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EVALUATION OF ANTI-INFLAMMATORY AND ANALGESIC PROPERTIES OF *JUSTICIA CARNEA*LEAFEXTRACT

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ABSTRACT

Background: There is need for scientific data to support the folkloric use of *J. carnea* leaf extract in the management and treatment of pain and inflammation. **Method:** The shade-dried and grinded leaves of *J. carnea* were cold macerated in 80% ethanol. The acute toxicity and qualitative phytochemical screening were carried out using the leaf dried extract. Two sets of 20 albino rats of both sexes divided into 4 groups (n=5) were used for the anti-inflamatory and analgesic investigations respectively. The same mode of animal grouping and dosing was adopted for each study. Groups 1 and 2 served as negative and positive control respectively having received 10 ml/kgbw of distilled water and 10mg/kgbw of diclofenac sodium respectively while groups 3 and 4 received 250 and 500 mg/kgbw of the extract of respectively. The carrageenan induced paw edema and the hot plate methods were used for the anti-inflammatory and the analgesic evaluations respectively. The data were analyzed with Statistical Package for Social Sciences using one way ANOVA. **Results**: The LD_{50} for the extract was found to be higher than 5000 mg/kgbw. At the doses of 250 and 500 mg/kg, the extract showed a time and dose-dependent significant ($p<0.05$) anti-inflammatory and analgesic effects when compared to the controls. **Conclusion:** *Justicia carnea* leaf extract was found to be relatively safe with both anti-inflammatory and analgesic effects. Its folkloric uses as an anti-inflammatory and analgesic agent are therefore justified by the findings of this study.

KEYWORDS: Anti-inflammatory effect, analgesic effect, pain latency period, peak effect, *Justicia carnea*.

1. INTRODUCTION

1.1 Background of the study

There is an increase in the use of herbal medicines in developed countries.[1] About 325 species and 95 families of medicinal plants were recognized as being used by most people in Nigeria for the treatment of various diseases.[2] Herbal medicines include herbs, herbal materials, herbal preparations, and finished herbal products that contain parts of plants or other plant materials as active ingredients.[3]

1.2. Inflammation

Inflammation is a complex biological response of body's tissue to harmful stimuli like infections, toxins and injuries, in an attempt to heal itself.[4].Inflammation is presented by five cardinal signs of heat (calor), redness (rubor), swelling (tumor), pain (dolor) and loss of function[5].Studies have demonstrated inflammation as being caused by pathogenesis of some disease like cancer, atherosclerosis, cardiovascular disease, arthritis and diabetes mellitus.[6]Inflammation can also be caused by physical factors such as burns, trauma, and foreign bodies or biological factors like immune reactions.[7]One of the major signs of inflammation is pain which can be as a result of direct stimulation of noiceptors or because of the action of inflammatory mediators like cytokines, histamines and serotonin.[8]

1.3. Ethnomedicinal uses of different parts *Justicia carnea*

Justicia carnea is from the family of *Acanthaceae Justicia* and it is the largest genus of *Acanthaceae* having about 700 species. It is cultivated in West and Central Africa especially in countries like Nigeria, Ghana, Guinea and Togo.[9] *Justicia carnea* is commonly called "Hospital is too far" or "blood of Jesus". It is known as "Ogwu obara" (blood tonic) by the Igbo speaking tribe of Nigeria while the Yoruba people of Nigeria call it "ewe eje" (blood leaf) as a reminder of the fact that the leaf is a substitute for blood transfusion. In folkloric medicine

the decoction from the leaves of *J.carnea* is employed in the treatment of muscle spasms and anemia.[10] The decoction from boiled root of *J. carnea* is used in the treatment of menstrual pain.[11] *Justicia carnea* plant is also generally considered as an ornamental plant.[12] In some parts of Nigeria, the leaves of *J. carnea* are used as vegetable in the preparation of edible soup.[10]

Fig.1: Fresh leaves of *Justicia carnea.*

1.4 Management of inflammation and pain

Nonsteriodal anti-inflammatory drugs (NSAIDs) is a class of drug that are used mostly as anti-inflammatory and analgelsic agents.[13]Corticosteroids are another class of drugsthat are used for the management of pain and inflammation.[14]Disease modifying anti-rheumatic drugs (DMARDs) are commonly used to treat inflammation and pain that are associated with rheumatoid arthritis. Some conventional DMARDs include methotrexate and sulfasalazine.[15] Amino salicylatesare a group of medications that are also employed in the treatment of inflammatory bowel disease such as ulcerative colitis and Crohn's disease; they include balsalazide and mesalamines.[15] Non-pharmacological management of inflammation and pain includes heat therapy, cold therapy, electrical stimulation and massaging.[16]

Studies have also revealed the anti-inflammatory activities of the aqueous extract of *Curcuma longa* (turmeric).[17] Likewise, the aqueous leaf extract of *Persea Americana* (Avocado pear)[18]have been attributed with anti-inflammatory and analgesic properties. Although synthetic non-steroidal anti-inflammatory drugs have provided effective management for pain and inflammation, there is risk of cardiovascular problems,[19] kidney, hepatic and gastrointestinal damage.[20] There is therefore the need for the development of medications that would have lesser side effects.

MATERIALS AND METHODS

2.1 Materials

2.1.1 Collection and authentication of plant materials

Fresh leaves of *Justicia carnea* were collected from Ukpor, Dunukofia local government area, Anambra state, Nigeria, in the month of August 2021. The leaves were identified and authenticated in the department of Botany, Nnamdi Azikiwe University, Anambra state, Nigeria, by a taxonomist and the voucher specimen number NAUH-203Awas assigned to it. The leaves were kept in the University's herbarium for future references.

2.1.2 Chemicals, reagents and drugs

Ethanol (JHD, Guangdong Guanghua Schi-Tech. Ltd China), Carrageenan (Zhejiang, China), chloroform (Sigma Aldrich, USA), Diclofenac sodium, (Greenlife Pharmaceutical company Nigeria)

2.1.3 Animals

Albino rats of both sexes with weight range of120-150g were purchased from the Animal house of the Department of Pharmacology and Toxicology, Chukwuemeka Odumegwu Ojukwu University (COOU), Anambra state Nigeria. They were housed in clean plastic cages, supplied with clean drinking water and fed with commercial pelleted feed (Guinea Feed®, Nigeria) and ethical approval number PHACOOU/AREC/2021/006 was assigned to attest the animals were cared for according to the Faculty of Pharmacy COOU Animal Research Ethics Committee guidelines (PHACOOUAREC) which are in line with the National Institute of Health (NIH) guidelines for the care and use of laboratory animals.

2.2 Methods

2.2.1 Extraction of plant material

The collected leaves of *Justicia carnea* were shade dried for seven days and were pulverized into powder with Binatone blender (Model BLG-401, China). Then, 500 g of the powdered leaves material was cold macerated with 80% ethanol. The filtrate so obtained was dried using rotary evaporator at 40^0C and stored in a refrigerator for further use. The percentage yield of the extract was also calculated.

2.2.2 Phytochemical analysis

Justicia carnea leaves extract was screened for the presence of secondary metabolites like proteins, carbohydrates, phenols, tannins, flavonoids, saponins, cardiac glycosides, steroids, terpenoids, anthroquinone and alkaloids using the revised methods of Trease and Evans.[21][22]

2.2.3. Acute toxicity study

Acute toxicity study (LD_{50}) was carried out using up and down method[23, 24] that was revised.[25] For this method, the animals were dosed one at a time and the doses were dependent on the response of the first animal to the initial dose. The second animal received a lower dose if the first animal died (the initial dose is decreased by a factor of 3.2) or the second animal received a higher dose if the first animal survived (the initial dose was increased by a factor of 3.2). Three rats weighing 150-200 g were used. Two rats served as negative control having received 10 ml/kg of distilled water orally while the test

animal received a default oral dose of 5000 mg/kg of the extract[23]. The animals were then observed for changes in behavior and any other obvious signs of toxicity continuously for 4 hours and subsequently daily for a total of 14 days for delayed toxicity.

2.2.4 Anti-inflammatory study of ethanol leaf extract of *J. carnea*

Animal grouping

Two sets of 20 albino rats (120-150 g), for anti-inflammation and analgesic evaluations, respectively, were used. Each set of animals, comprising of 20 albino rats of both sexes were divided into 4 groups (5 rats per group). Same treatments were carried out for both anti-inflammatory and analgesic studies. They were first starved for 24 hours, and then treated orally as follows:

Group 1: 10 ml/kgbw of distilled water

Group 2: 10 mg/kgbw of diclofenac sodium

Group 3: 250 mg/kgbw of ethanol leaf extract of *J. carnea*

Group 4: 500 mg/kgbw of ethanol leaf extract of *J. carnea*

The dose used for the study was determined from the result of the acute toxicity study.[26]The Carrageenan-induced rat paw edema method[27] and its revised version[28] were used. One hour after the oral treatment of the rats with the extract as described above, the paw volumes were measured. This was followed by the injection of carrageenan (0.1 ml of 1% w/v suspension of carrageenan) using plethysmometer into the sub-plantar region of the right hind paw. The paw volumes were subsequently measured again at the following intervals: 1, 2, 3, 4, 5 and 6hrs after the carrageenan injection. The mean increase in paw volume was noted. The edema volumes in control (Vc) and in the test groups (Vt) were calculated. The percentage inhibition was calculated using the formula: % Inhibition = 100(1-Vt/ Vc). Where, Vc = Edema volume of control at corresponding time and Vt = Edema volume of test at corresponding time

2.2.5Analgesic study of ethanol leaf extract of *J. carnea*

The hot plate method[29] that was revised[30] was adopted for this study. The twenty albino rats (120-150g) were grouped and treated as described above (section 2.2.4). After administration of the extract the rats were allowed to stay for 30, 60, 90, 120, and 150 minutes before they were placed on the hot plate in order to observe the effect of the administered leaf extract on them. The latency period was recorded. The latency period is the time until the animal licked the paw, fluttered any of the paws or jumped out of the hot plate. It was noted as reaction time. A cut-off time of 20 seconds was used to avoid paw tissue damage.

2.2.6 Statistical Analysis

The data were analyzed by Statistical Package for Social Sciences (SPSS version 20) using one way ANOVA, followed by post-hoc turkey's test for multiple comparisons. The data were expressed as mean ± standard error of mean (SEM). Graphical representation was done using Microsoft excel 2010 and the difference between mean were considered significant at $p<0.05$.

3.1. RESULTS

3.1. The weight of the crude extract obtained was 480g representing a percentage yield of 96%w/w

3.2. Qualitative phytochemical screening of ethanol leaf extract of *J. carnea*

The phytochemical analysis of *J. carnea* leaves revealed the abundance of alkaloids, flavonoids and saponins while terpenoids, tannins and phenols were in moderate amount. Traces of steroids, anthraquinone and proteins were also observed (Table 1)

3.3Acute Toxicity test (LD_{50}) of ethanol leaf extract of *J. carnea*

Oral administration of the extract up to 5000mg/kgbw dose produced no change in behavior, neither was there any mortality in any of the groups. Therefore, the LD_{50} of ethanol leaf extract of *Justicia carnea* was above 5000mg/kgbw.

Table 1: Phytochemical screening of ethanol leaf extract of J. *carnea.*

Const.	Alk	flav	Ste	Terp	Anthr	Phe	Sp	Tan	Prot	Card
Presence	+++	+++	+	++	+	++	+++	++	+	++

Key: Alk = alkaloids, Flav =flavonoids, Ste= steroids, Terp= terpenoids, Anthr= anthraquione
Phe=phenol, Sp=saponin, Tan=tannins,Prot=proteins, Card =cardiac glycosides.
(-) = not present, (+) =faintly present, (++) = moderately present, (+++) = abundance

3.4. Anti-inflammatory effects of ethanol leaf extract of*J. carnea*

At 250 and 500 mg /kg, the extract exerted a significant ($p<0.05$) anti-inflammatory effect in a dose-dependent manner. There was no significant ($p<0.05$) reduction in paw volume after 1 hrin all the groups when compared to control. However at the 2^{nd} ,3^{rd} , 4^{th} and 5^{th} hour, the percentage inhibition in paw size for 500 mg/kg dose were37.46%, 45.39%, 54.51% and 63.30%respectively. These percentage reductions in paw size volume were greater than that of standard drug, diclofenac sodium (23.73%, 33.00%, 47.67% and 60.52%respectively) (Table 2).

Table 2. Anti inflammatory effects of ethanol leaf extract of J. *carnea.*

		1hr	2hr	3hr	4hr	5hr	6hr
TRT	**Dose/kg**	**Change in paw diameter(mm) after treatment**					
D/H_2O	10 ml	100.00±0.00	104.30±0.11	103.16±0.32	104.58±0.65	102.17±1.54	104.29±1.32
Diclo	10 mg	100.0±0.00 (0.00)	79.54±1.21* (23.73%)	69.11±0.98* (33.00%)	54.72±0.66* (47.67%)	40.33±1.98* (60.52%)	21.49±0.15* (79.39%)
ELEJ	250 mg	100.00±0.00 (0.00)	92.54±0.55* (11.27%)	79.31±0.79* (23.11%)	77.22±0.89* (26.16%)	65.76±0.41* (35.60%)	60.79±0.46* (41.71%)
	500 mg	100.00±0.00 (0.00)	65.22±1.54* (37.46%)	56.33±0.21* (45.39%)	47.57±1.76* (54.51%)	37.49±0.18* (63.30%)	25.19±0.18* (75.84%)

Values are represented as mean ± standard error of mean (n=5). *p<0.05: Statistically significantly different from the control group. Key: ELEJ= ethanol leaf extract of *Justicia carnea*, D/H_2O = Distilled water, Diclo = Diclofenac sodium, TRT= treatment

3.5 Analgesic effects of ethanol leaf extract of J. *carnea*

At 250 and 500 mg/kg there was a significant (p<0.05) dose-dependent increase in pain latency when compared to the control beginning from 60th minute. Furthermore, at150 minutes the 500mg/kg dose was able to produce a peak analgesic effect of 15.75± 1.59 while that for diclofenac sodium was 17.88±0.39 (Table 3).

Table 3: Analgesic effects of ethanol leaf extract of J. *carnea.*

Treatment	Dose/kg	Time (minutes)					
		0	**30**	**60**	**90**	**120**	**150**
Dist.water	10 ml	0.00	1.05 ± 0.44	1.02± 0.76	1.09 ± 1.90	1.00± 0.74	1.00±0.55
Diclofenac	10 mg	0.00	1.45 ± 0.77	8.16±0.42*	12.33±1.00*	15.00±2.44*	17.88±0.39*
ELEJ	250 mg	0.00	0.88± 2.00	4.22± 1.41*	6.15± 0.76*	8.32 ± 0.21*	9.98 ± 1.44*
	500 mg	0.00	1.00 ± 0.76	6.29± 1.54*	10.11±1.00*	13.70±0.65*	15.75±1.59*

Values are represented as mean ± standard error of mean (n=5). *p<0.05: Statistically significantly different from the control group. Key: ELEJ= ethanol leaf extract of *Justicia carnea*

3.2. DISCUSSION

The focus onplants as sources of discovery for new drugsisincreasing[31]. *Justicia carnea* is a folkloric herbal medicine which has been used for decades for the treatment of pain and inflammation and for its blood boasting effects.[32]

The acute toxicity study of the leaf extract had revealed the relative safety of the extract. The oral administration of the extract up to 5000 mg/kg produced no signs of toxicity in all the treated animal groups. The qualitative phytochemical screening revealed the abundant presence of alkaloids, flavonoids, saponins and moderate presence of terpenoids and phenols. Alkaloids from plants are important class of molecules with anti-inflammatory activity.[33] Flavonoids on the other hand, are anti-oxidant that presents anti-inflammatory activity by inhibiting pro-inflammatory cytokines.[34]The anti-inflammatory, analgesic and antioxidant effects of phenolic compound have been reported.[35]Spooning have analgesic and anti-inflammatory properties.[36] Acute inflammation is a process that involves the overproduction of free radicals, activation of a complex enzymes, and release of several inflammatory and pro-inflammatory mediators.

The carrageenan-induced paw edema is a well-known acute model of inflammation that is widely used for screening novel anti-inflammatory compounds. Carrageenan is an agent of choice for testing anti-inflammatory drugs since it is devoid of antigenic reaction and systemic effects with high degree of reproducibility.[36] Carrageenan injection into the subplantar surface of rat paw induced a biphasic edema. However, the delayed phase (after 1hour) is attributed to neutrophil infiltration, and the continued generation of prostaglandins.[37] The early phase observed around 1hour is related to the release of histamine, serotonin, bradykinin.[38] This second phase is attributed to the overproduction of prostaglandin in tissues and also the release of bradykinin, protease and lysosomal enzymes, which cause pain and fever.[36]

The anti-inflammatory potential of the ethanol leaf extract of *J carnea* was evaluated using carrageenan induced model. As was observed, the sub-plantar injection of carrageenan led to a time dependent development of inflammation. There was a time dependent inflammation in all the groups after 1hour. One hour after inducement of inflammation, no significant (p<0.05) reduction in paw edema volume was observed in all the groups. This could be attributed to the fact that at the first 1 hour, inflammation was still in its early phase that even diclofenac sodium, which served as the standard drug, could not exert any significant(p<0.05) reduction in paw edema volume. This could probably be because diclofenac sodium exerts its anti-inflammatory action by inhibiting prostaglandin synthesis which only occurs in the second phase of

inflammation.[38]Hence, from the second hour there was significant ($p<0.05$)dose-dependent reduction in paw edema thickness for the extract treated groups (250 and 500 mg/kg) and the standard group when compared to the control. Moreover, at the 6^{th} hour, the anti-inflammatory effect observed for diclofenac sodium (79.39%) was comparable to that of the leaf extract at 500 mg/kg dose (75.84%).

The screening model that was employed for the analgesic activity study was a pain state model of thermal stimuli which is the hot plate method. The analgesic effect of the leaf extract was measured by the latency period. No significant ($p<0.05$) analgesic effect was observed when compared to control in all the groups during the first 30 minutes. However, on the 60^{th}, 120^{th}, 150^{th} minutes of the extract administration, there were significant ($p<0.05$) analgesic effect when compared to control. The extract was able to cause a dose-dependent significant ($p<0.05$) increase in latency period as was observed. We noted the analgesic effect produced by diclofenac sodium 10 mg/kg was higher than that of the extract throughout the study. Since latency period is a measure of tolerance to pain, the ethanol leaves extract of *J. carnea* therefore possesses a significant ($p<0.05$) analgesic effect when compared to the control. The abundance of alkaloids, flavonoids and saponins may have contributed to the observed anti-inflammatory and analgesic effects of the extract. These metabolites are well known for their ability to inhibit pain perception due to inhibition of enzymes involved in inflammation, especially in the arachidonic acid metabolic pathway and the synthesis of prostaglandins.[38]

4. CONCLUSION

Justicia carnea leaf extract is relatively safe as both analgesic and anti-inflammatory agent, hence its folkloric use in the management of pain and inflammation are justified by the findings of this study.

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