Archives of Pharmaceutical Sciences and Biotechnology Journal

Volume 1 Issue 1 June, 2021 Page 38-47 ISSN: 2971_611X

APSBJournal

APSB-04

ANALGESIC AND ANTI-INFLAMMATORY ACTIVITY OF THE AERIAL PART OF *DYSCHORISTE PEDICELLATA* C.B CL. (ACANTHACEAE)

Shehu S*, Ibrahim AB, Iliyasu U, Turaki RM, Abah JO

Department of Pharmacognosy and Drug Development, Faculty of Pharmaceutical Sciences, Kaduna State University, Kaduna-Nigeria

***Corresponding author: Shehu S* Email:** *pharmsani@gmail.com*, Phone: *+2348036617718*

ABSTRACT

Aim: To investigate the analgesic and anti-inflammatory activities of the aerial part of 70% aqueous ethanol extract of *Dyschoriste pedicellata*.

Background of the study: *Dyschoriste pedicellata* plant is used in ethno-medical practice in Africa for the treatment of fever and pains. No scientific data confirming its use in the treatment of pain and inflammation has been reported.

Place and duration: This study was conducted in the Faculty of Pharmaceutical Sciences, Kaduna State University from April to December 2019.

Methodology: The preliminary phytochemical screening of the 70% aqueous ethanol extract of *D. pedicellata* was performed according to standard procedures. The acute toxicity studies was carried out using OECD method. : The analgesic activity study was carried out using acetic acid-induced abdominal writhing in mice, hot plate test in mice and formalin-induced hind paw licking in rats, while the effect of the extract on inflammation was carried out using Carrageenan-induced paw oedema in rats.

Results: 70% aqueous extract of *D. pedicellata* was found to contain flavonoids, tannins, triterpenoids and deoxysugar. The extract produced a significant ($p < 0.001$) and dose-dependent inhibition of the acetic acid-induced abdominal constriction and increased the threshold for pain perception dose-dependently in the hot plate test in mice. The extract also produced a significant ($p < 0.05$) decrease in a dose-dependent in paw oedema in Carrageenan-induced oedema. There is also a significant ($p<0.05$) inhibition in nociceptive response especially in the late phase in formalin-induce paw licking at dose-dependent manner.

Conclusion: The aqueous ethanol extract of *D. pedicellata* possess analgesic and anti-inflammatory properties which may be attributed to the phytochemical constituents.

Keywords: *Dyschoriste pedicellata*, Acanthaceae,; Analgesia; Anti-inflammatory; Ethnomedicine.

INTRODUCTION

Traditional medicine has been a primary source of healthcare for more than 80% of sub-Saharan Africans [1] which may be attributed to ease of accessibility and affordability [2]. Plants are widely use in traditional systems of medicine, and in several communities of the developing world and it is also used by a large proportion of the global population as complementary and alternative medicines [3]. The use of medicinal plants for various ailments, may be as a result of increased costs of western medicines, their enhanced acceptability in human society, better compatibility with the body and their natural power to treat ailment via synergistic effects and neutralizing combinations to lessen adverse effects [4].

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Analgesia also known as "pain" defined by the International Association for the Study of pain (IASP) is an unpleasant, sensory and emotional experience associated with actual or potential tissue damage [5]. Inflammation is a complex biological response of vascular tissue to harmful stimuli caused by injury, infection, environmental agents, malignancy and cellular changes. It is a protective attempt by the body to remove the injurious stimuli as well as initiate the healing process for the tissue [6].

In treatment of pain and inflammation the most wildely prescribed agents are nonsteroidal anti-inflammatory drugs (NSAIDs). However, long-term usage of these NSAIDs are associated with significant side effects, such as gastrointestinal lesion, bleeding, and peptic ulcer [7]. Herbs have been shown to possess anti-inflammatory and analgesic effects and such plants can be used as sole therapy in managing inflammatory conditions or as complementary therapy allowing patients to take smaller doses of conventional anti-inflammatory drugs, thereby minimizing the side effects [8]. Hence the need to carry out studies on *plants* which possibly offers good analgesic and anti-inflammatory actions with potentially less adverse effects, easy accessibility and at a lower cost.

Dyschoriste pedicellata C.B.Cl.is a member of the genus referred to as "snake herb" in the family "Acanthaceae" and locally called Fidda-hakukuwa in Hausa [9]. The leaves are narrow elliptic with pubescent on both surfaces [10]. The flowers are stalked cymes, low shrublet, shortly pubescent, with pinkish or light purplish colour [11].

The leaf-infusion of *D. pedicellata* is given to children in the Gambia as a febrifuge [12]. The seeds were reported to be soaked in water and applied as eye drop for treatments of eye allergens [9]. The phytochemical screening of petroleum ether, ethyl acetate and methanol extracts revealed the presence of alkaloids, flavonoids, glycosides and triterpenes which may be attributed to the bacteriostatic and bactericidal/fungicidal activity of the extracts [12].

MATERIALS AND METHODS

Identification, Collection and Preparation of *D. pedicellata*

Fresh whole plant of *D. pedicellata* was collected from Kakiyayi Zaria, Kaduna State, in April 2019, identified and authenticated by U.S. Gallah a taxonomist at the Herbarium unit of the Department of Pharmacognosy and Drug Development, Faculty of Pharmaceutical Sciences, Kaduna State University with a voucher number KASU/PCG/094. The plant material was shade dried. The dried aerial parts were grounded to powder and stored in air tight container for future use.

Extraction of *D. pedicellata*

The air-dried powdered plant material (250 g) of *D. pedicellata* was macerated with 1.5 liters of 70%v/v ethanol in a glass bottle for 72 hours. The extract was filtered through a filter paper afterwards. The filtrate was concentrated to dryness on a water bath at 50 ^{o}C. The extract was then weighed and kept in a closed container in a dessicator at room temperature.

Preliminary phytochemical screening

70% aqueous ethanol extract of *D. pedicellata* was investigated for possible presence of phytochemicals. Alkaloids test was carried out using standard method described by Saleli Surmaghi *et al.*, [13] while flavonoids, triterpenoids, anthraquinones, tannins, and saponins tests were carried out as described by Evans [14].

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Animals

Wistar rats (150–250 g) and Albino mice (20–25 g) of both sexes were used for studies. The animals were obtained from Nigerian Institute for Trypanosomiasis Research (NITR) Kaduna. The animals were acclimatized in the animal house of Department Pharmacology Kaduna State University for seven days before the start of experiments. The animals were kept in cages under standard environmental conditions (12:12 h light: dark cycle at room temperature) and were provided with standard pellet diet and water given ad libitum. Food was withdrawn 12 hours before experiments.

Drugs

Ketoprofen (Lek Pharmaceutical Company), Morphine injection (Martindale USA), Piroxicam (Pfizer Pharmaceuticals), Aspirin (Dana Pharmaceutical Company).

Acute Toxicity Study

Acute toxicity of the ethanol extract of *D. pedicellata* was carried out using OECD (2008) Guideline [15]. The studies were carried out using oral route of administration. Five (5) female mice weighing 20-23 g were housed individually in a clean plastic cage in the laboratory. The first mouse was starved of food (and not water) for 3 hours before dosing with the 5000 mg/kg extract. After the extract was administered, food was withheld for another 1-2 hours and was observed any signs of toxicity (tremors, convulsions, salivation, diarrhea, lethargy, sleep and coma) and mortality within 24 hours. Four other mice were dosed with the extract 5000 mg/kg orally after the first mouse did not die after 24 hours. The dosed mice were observed for signs of toxicity and mortality. After 24 hours, the four mice survived and the limit test was determine and the mice were observed for 14 days for any sign of toxicity. The same procedure was carried out on female rats weighing (120-170 g).

Analgesic Studies of 70% Ethanol Extract of *D. pedicellata* extract

Acetic acid induced writhing test in mice

The method described by Correa *et al*. [16] was adopted. Twenty-five (25) mice were divided into five groups of five (5) mice each. Group one mice served as the negative control and were administered 10 mL/kg body weight of distilled water. Mice in groups II, III and IV were given 250, 500, and 1000 mg/kg body weight of the extract via oral route respectively. The group V mice received 1.5 mg/kg body weight ketoprofen orally. Thirty minutes after pre-treatment, all the mice were administered 0.6% v/v acetic acid at 10 mL/kg body weight, intraperitoneally (i.p.). The numbers of writhing/constrictions were counted for 30 minutes, starting after 5 minutes of administration of extract/drug. Analgesic response was expressed as the reduction of the number of abdominal constrictions between control animals and mice pre-treated with the extract.

Hot plate induced pain test in mice

The effect of the extract on hot plate-induced pain in mice was carried out according to the method described by Turner [17] as used by Vongtau *et al.*, [18]. The hot plate was maintained at 55 ± 1°C and each mouse was placed on the heated surface. The time(s) taken to elicit nociceptive responses (after placement on hot plate) shown by lifting, shaking, sucking, licking of the paw or jumping off was recorded as the index of response latency. An automatic 30 second cut-off was used to prevent tissue damage in the absence of any

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response. Mice that showed these responses within 20 seconds were selected and randomly divided into five groups of five mice each, and were fasted for 24 hours but allowed access to water *ad libitum*. Group I mice (negative control) received 10 mL/kg of distilled water. Mice in groups II, III and IV were pre-treated with 250, 500 and 1000 mg/kg extract orally respectively, while Group V mice were pre-treated with morphine 4 mg/kg intraperitoneally (i.p.) one hour prior to their placement on the hot plate.

Formalin-induced hind-paw licking in rats

The procedure described by Dubuisson and Dennis [19] as modified by Tjolsen *et al*. [20] was adopted for the study. The amount of time spent licking the injected paw was noted and considered as an indication of pain. The response was measured and recorded for five minutes after the formalin injection and 15-30 minutes after Formalin injection (second inflammatory phase). The albino rats were randomized into 5 groups containing five rats each. The animals were fasted for 24 hours before the experiment, but were allowed access to water. The rats in group 1 (negative control) received 5 ml/kg distilled water, while those in group II, III and IV received 250, 500 and 1000 mg/kg body weight of the extract orally respectively, while group V rats (positive control) received 300 mg/kg aspirin intraperitoneally. Thirty minutes after extract and drug administration, 0.05 mL solution of formalin (2.5% formaldehyde) was injected subcutaneously under the plantar surface of the left hind paw. The response for each rat was graded as follows [2];

0= Rat unaffected, stands or walk around on injected paw.
1= Injected paw favored or partially lifted.
2= Complete lifting of injected paw.
3= Licking, biting, or chewing of injected paw.

Percentage inhibition of inflammation was calculated using;

$$\text{Percentage inhibition} = \frac{\text{Mean paw licking (control)} - \text{Mean paw licking (test)}}{\text{Mean paw licking (control)}} \times 100$$

Anti-inflammatory Studies

Carrageenan-induced paw edema

The method described by Winter *et al.*, [21] was used. Thirty rats were divided into 5 groups each consisting of five rats per group. Group I received 1 mL/kg normal saline (negative control), group II, III and IV received extract at dose of 250, 500 and 1000 mg/kg respectively while the last group received piroxicam 20 mg/kg (positive control). Thirty minutes later, 0.1 mL of sterile saline solution of 1% carrageenan was injected the sub-plantar surface of the left hind paw. Paw diameter was measured using Vernier Caliper at time 0, 1, 2, 3, 4, and 5 hours after carrageenan administration [22].

The percentage of anti-inflammatory activity was calculated using the formula given below:

$$Antiinflammatory\ activity = 100 \times (1 - {}^{Dt}/_{Dc})$$

Dc is the mean paw diameter of control group; Dt is the mean paw diameter of the test group.

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Statistical Analysis

The results were presented as a mean ± Standard Error of Mean (SEM) of the indicated number of animals. One-way analysis of variance (ANOVA) followed by Dunnett's test or by two-way ANOVA by Bonferroni's test were used to find the statistical differences between control and tested groups. All statistical analyses were performed using Statistical Package for Social Science (SPSS) version 15.0).

RESULTS AND DISCUSSION

The macerated 250 g of the powered plant of *D. pedicellata* yielded 33.03 g of the extract with a percentage yield of 13.21%.w/w. The phytochemical screening of extract showed the presence of tannins, steroids/triterpeniods, flavonoids, saponins and deoxy sugars (Table 1). These phytochemical constituents are responsible for the biological activities of plant. The median lethal oral dose (LD_{50}) of the extract was found to be greater than 5000 mg/kg with no observable sign of toxicity which suggested that the extract was practically non-toxic based on the toxicity classification [23].

Table 1: Phytochemical Compound present in the 70% Aqueous Ethanolic Plant Extract of *D. pedicellata*

Phytochemicals	Test	Inference
Alkaloids	Mayer's	-
	Dragendorff's	-
Flavonoids	Shinoda	+
	Sodium hydroxide	+
Tannins	Lead acetate	+
	Ferric chloride	+
	Gold beater's skin	+
Saponins	Frothing	+
	Heamolysis	+
Terpenoids/steroids	Liebermann-Burchard's	+
Anthraquinones	Bontrager's	-
Deoxy sugars	Keller-Kiliani	+

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Analgesic activity

The abdominal constriction response induced by acetic acid is a sensitive procedure to establish peripherally acting analgesics. The response is thought to involve local peritoneal cells and is mediated by the prostaglandin pathways [24]. Treatment mice with extract at doses of 250, 500 and 1000 mg/kg caused a significant ($p< 0.001$) and dose dependent reduction in the number of abdominal constrictions in mice when compared to the control group. Extract (1000 mg/kg) had the highest reduction in the number of abdominal constrictions of all the doses used in the study (Table 2).

Table 2: Analgesic activity of 70% aqueous ethanol extract of *D. pedicellata* on acetic acid-induced writhing response in mice

Treatment	Dose(mg/Kg Body weight)	No. of Abdominal Constriction	% Inhibition
Normal saline		85.00 ± 0.12	-
Extract	250	59.6 ± 0.17**	29.88
Extract	500	55.8 ± 0.16**	34.35
Extract	1000	36.8 ± 0.9***	56.71
Ketoprofen	1.5	47.2 ± 0.9***	44.47

Value indicates mean ± SEM significant variation against control group at **$p< 0.01$ and ***$p< 0.001$, n=5(ANOVA, with multiple comparison method by Dunnett)

Oral administration of 70% ethanol extract of *D. pedicellata* in three different doses showed a significant reduction in pain threshold as compared to the control group. The time of unset of pain reaction increases as the dose of extract increase. At 250, 500 and 1000 mg/kg of the extract the unset of pain reaction was 6, 11.8 and 15.8 minutes respectively a shown in Table 3.

The highest doses of extract (1000 mg/kg) significantly ($p < 0.01$) raised the pain threshold of the mice as indicated by the increase in reaction time. Morphine 4 mg/kg had the highest analgesic activity of all the extract doses used in the study ($p< 0.05$). Morphine caused significant elevation of pain threshold that were more than those caused by the extract groups.

Table 3: Effect of 70% aqueous ethanol extract of *D. pedicellata* on Hot-plate induced pain in mice

Groups	Dose (mg/kg body weight)	Pain Reaction (min)
Control	10 mL/Kg	5.60 ± 4.40
Extract	250	6.00 ± 4.18
Extract	500	11.8 ± 2.74*
Extract	1000	15.80 ± 2.36*
Morphine	4.00	68.50 ±9.34*

Value indicates mean ± SEM significant variation against control group at *$p< 0.05$ (ANOVA, with multiple comparison method by Dunnett), n=5.

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Anti-Inflammatory Activity

The result in Carrageenan-induced paw oedema shows that the ethanol extract of *D. pedicellata* produced a significant ($p< 0.05$) decrease in the paw edema in rat when compared to the control group (Table 4). Carrageenan-induced paw oedema is a suitable model for evaluating anti-inflammatory activities of natural products and is a significant predictive test for anti-inflammatory agents acting by mediators of acute inflammation [25]. In the carrageenan-induced rat paw edema test (Table 4) for acute inflammation, the ethanol extract of *D. pedicellata* in doses of 250, 500 and 1000 mg/kg showed 50.93, 59.26 and 66.67 % inhibition of edema, respectively, at the end of 4h. The results were statistically significant ($p< 0.01$). The result also showed that the activity increases with increase in dosage across the hours (1^{st} to 5^{th} hour). Thus, the effect was dose dependent. Therefore, the results of this study are indication that the extract can be effective in acute inflammatory disorders. It also showed that the edema paw size reduction of the extract at all doses increases with increase in time. The carrageenan-induced paw edema in rats is believed to be biphasic in which the first phase is due to the release of histamine or serotonin, and the second phase is caused by the release of bradykinin, protease, prostaglandin, and lysosome [26]. Therefore, it can be assumed that the inhibitory effect of the extract of *D. pedicellata* on carrageenan-induced inflammation could be due to the inhibition of the enzyme cyclooxygenase, which plays an important role in conversion of arachidonic acid into prostaglandins in the later inflammation phase in the carrageenan-induced oedema model and this enzyme is considered to be a known target for a variety of NSAIDs. So, it is possible that the constituents found present in the extract such as steroids and triterpenes, saponins and flavonoids might be influencing both stages of carrageenan. Usually most anti-inflammatory drugs produced antipyretic action through the inhibition of prostaglandin [27].

Table 4: Anti-inflammatory Activity of 70% Aqueous Ethanol Extracts of *D. pedicellata* on Carrageenan-induced Rat Paw edema

Groups	Dose (mg/kg body weight)	Paw edema volume (mm)				
		1 hr	2 hr	3 hr	4 hr	5 hr
Normal Saline (Control)	1 mL	1.80 ± 0.07	1.92 ± 0.16	2.12 ± 0.11	2.16 ± 0.17	1.74 ± 0.42
Extract	250	1.24 ± 0.13 (31.10)	1.06 ± 0.15 (44.79)	1.12 ± 0.39 (47.17)	1.06 ± 0.42* (50.93)	1.02 ± 0.27 (41.38)
Extract	500	1.12 ± 0.19 (37.78)	1.00 ± 0.14* (48.00)	1.02 ± 0.22* (52.89)	0.88 ± 0.21** (59.26)	0.84 ± 0.27* (51.72)
Extract	1000	1.05 ± 0.14* (39.44)	0.86 ± 0.13* (55.21)	0.85 ± 0.33** (59.90)	0.72 ± 0.50** (66.67)	0.76 ± 0.50* (56.32)
Piroxicam	20	1.03 ± 0.10* (42.78)	0.82 ± 0.18** (57.29)	0.74 ± 0.42** (65.09)	0.65 ± 0.11** (69.91)	1.74 ± 0.09** (57.47)

Values are expressed as Mean ± SEM (n=5), at **$p< 0.01$ and *$p< 0.05$, Two-way ANOVA followed by Bonferroni's test. Figures in parenthesis indicate percentage inhibition of oedema development.

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The results of formalin-induced hind paw licking in rats administered with extract are presented in figure 1 below. The extract at different doses of showed slight inhibition of inflammation when compared with the negative control group in the first phase. In the second phase, the different doses demonstrated a significant anti-inflammatory activity when compared with the negative control. At the higher doses of 500 mg and 1000 mg/kg, the extract showed significant inhibition of nociceptive responses in both phases respectively, dose and time-dependently in comparison with the control group. Asiprin (300 mg/kg) showed the highest level of anti-inflammatory activity of all the doses used in the study at the second phase ($p< 0.001$).

Formalin test is a model for chronic pain that is useful in identifying both centrally acting anti-nociceptive agents such as narcotics, and peripherally acting analgesics such as acetyl salicylic acid [28, 29]. This test also distinguishes between non-inflammatory (early phase) and inflammatory (late phase) pain episodes according to the site and mechanism of action [30]. Formalin induces pain based on two different processes by: stimulation of nociception in the paw that is centrally mediated (early phase); and secondly by activation of local inflammatory processes that stimulates pain sensation and to some degree, sensitization of nociceptive neurons [20]. Suppression of both phases of pain by the extract provides further evidence of dual activity involving both the centrally mediated and peripherally localized pain mechanisms.

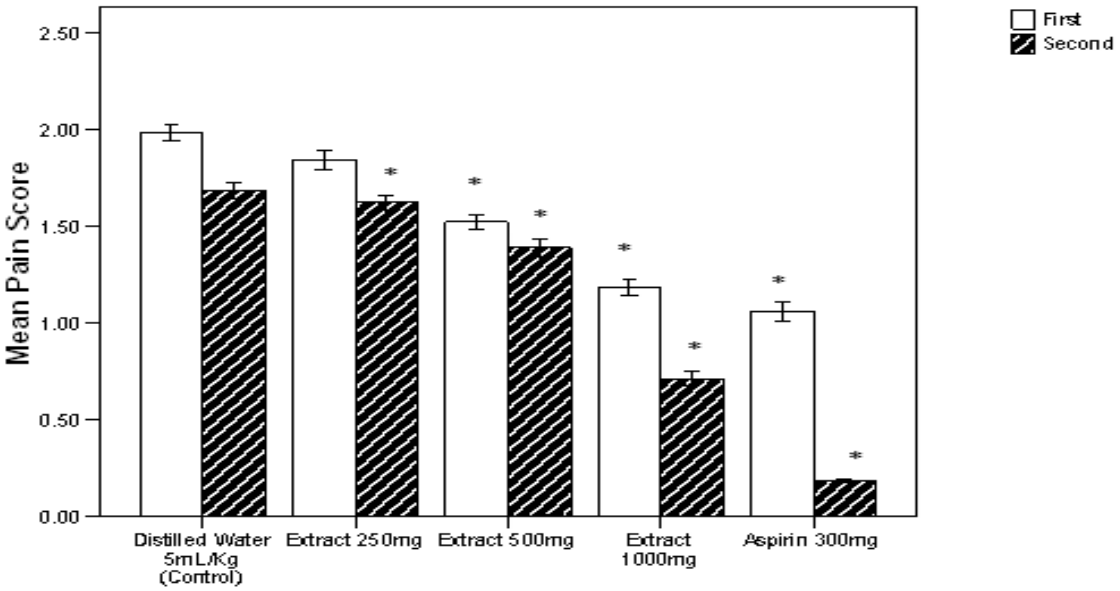

Figure 1: Effect of aqueous ethanol extract of *D. pedicellata* on anti-inflammatory activity in rats using Formalin-induced hind paw licking *

*$p< 0.05$ as compared with control group (n = 5); First Phase means 0-5 minutes, Second Phase means 15-30 minutes.

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CONCLUSION

The present study indicates that 70% aqueous ethanolextract of *D. pedicellata* exhibited analgesic and anti-inflammatory properties which may be attributed to its phytoconstituents. Hence an effort should be made to fractionate, isolate and characterize the active agents responsible for the observed pharmacological activity.

Authors Contribution

Conceptualization, S.S; methodology, S.S and U.I; Writing—Original draft preparation, A.I.B.; Writing—Review and editing, S.S and R.M.T.; supervision, S.S and J.O.A. All authors have read and agreed to the published version of the manuscript.

Conflict of Interests

The authors report no conflict of interests.

Acknowledgement

The authors are grateful to the Department of Pharmacology and Therapeutics, Faculty of Pharmaceutical Sciences, Kaduna State University, Kaduna, Nigeria for providing an enabling environment for the work.

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