

Evaluation of Anti-Inflammatory, Anti-Nociceptive and Antipyretic Properties of the Ethanol Leaf Extract of *Celosia leptostachya* in Rats and Mice

IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Eugene Ohams Ohanme[1,*], Kenneth Eze Etu[1], Godwin C. Akuodor[2], Benjamin Nwakelu[3] Casimir Chijioke Ofor[1]

[1]*Department of Pharmacology and Therapeutics, Faculty Basic Clinical Sciences, Ebonyi State University , Abakaliki, Nigeria*
[2] *Department of Pharmacology and Therapeutics, Faculty of Basic Clinical Sciences, College of Health Sciences, Nnamdi Azikiwe University Nnewi Campus, Nigeria*
[3]*Department of Pharmacology and Therapeutics, Faculty of Basic Clinical Sciences Alex Ekwueme Federal University Ndufu Alike, Ikwo, Ebonyi State, Nigeria*

Submitted: 25 October 2022
Accepted: 31 October 2022
Published: 30 November 2022

Keywords: Anti-inflammatory, anti-nociceptive, analgesic, antipyretics and *Celosia leptostachya*.

ABSTRACT

The decoction of the leaf of *Celosia leptostachya* has been widely used in traditional medicine in various part of Nigeria as anti-inflammatory, antipyretics and analgesic. This study was done on the plant to confirm these claims using established experimental models. Phytochemical screening and acute toxicity tests were determined using the ethanol leaf extract of the plant. Anti-inflammatory activity using egg albumin-induced rat paw oedema and *Carrageenan*-induced rat paw oedema, acetic acid-induced writhing in mice and hotplate- induced pain in mice, antipyretic activity using Brewer's yeast and D-amphetamine induced pyrexia in rats were determined at 100mg/kg, 200mg/kg and 300mg/kg doses of the leaf extract. The median lethal dose (LD_{50}) of the ethanol leaf extract of *Celosia leptostachya (orally)* was found to be more than 5000mg/kg. The extract contains alkaloids, flavonoids, carbohydrates, terpenoids, steroids, saponins, balsam, and resins. The ethanol leaf extract of *Celosia leptostachya* elicited significant ($p<0.05$, $p<0.01$and $p<0.001$) dose dependent inhibition of oedema comparable to *dexamethasone* .in egg albumen induced oedema model. Also, in carrageenan induced oedema model, the extract significantly ($p<0.05$, $p<0.01$and $p<0.001$) showed strong anti-inflammatory effects comparable to the standard drug: diclofenac. The extract and the referenced drug (Aspirin) significantly ($p<0.05$) reduced the number of writhes caused by acetic acid. The extract (dose dependent) and morphine significantly prolonged reaction time in hotplate induced pains in mice at ($p<0.05$, $p<0.01$and $p<0.001$). The extract significantly and dose dependently decreased high temperature in both Brewer's and D-amphetamine induced pyrexia in rats at ($p<0.05$). The findings from this study show that *Celosia leptostachya* ethanol leaf extract contains anti-inflammatory, anti-nociceptive and antipyretic properties which has validated for its use in traditional medicine for inflammation, analgesic and pyrexia.

HUMAN JOURNALS
www.ijppr.humanjournals.com

INTRODUCTION

Plants with medicinal values have always form alternative or local systems of medicine. Hence, plants with medicinal values remain the major basis upon which new chemical compounds are discovered and this eventually results into new drugs.

Therefore, this pant: *Celosia leptostachya* plant is erect and occasionally straggling up to 60cm high and reproduces seeds. It has an angled, slender and hairless stem with the basal part decumbent. It is usually about 30cm high, but up to 60cm when straggling up on supports. The leaves are smooth, simple and alternate, ovate, 2-8cm long and 1-3cm wide. The petioles are about 3cm long. The inflorescence is long and slender, consisting of axillaries spikes. This particular specie of *C. Leptostachya* has a brownish flower and occurs in distantly spaced clusters on the axis. The fruits are utricles with many black, shiny seeds.

It is a common weed of cultivated fields in both forest and Savanna zones of West Africa. In Nigeria, it is commonly found in the eastern part of the country with Isiangwa South and North having the major distributions. Specifically, it is found more in number in a particular village called *Umuhu Nvosi in Isiangwa* South Local Government Area of *Abia* state during dry and raining season.

Celosia leptostachya leaves and plants (before they come into flower) are occasionally eaten as a cooked vegetable by *Ngwa* people. It is used by traditional healers to treat boils, fever and convulsion in children. They also applied it through the eye, to treat high fever. My personal interaction with traditional healers revealed that they used it to treat scorpion and snake bites. It is also used to treat wounds and pains. There is no scientific work done so far to confirm all these claims except its antimicrobial activity reported by Umar *et al.* (2011) in research titled "*Antibacterial and phytochemical screening of methanolic extract of Celosia leptostachya Benth leaves on some selected clinical isolates.* Due to lack of comprehensive work on this plant with promising medicinal properties, this work was birthed.

MATERIALS AND METHODS

Plant materials

The fresh plants of *Celosia leptostachya* leaves were collected within the vicinity of *Umuhu Nvosi* in *Isiala-Ngwa* South Local Government Area of Abia State, Nigeria. The plant was identified and authenticated by Mr. Ibe K. Ndukwe of Herbarium unit, Department of

Forestry Micheal Okpara University of Agriculture *Umudike*, Abia State Nigeria with the Herbarium number: FHI3081 and International Plant Names Index:59904-1 (urn:lsid:ipni.org:names:59904-1).

Preparation of plant and extraction procedure

The fresh leaves of *Celosia leptostachya* collected was washed under a running clean tap water to remove any possible dirt and thereafter were dried up under room temperature. The dried leaves were pounded into powdered form using mortar and pestle. A portion of the resulting powdered leaves (800g) were subjected to extraction using ethanol 97% for 72 hours using maceration method. The extract was collected from the separating funnel by filtration using Whatmann filter No. 25. The solvent was evaporated from the resulting filtrate on a water bath set at low temperature (40^0C) to preserve the metabolites. The solution of the extract was always freshly prepared for each study by dissolution of the appropriate amount required in deionised water under standard laboratory conditions.

Phytochemical analysis

The ethanolic leaf extract of *Celosia leptostachya* was screened qualitatively for the presence of different categories of plant's secondary metabolites using standard methods (1).

Animals

Albino mice and rats which weigh between 20-25g and 150-200g of either sex respectively were employed in this work. These animals were got from animal house of Ebonyi State University, Abakaliki, Nigeria. Also, these animals were kept within the standard of environmental conditions. This means that the animals experienced 23-25^0C, 12 hours light and 12 hours darkness with access to standard dietary feed meant for rodents (Grancereal Vita feed Jos Plateau State, Nigeria) and water *ad libitum.* Also, the procedures used for this experiment was in line with global guideline on the use of laboratory animals in biomedical research (2). The male and female animals used in this study were even and two weeks were used to get the animals acclimatized before carrying out any experimental procedures on them.

Acute toxicity test

The acute toxicity test of the ethanol leaf extract of C. *leptostachya* was done both in mice and rats using modified methods in two phases (3). The animals were starved overnight before

the administration of plants extract. During phase1, there were 3 groups of animals containing 3 animas each in each cage. Graded doses of 10mg/kg, 100mg/kg and 1000mg/kg were given orally in that order. The animals were then kept under close watch for specific signs of toxicity for 4 hours and possible deaths over 24hours. When there was no lethality, phase2 was set in. Here, one animal was used in each case of 3 groups with the administration of increasing doses of 1600mg/kg, 2600mg/kg and 5000mg/kg in that order. The animals were also observed for noticeable signs of toxicity in the first 4hours and possible mortality for 24hour (4).

Evaluation of anti-inflammatory activity

The following models of experimental designs were used to determine the anti-inflammatory activities of the ethanol leaf extract of *Celosia leptostachya*.

Egg albumin-induced rat paw oedema

Here, fresh egg albumin was used to induce inflammation as recorded by Ajayi *et al* (2016) (5). Five groups of rats containing five rats of equal sexes each which were kept out of food over 12 hours were selected for this study. Group one and two served as negative and positive controls of which they were respectively pre-treated *intraperitoneally* with 10ml/kg normal saline and 4mg/kg of Dexamethasone. On the other hand, the other three groups were pre-treated with 100mg/kg, 200mg/kg and 300mg/kg of the ethanol leaf extract of *C. leptostachya* 30minutes before 0.1ml of egg albumin was used to induced inflammation (6). Thereafter, the increase in linear paw circumference was taken with the aid of cotton method of Bambgose and Noamesi modified by Akindele (7) at 30minutes intervals for 3 hours.

Carrageenan*-induced rat paw o*edema

Carrageenan induced rat paw o*edema* was used with slight modifications from the work of Bamgbose and Noamesi (1981) by Akuodor *et al.(*2021) (8). Rats used here where grouped into five of five animals each. Group 1 and 2 were treated with normal (10ml/kg) and Diclofenac (10mg/kg) respectively. The remaining groups were treated with 100mg/kg, 200mg/kg and 300mg/kg of the ethanol extract of *C. leptostachya* leaf. One hour later after the administration of different agents, acute inflammation was induced by injecting 0.1mL of 1% suspension of carrageneenan into the subplantar tissue of the right hind paw of the rats. Thereafter, the linear paw circumference was recorded with the aid of cotton thread method

(9). Also, the records of rats' paw circumferences were taken prior to administration of the *phlogistic* agent and it was done at one hour intervals for six hours.

Anti-nociceptive test

Acetic acid-induced writhing in mice

The analgesic properties of the ethanol leaf extract of *C. leptostachya* was determined by the use of acetic acid-induced writhing method (10). Albino mice weighing between 20 -25g comprising 5 mice of both genders were collated randomly into different cages. Group one which served as negative control received 10ml/kg of normal saline whereas, group two which served as the positive control received 100mg/kg of acetylsalicylic acid (ASA). The extract (100mg/kg, 200mg/kg and 300mg/kg) were given to the remaining groups: 3, 4 and 5.

30 minutes later, 10mg/kg, 0.7% of acetic acid was injected *intraperitoneally* to each mouse. They were carefully separated into a transparent cage and the number of writhing (usually noticed by the contraction of the abdominal musculature and limbs' extension) was counted for 30minutes at the interval of 5minutes. Inhibition was calculated in percentage using the data obtained.

Hot plate test

Mice of both sexes were screened by first placing them one after the other on the hot plate set at 55ºC, and the animals which could not lick the hind paw or jump within 15 seconds (nociceptive responses) were excluded from the work (11). Thereafter, the qualified animals were divided into five groups of five animals each. The controlled group received 10ml/kg of normal saline while the remaining groups received 100mg/kg, 200mg/kg and 300mg/kg of the ethanol leaf extract of *Celosia leptostachya.* The reaction time of each mouse was noted and this was the period between when the animal was placed on the hot plate and the time it began to lick its paw or jump around.

Antipyretic activity

Brewer's yeast induced pyrexia

The animal experimental model of brewer's yeast induced pyrexia was applied to evaluate the antipyretic activity of the ethanol leaf extract of *Celosia leptostachya (12).* Here, *wistar* rats of both sexes were randomly selected after 24 hours of fasting. They were grouped into five

with each group containing five rats. The basal temperature of each animal was recorded at zero hour using infra-red thermometer. Thereafter, the temperature of each animal was raised following the administration of 20ml/kg of 20% aqueous suspension of Brewer's yeast subcutaneously. Group 1 received 10ml/kg of normal saline and it served as negative control. Group 2, 3and 4 respectively received orally 100mg/kg, 200mg/kg and 300mg/kg of ethanol leaf extract of *Celosia leptostachya* while group 5 were injected 100mg/kg of standard drug; aspirin. The rectal temperature of the animals was then noted at 1, 2, 3, 4, and 5 hours.

D-amphetamine induced pyrexia test

The antipyretic activities of the secondary metabolites of the ethanol leaf extract of *Celosia letostachya* was screened using D-amphetamine induced pyrexia method (13). The animals used (wistar rats) of sexes were subjected to 24 of hours fasting. The initial temperatures of the selected rats were recorded. The rats were grouped into 5 with 5 rats in each cage and each rat was given 5mg/kg of D-amphetamine *intraperitoneal* in order to induce pyrexia. 24 hours after, the temperatures of the animals were noted for increase and any rat that the temperature was less than 0.6ºC was avoided. Group 1 and 5 were negative and positive controls respectively. Whereas, group 2, 3 and 4 received orally, 100mg/kg, 200mg/kg and 300mg/kg of *Celosia leptostachya* ethanol leaf extract.

Statistical analysis

The outcomes of this study was presented as mean ±standard error of mean (SEM) and analyzed with statistical package for social science (SPSS version 20) using one-way analysis of variance (ANOVA) followed by Turkey's post hoc test. Difference in mean $P<0.05$ was statistically difference.

RESULTS AND DISCUSSION

Phytochemical analysis

The result of the phytochemistry of the ethanol leaf extract of *Celosia Leptostachya* confirmed that alkaloids, flavonoids, carbohydrates, terpenoids, steroids, saponins, balsam, and resins were actually available.

Acute toxicity studies

It was observed that at dose of 5000mg/kg of the extract, there was no mortality or clinical evidence of toxicity within 72 hours after the oral administration of ethanol leaf extract of *Celosia leptostachya.* This means that the animals were healthy and active throughout the study. The median lethal dose (LD_{50}) was found to be more than 5000mg/kg.

Anti-inflammatory studies

Egg albumin-induced rat paw oedema

The anti-inflammatory activity of the ethanol extract of *Celosia leptostachya* was seen to be dose dependent. This is because, there was significant activity at $p<0.05$ with 100mg/kg and 200mg/kg and more significant occurred with the highest dose of 300mg/kg at $p<0.001$. It was also observed that the percentage inhibition increased more significantly as the dosage increases. The reference drug (*Dexamethasone*) used also showed a more significant effect in this study as its anti-inflammatory activity started at 0.5hr after administration and lasted for 3 hours before disappearing. This can be seen on Table 1.

The anti-inflammatory effect of *Celosia leptostachya* ethanol leaf extract against *carrageenan*-induced oedema in rat can be seen displayed in Table 2. The extract exerted significant activity against oedema induced by carrageenan in rat at $p<0.05$. But it showed more anti-inflammatory activity at dose of 200mg/kg and 300mg/kg at $p<0.01$ and $p<0.001$ respectively comparable with standard drug. Also, the percentage inhibition was seen to increase at higher doses suggesting that the extract is dose dependent.

Anti-nociceptive studies

The ethanol leaf extract of *Celosia leptostachya* really exerted anti-nociceptive activity significantly at various graded doses against acetic acid-induced writhing in mice at $p<0.05$. The inhibitory effect was seen to be more potent at 300mg/kg (99.10%) comparable to reference drug 100mg/kg (92.00%). This result shows that the extract is also dose dependent (Table 3).

On the other hand, the ethanol leaf extract of *C. leptostachya* significantly and dose dependently at $p<0.05$, $p<0.01$and $p<0.001$ reduced the thermal stimuli in mice. This protection was observed to be more potent at higher dose of 300mg/kg. However, morphine

(reference drug) showed slightly stronger protection at 28.78±0.76compared to the protection the extract showed at 300mg/kg at (28.39±0.15) and 200mg/kg at (14.35±0.32) (Table 4).

Antipyretic studies

The antipyretic effect of the ethanol leaf extract of *C. leptostachya* against Brewer's yeast induced pyrexia in rat showed significant and dose dependent effects at $p<0.05$ similar to the referenced drug: aspirin as shown in Table 5.

Similarly, the result of the effect of the ethanol leaf extract of *C. Leptostachya* against D-amphetamine induced pyrexia is presented in Table 6. The result shows a progressive dose dependent reduction at $p<0.05$ in the temperature of the rats treated with the extract and is comparable to the standard drug: PCM.

This work was designed to investigate the traditional claims regarding the use of *Celosia leptostachya* in inflammation, pyrexia and pain disorders or similar conditions. Inflammation is as result of the release of several mediators. These released mediators alter the functions of epithelia surfaces and some other elements of the nervous system (14). Non-steroidal anti-inflammatory drugs (NSAIDs) and steroidal anti-inflammatory drugs are the two widely classified anti-inflammatory agents (15).

In this current study, the findings of anti-inflammatory study of *C. leptostachya* leaf on egg albumin-induced oedema in rats paw revealed that the extract displayed considerable anti-inflammatory action which is similar to the referenced drug: Dexamethasone. However, at higher dose (300mg/kg), the extract exerted more superior anti-inflammatory effect with a 94.50% inhibitory effects (0.02±0.02 (94.50)) compared to 73.50% inhibitory effect of the standard drug(0.06±0.06(73.50)) used. The result of this study revealed the ability of the ethanol extract of *Celosia leptostachya* leaf to suppress the diametric rat paw induced oedema by egg albumen. Dexamethasone being a steroidal anti-inflammatory agent that inhibits arachidonic acid cascade by inhibiting phospholipase A_2. This suggests that *C. leptostachya* exerted its activity against egg albumen induced inflammation by the same mechanism of action.

Carrageenan induced paw oedema has been a reliable method of testing anti-inflammatory agents and this has been widely employed for screening oedematous activity of herbal products (16). However, the process leading to the formation of oedema depends on the presence of bradykinin and polymorphonuclear leucocytes that have pro-inflammatory factor

such as prostaglandin (17). *Diclofenac* as a Non-steroidal anti-inflammation drug may not inhibit the initial stage of oedema, produced by carrageenan while the second accelerating stage could be antagonised by the drug (18). But the ethanol leaf extract of *C. leptostachya* elicited biological effects on both initial and second accelerated stages of inflammation induced by *carrageenan*. The extract may have done this by inhibiting the release of prostaglandins.

On the other hand, the findings of this study revealed that ethanol leaf extract of *Celosia leptostachya* showed strong anti-nociceptive effects against chemical pains (writhing) induced by acetic acid (19). This chemical is commonly used to determine the anti-nociceptive properties of plant extract and drugs. Some experimental reports really support that the response of rodents (mice) to acetic acid induction is quick and reliable method of testing peripheral anti-nociceptive effects of herbal preparations (20).

However, the extract of *C. Leptostachya* and the referenced drug: Aspirin (acetylsalicylic acid) inhibited acetic acid induced writhing in mice. Hence, these findings showed that the extract has peripheral anti-nociceptive effects suggesting its activity maybe through direct inhibition of local peripheral receptors (21). Furthermore, whether the result of this experimental model evaluates peripheral anti-nociceptive action only or non-specific, the result has confirmed the use of this plant as analgesic in Nigeria. It is on record that that injection of acetic acid triggers the release of pain's mediators such as prostaglandins and other cyclokinase (22). This phenomenon however, suggests that the extract of *C. Leptostachya* exerted its effect by inhibiting action of cycloxygenase known to be responsible for the release of prostaglandin from arachidonic acid (23). Consequent upon this, the analgesic effect elicited by this plant: *C. Leptostachya* has validated its use by the traditional healers as analgesics.

To affirm if this plant extract analgesic activity acts centrally, analgesic model that act centrally (hotplate induced pains) was employed. This model of analgesic assay that is used to determine the involvement of central analgesic mechanism is believed to involve spinal reflex (24).

There is a record which support that centrally acting agents such as morphine possess this activity in both central and peripheral whereas, peripherally acting agent like acetyl salicylic acid have been credit to elicit anti-nociceptive action only in writing test (25). It is vital to note that the action of acetylsalicylic acid can only be linked with its ability to directly inhibit

prostaglandin activity or by indirectly inhibit prostaglandin secretion by inhibition of cyclo-oxygenase activity (26). This study shows that ethanol leaf extract of *C. leptostachya* successfully block pain receptors on hotplate induced pains in mice at higher dose suggesting that the extract has both central and peripheral analgesic activity similar to morphine. Antipyretic drugs have been shown to elicit their biological activity by antagonizing cyclo-oxygenase activity through increase in prostaglandin E_2 and in turn suppress high temperature (27). Different diseases such as infections, damaged tissues and many others can trigger rise in temperature. By this process, mediators such as interleukins can be activated which could progress to prostaglandin E_2 formation of elevated body temperature (28). This study showed that the ethanol extract of *C. Leptostachya* leaf reduced rats' rectal temperature similar to aspirin effect. This suggests that ethanol leaf extract of *C. Leptostachya* could bring fever under control as it terminate inflammatory symptoms centrally (at thermoregulatory zone) and peripherally. They could breakdown pyrogenic secreting cytokines as they also reduce synthesis of prostaglandin E_2 from cyclo-oxygenase maybe via the same mechanism of action of paracetamol.

The plants bioactive components are credited for the therapeutic potentials of plants with medicinal values. It was reported that Flavonoids as a plant secondary metabolite target prostaglandins involved in late stage of acute inflammation, pain and have been associated with anti-nociceptive, anti-inflammatory and antipyretics (29). As a matter of fact, it does not beat imagination to have seen these activities in the ethanol leaf extract of *Celosia leptostachya.* Also, it is fair to say the result of the LD_{50} of this study was safe to be used as herbal agent as there was no death recorded.

Table No. 1: Effect of *Celosia leptostachya* ethanol leaf extract on egg albumin induced rat paw oedema

Time (hours)	**Control Normal saline 10ml/kg**	***Dexamethasone* 4mg/kg**	***Celosia L* 100mg/kg**	***Celosia L* 200mg/kg**	***Celosia L* 300mg/kg**
0.5	0.88±0.02(0.00)	0.46±0.02**(50.00)	0.40±0.07(57.50)	0.36±0.04(62.50)	0.34±0.02**(65.00)
1.0	0.74±0.02(0.00)	0.36±0.05**(54.00)	0.32±0.03(60.00)	0.29±0.02*(63.00)	0.27±0.06***(66.00)
1.5	0.46±0.02(0.00)	0.21±0.05***(57.23)	0.17±0.04*(62.04)	0.015±0.03*(68.82)	0.14±0.02***(71.59)
2.0	0.36±0.05(0.00)	0.15±0.03***(61.76)	0.12±0.05*(74.12)	0.11±0.05**(75.50)	0.08±0.04***(80.29)
2.5	0.29±0.07(0.00)	0.11±0.04(67.50)	0.07±0.04(75.00)	0.05±0.03**(82.50)	0.04±0.05***(90.00)
3.0	0.21±0.02(0.00)	0.06±0.06(73.50)	0.04±0.02(82.20)	0.03±0.08*(84.00)	0.02±0.02(94.50)

*Values represent mean ± SEM (n=5) Figures in brackets represent percentage (%) inhibition of edema development. *P<0.05, **P<0.01, ***P<0.001 values compared with control (one-way ANOVA, followed by Turkey's post hoc test)*

Table No. 2: Effect of *Celosia leptostachya* ethanol leaf extract on carrageenan-induced rat paw oedema

Increase in circumference (cm)					
Treatment period (hr)	Normal saline (10mg/kg)	*Diclofenac* (25mg/kg)	*Celosia L* (100mg/kg)	*Celosia L* (200mg/kg)	*Celosia L* (300mg/kg)
1.0	0.33±0.06(0.00)	0.13±0.01***(40.00)	0.11±0.02***(50.00)	0.09±0.01***(60.01)	0.07±0.03***(70.00)
2.0	0.54±0.04(0.00)	0.13±0.02**(67.22)	0.16±0.02* (55.00)	0.12±0.02**(67.22)	0.11±0.04**(73.34)
3.0	0.75±0.03(0.00)	0.14±0.06***(74.80)	0.22±0.03*(57.20)	0.16±0.0*** (70.40)	0.14±0.04***(74.80)
4.0	0.51±0.08(0.00)	0.07±0.02***(84.12)	0.12±0.02* (64.70)	0.09±0.04** (77.65)	0.07±0.02***(84.12)
5.0	0.51±0.08(0.00)	0.05±0.01***(90.59)	0.11±0.05** (71.18)	0.07±0.02***(77.65)	0.05±0.01***(90.59)
6.0	0.51±0.08(0.00)	0.04±0.02***(97.06)	0.07±0.03**(84.12)	0.05±0.05***(90.59)	0.04±0.02***(97.06)

*Values represent mean ± SEM (n=5). Figures in brackets represent percentage (%) inhibition of edemadevelopment.*P<0.05, **P<0.01, ***P<0.001 values versus control (one-way ANOVA, followed by Turkey's post hoc test)*

Table No. 3: Effect of ethanol leaf extract of *Celosia leptostachya* on acetic acid-induced writhing in mice

Treatment	Dose (mg/kg)	Mean number of writhing	Inhibition (%)
Normal saline	10ml/kg	57.98±0.18	**0.00**
Celosia L	100	5.41±0.11*	**88.30**
Celosia L	200	2.71±0.06*	**94.80**
Celosia L	300	0.51±0.50*	**99.10**
ASA	100	2.8±0.14*	**92.00**

*Values represent mean ±SEM. (n=5)*Denotes P<0.05 as compared with the control (one-way ANOVA, followed by Turkey's post hoc test)*

Table No. 4: Effect of ethanol extract of *Celosia leptostachya* leaf on hotplate-induced pain in mice

Treatment	Dose mg/kg	Mean time spent on hotplate (Secs)
Normal saline	10ml/kg	1.69±0.01
Celosia L	100	8.98±0.07*
Celosia L	200	14.35±0.32**
Celosia L	300	28.39±0.15***
Morphine	10	28.78±0.76***

*Values represent mean ± SEM (n=5)*P<0.05, **P<0.01, ***P<0.001 values compared with control (one-way ANOVA, followed by Turkey's post hoc test)*

Table No. 5: Effect of antipyretic activity of the ethanol extract of *Celosia leptostachya* leaf using brewer's yeast induced pyrexia test in rats

Treatment Dose(mg/kg)	Body temperature at various degree of time (ºC)						
	Ohr	24hr	1hr	2hr	3hr	4hr	5hr
Normal Saline10ml/kg	35.35±0.30	37.52±0.21	37.82±0.30	37.76±0.26	37.65±0.22	37.50±0.20	37.50±0.25
EECL100	35.28±0.16	37.30±0.30	36.48±0.26	36.20±0.42	35.63±0.70	35.36±0.32	35.22±0.23***
EECL200	35.25±0.16	37.32±0.22	36.20±0.36	36.18±0.20	35.60±0.29	35.34±0.80	35.20±0.23***
EECL300	35.22±0.20	37.35±0.26	36.18±0.26	36.16±0.33	35.59±0.25	35.31±0.26	35.16±0.10***
ASPIRIN 150	35.18±0.15	36.70±0.10	35.82±0.28	35.75±0.50	35.39±0.10	35.52±0.21	35.24±0.19**

*Values represent mean ±SEM. (n=5)*Denotes P<0.05 as compared with the control (one-way ANOVA, followed by Turkey's post hoc test)*

Table No. 6: Effect of antipyretic activity of the ethanol extract of *Celosia leptostachya* leaf using d-amphetamine induced pyrexia test in rats

Treatment Dose(mg/kg)	Body temperature at various degree of time (ºC)						
	Ohr	24hr	1hr	2hr	3hr	4hr	5hr
Normal Saline10ml/kg	35.22±0.10	37.38±0.16	37.82±0.30	37.68±0.19	37.47±0.31	37.24±0.29	37.72±0.18
EECL100	35.20±0.15	37.27±0.12	36.42±0.15	36.24±0.14	35.60±0.11	35.31±0.21	35.23±0.14***
EECL200	35.25±0.11	37.29±0.18	36.39±0.11	36.22±0.12	35.58±0.12	35.29±0.14	35.20±0.18***
EECL300	35.24±0.12	37.30±0.17	36.35±0.26	36.20±0.20	35.45±0.14	35.24±0.12	35.18±0.15***
PCM 150	35.21±0.12	37.32±0.17	36.37±0.14	36.21±0.20	35.40±0.16	35.30±0.13	35.20±0.15***

*Values represent mean ±SEM. (n=5)*Denotes P<0.05 as compared with the control (one-way ANOVA, followed by Turkey's post hoc test)*

CONCLUSION

The results of this current study show that the ethanol leaf extract of *Celosia leptostachya* possess anti-inflammatory, anti-nociceptive and antipyretic properties. This has authenticate the folklore uses of this plant for the treatment of various ailment.

ACKNOWLEDGEMENT

Authors express their profound gratitude to Mr. Diyen Bulus Auta and Mr. Sunday Azi of University of Jos Nigeria for their unquantifiable technical assistance during this work.

REFERENCES

1. Ezeonu CS, Ejikeme CM. Qualitative and Quantitative Determination of Phytochemical Contents of Indigenous Nigerian Softwoods. New Journal of Science. 2016;2016:5601327.
2. Guillén J, Vergara P. Chapter 1 - Global Guiding Principles: A Tool for Harmonization. In: Guillén J, editor. Laboratory Animals (Second Edition): Academic Press; 2018. p. 1-13.
3. Kyhoiesh HAK, Al-Hussainawy MK, Waheeb AS, Al-Adilee KJ. Synthesis, spectral characterization, lethal dose (LD50) and acute toxicity studies of 1,4-Bis(imidazolylazo)benzene (BIAB). Heliyon. 2021;7(9):e07969.
4. Macias CI, Quintanar V, Sanchez-Huesca R, Vergara-Castañeda A, Martino-Roaro L. LD_{50} determination of caffeine-alginate methylsilanetriol in mice. Toxicology Letters. 2016;259:S153.
5. Ajayi AM, Tanayen JK, Magomere A, Ezeonwumelu JOC. Antinociceptive and anti-inflammatory effects of aqueous extract of *Chenopodium opulifolium* schrad leaves. J Intercult Ethnopharmacol. 2016;6(1):14-21.
6. Anosike CA, Obidoa O, Ezeanyika LUS. The anti—inflammatory activity of garden egg (*Solanum aethiopicum*) on egg albumin—induced oedema and granuloma tissue formation in rats. Asian Pacific Journal of Tropical Medicine. 2012;5(1):62-6.
7. Akindele AJ, Oladimeji-Salami JA, Usuwah BA. Antinociceptive and Anti-Inflammatory Activities of *Telfairia occidentalis* Hydroethanolic Leaf Extract (Cucurbitaceae). Journal of medicinal food. 2015;18(10):1157-63.
8. Akuodor GC, Ohadoma SC, Ofor CC, Megwas AU, Chukwu LC, Ramalan MA, *et al.* Anti-nociceptive, anti-inflammatory and antipyretic activities of the ethanol root bark extract of *Salacia lehmbachii* in rats and mice. International journal of basic and clinical pharmacology. 2021;10(6):7.
9. Su JY, Tan LR, Lai P, Liang HC, Qin Z, Ye MR, et al. Experimental study on anti-inflammatory activity of a TCM recipe consisting of the supercritical fluid CO2 extract of *Chrysanthemum indicum*, Patchouli Oil and Zedoary Turmeric Oil *in vivo*. Journal of ethnopharmacology. 2012;141(2):608-14.
10. Choi J, Jung HJ, Lee KT, Park HJ. Antinociceptive and anti-inflammatory effects of the saponin and sapogenins obtained from the stem of Akebia quinata. Journal of medicinal food. 2005;8(1):78-85.
11. Yam MF, Loh YC, Oo CW, Basir R. Overview of Neurological Mechanism of Pain Profile Used for Animal "Pain-Like" Behavioral Study with Proposed Analgesic Pathways. International journal of molecular sciences. 2020;21(12):4355.
12. Muhammad N, Saeed M, Khan H, Raziq N, Halimi SMA, Abass M. Antipyretic and anticonvulsant activity of n-hexane fraction of *Viola betonicifolia.* Asian Pacific journal of tropical biomedicine. 2013;3(4):280-3.
13. Tarkang PA, Okalebo FA, Siminyu JD, Ngugi WN, Mwaura AM, Mugweru J, *et al.* Pharmacological evidence for the folk use of Nefang: antipyretic, anti-inflammatory and antinociceptive activities of its constituent plants. BMC complementary and alternative medicine. 2015;15:174-.
14. Abdulkhaleq LA, Assi MA, Abdullah R, Zamri-Saad M, Taufiq-Yap YH, Hezmee MNM. The crucial roles of inflammatory mediators in inflammation: A review. Veterinary world. 2018;11(5):627-35.

15. Nissen CV, Bindslev-Jensen C, Mortz CG. Hypersensitivity to non-steroidal anti-inflammatory drugs (NSAIDs): classification of a Danish patient cohort according to EAACI/ENDA guidelines. Clinical and Translational Allergy. 2015;5(1):10.
16. Xu Z, Zhou J, Cai J, Zhu Z, Sun X, Jiang C. Anti-inflammation effects of hydrogen saline in LPS activated macrophages and carrageenan induced paw oedema. Journal of Inflammation. 2012;9(1):2.
17. Souza DG, Pinho V, Pesquero JL, Lomez ES, Poole S, Juliano L, *et al.* Role of the bradykinin B2 receptor for the local and systemic inflammatory response that follows severe reperfusion injury. British journal of pharmacology. 2003;139(1):129-39.
18. Moilanen LJ, Laavola M, Kukkonen M, Korhonen R, Leppänen T, Högestätt ED, *et al.* TRPA1 contributes to the acute inflammatory response and mediates carrageenan-induced paw edema in the mouse. Scientific reports. 2012;2:380-.
19. de la Puente B, Romero-Alejo E, Vela JM, Merlos M, Zamanillo D, Portillo-Salido E. Changes in saccharin preference behavior as a primary outcome to evaluate pain and analgesia in acetic acid-induced visceral pain in mice. Journal of pain research. 2015;8:663-73.
20. Gupta AK, Parasar D, Sagar A, Choudhary V, Chopra BS, Garg R, *et al.* Analgesic and Anti-Inflammatory Properties of Gelsolin in Acetic Acid Induced Writhing, Tail Immersion and Carrageenan Induced Paw Edema in Mice. PloS one. 2015;10(9):e0135558-e.
21. al-Swayeh OA, Clifford RH, del Soldato P, Moore PK. A comparison of the anti-inflammatory and anti-nociceptive activity of nitroaspirin and aspirin. British journal of pharmacology. 2000;129(2):343-50.
22. Booker L, Naidu PS, Razdan RK, Mahadevan A, Lichtman AH. Evaluation of prevalent phytocannabinoids in the acetic acid model of visceral nociception. Drug and alcohol dependence. 2009;105(1-2):42-7.
23. Shoeb M, Ramana KV. Anti-inflammatory effects of benfotiamine are mediated through the regulation of the arachidonic acid pathway in macrophages. Free radical biology & medicine. 2012;52(1):182-90.
24. Alhadeff AL, Su Z, Hernandez E, Klima ML, Phillips SZ, Holland RA, *et al.* A Neural Circuit for the Suppression of Pain by a Competing Need State. Cell. 2018;173(1):140-52.e15.
25. Komla E, Stevens DL, Zheng Y, Zhang Y, Dewey WL, Akbarali HI. Experimental Colitis Enhances the Rate of Antinociceptive Tolerance to Morphine via Peripheral Opioid Receptors. The Journal of pharmacology and experimental therapeutics. 2019;370(3):504-13.
26. Mitchell JA, Warner TD. Cyclo-oxygenase-2: pharmacology, physiology, biochemistry and relevance to NSAID therapy. British journal of pharmacology. 1999;128(6):1121-32.
27. Abotsi WKM, Lamptey SB, Afrane S, Boakye-Gyasi E, Umoh RU, Woode E. An evaluation of the anti-inflammatory, antipyretic and analgesic effects of hydroethanol leaf extract of *Albizia zygia* in animal models. Pharmaceutical biology. 2017;55(1):338-48.
28. Kita Y, Yoshida K, Tokuoka SM, Hamano F, Yamazaki M, Sakimura K, *et al.* Fever Is Mediated by Conversion of Endocannabinoid 2-Arachidonoylglycerol to Prostaglandin E2. PloS one. 2015;10(7):e0133663-e.
29. Ullah A, Munir S, Badshah SL, Khan N, Ghani L, Poulson BG, *et al.* Important Flavonoids and Their Role as a Therapeutic Agent. Molecules (Basel, Switzerland). 2020;25(22):5243.