https://dx.doi.org/10.4314/jpb.v14i2.8
Vol. 14 no. 2, pp. 155-161 (September 2017)
http://ajol.info/index.php/jpb

Journal of
PHARMACY AND BIORESOURCES

Analgesic and anti-inflammatory studies on crude ethanol extract of *Cadaba farinosa* Forssk leaf in mice and rats

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Received 2nd April 2017; Accepted 12th July 2017

Abstract
The plant *Cadaba farinosa* Forssk. is used in the treatment of pains, dysentery, rheumatism, cough, and fever. Phytoconstituents include alkaloids, anthraquinones, cardiac glycosides, flavonoids, saponins and tannins. The intraperitoneal LD_{50} of the crude ethanol leaf extract (CEE) was found to be 2154.1 mg/kg body weight in mice. The analgesic activity was investigated using acetic acid-induced writhing and hot plate tests in mice while Anti-inflammation potential was investigated using carrageenan-induced hind paw oedema in rats. The extract at all the doses tested and piroxicam produced significant decrease in number of writhes at $p \leq 0.05$ for extract and piroxicam compared to normal saline group. There was a significant increase ($p \leq 0.01$) in the pain-reaction time for thermally induced pain at 30 min of both 300 mg/kg CEE and 20 mg/kg pentazocine (standard drug). At 60 min, the significant increase ($p \leq 0.05$) was at 75 mg/kg CEE and 20 mg/kg pentazocine when compared to the control. The highest significant increase in mean pain reaction time for 75, 150 mg/kg CEE and pentazocine was observed at 90 min compared to the normal saline group. There was no significant difference in the mean pain latency time for thermally-induced pain between CEE and pentazocine for doses tested at 30, 60 and 120 min except for pentazocine with significant increase of $p \leq 0.05$ compared to the control group. There was generally a significant reduction ($p \leq 0.01$) in mean paw diameter for both the extract and piroxicam treated groups compared to control group throughout the study.

Keywords: Analgesic activity, *Cadaba farinosa*, acetic acid, carrageenan, pain, writhes, hot plate, paw oedema

INTRODUCTION

Cadaba farinosa Forssk belongs to the family Capparidaceae (Capparaceae). It is distributed throughout the world, mostly in tropical and subtropical region. The plants are usually herbs, erect or scandent, shrubs and rarely trees in dry short grass savannas. The Leaves are entire, simple, silvery gray and with simple scales [1]. It is locally called *bagayi* or *hanza* in HAUSA while KANURIS call it *bultu* in Nigeria [2]. Pain is a kind of symptom and disease with emotional changes and tissue damage. Instant relief is essential since severe pain can cause metabolism disorder and other diseases [3]. Analgesics are mainly divided into two classes: opiate receptor agonists and non-steroidal anti-inflammatory drugs (NSAIDs). The former

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ISSN 0189-8442

may lead to drug dependence even though it is effective for various pains. NSAIDs are widely used in the treatment of pain, but they have side effects, especially on the gastrointestinal tract [4]. Inflammation, as one major cause of pain, is related to cancer, diabetes and cardiovascular disease [5]. It is the body's immediate response to damage, its tissues and cells by pathogens, noxious substances or physical injury. These instigators induce activation of inflammatory mediators such as kinins, cyclooxygenase products and cytokines, which have become key targets for therapeutic intervention in a range of diseases including pain [6]. The search for analgesics continues because there is still lack of potent analgesics with minimal or no side effects [7]. Currently, the most utilized drugs for the management of acute and chronic pain are opiates like morphine and its derivatives [8], which are frequently under-prescribed in apprehension of drug tolerance, dependence and addiction [9]. However, many natural products have the potential to be developed into new and effective analgesic drugs [10]. Hence, this study looked into the analgesic and anti-inflammatory effects of the crude ethanol leaf extract of *Cadaba farinosa* Forssk (Capparaceae).

EXPERIMENTAL

Collection, identification and preparation of plant material. Fresh leaf samples of *Cadaba farinosa* were collected from Maiduguri Metropolitan Council Area of Borno State, Nigeria. The plant specimen was identified and authenticated at the Herbarium section of the Department of Biological Sciences, Ahmadu Bello University, Zaria, Nigeria, which corresponded with that of voucher specimen number V/No: 2744. The leaves were air-dried under shade for several days and pulverised into fine powder for extraction.

Extraction of plant material. The air-dried ground powdered leaf material (1,500 g) was extracted exhaustively with 70 % ethanol using cold maceration method for several days with occasional shaking. The crude ethanol leaf extract was concentrated to dryness on water bath at 50^{o} C and coded CEE – crude ethanol leaf extract of *Cadaba farinosa*. The coded extract (CEE) served as the working sample for the chemical investigations, acute toxicity determination as well as pharmacological investigations of the plant.

Animals. Locally bred adult Swiss albino mice (16-30 g body weight) and Wistar rats (118-165 g body weight) of either sex were acquired from Animal House facility of the Department of Pharmacology and Therapeutics, Ahmadu Bello University Zaria, Nigeria. The animals were fed with laboratory diet and water *ad libitum* and maintained under standard conditions in propylene cages at room temperature.

Drugs

Acetic acid-induced writhing test in mice. CH_3COOH (96%) by Riedel-de Haën, Germany. Dose: 0.6 % v/v. Piroxicam by Hovid, Malaysia. Dose: 10 mg/kg.

Thermally-induced pain of hot plate in mice. Pentazocine by Rambaxy, India, Dose: 20 mg/kg; Temp. = 55.0 ± 1.0^{o}C (i.e. 55 ± 1^{o}C) of the water bath heating the hot plate not hot plate itself.

Phytochemical screening. A little quantity each of the CEE (crude ethanol leaf extract) was subjected to qualitative phytochemical screening to test for the presence of alkaloids, anthraquinones, carbohydrates, cardiac glycosides, flavonoids, glycosides, saponins and tannins as described by several authors [11-15].

Acute toxicity studies. The median lethal dose (LD_{50}) was determined using Lorke's method [16]. Briefly, in the first phase, three mice of both sexes each were divided into

three groups. The crude ethanol leaf extract of *Cadaba farinosa* (CEE) was administered intraperitoneally (*i.p.*) at doses of 10 mg/kg 100 mg/kg and 1,000 mg/kg per body weight respectively. The animals were observed for signs of toxicity and death within 24 hours (none of the animals died). In the second phase, a mouse each in the three groups was administered intraperitoneally (*i.p.*) with more specific doses of 1,600 mg/kg, 2,900 mg/kg and 5,000 mg/kg body weight of the crude extract based on result of the first phase and observed for signs of toxicity and death within 24 hours. The median lethal dose (LD_{50}) was estimated as geometric mean of lowest dose that caused death and the highest dose of survival (i.e. square root of the product of lowest lethal dose and highest non-lethal dose for which the animal survived).

Acetic acid-induced writhing test. The method of Koster *et al*. [17] was adopted. Mice in groups one, two, three and four received respectively 10 ml/kg normal saline, 75 mg/kg, 150 mg/kg and 300 mg/kg CEE of the plant intraperitoneally (*i.p.*). Group five was given 10 mg/kg piroxicam (standard drug). Mice in all groups were treated with 10 ml/kg 0.6 % of acetic acid intraperitoneally (*i.p*). Each mouse was individually placed into a cage. The number of writhes within 10 min after a five minutes latency period was recorded. Stretching of abdomen with simultaneous stretching of at least one hind limb is considered a "writhe" [18]. The percentage inhibition was calculated using:

$$\% \text{ Inhibition/ Protection} = \frac{\text{mean control} - \text{mean treated}}{\text{mean control}} \times 100$$

Hot plate test. The method of Eddy and Leimback [19] was adopted for the study. Mice in groups one, two, three and four received respectively 10 ml/kg normal saline, 75 mg/kg, 150 mg/kg and 300 mg/kg CEE of the plant intraperitoneally (*i.p.*). Group five received 20 mg/kg of pentazocine (standard drug) intraperitoneally (*i.p).* After 30 min each mouse in the groups was individually placed on a hot plate (Gallenkamp thermostat) at 55 ^{o}C ± 1 ^{o}C (water bath temperature) to record the pain response latency time subsequently using stop watch at 30 min, 60 min, 90 min and 120 min. The interval between the time a mouse was placed on hot plate and the time it started shaking/licking its paw or jumped off hot plate was considered "index of pain response latency" [20].

Carrageenan-induced rat paw oedema. The method described by Winter *et al*. [21] was adopted. Mice in groups one, two, three and four received respectively 10 ml/kg normal saline, 75 mg/kg, 150 mg/kg and 300 mg/kg CEE of the plant intraperitoneally (*i.p.*). Group five received 10 mg/kg piroxicam (standard drug) intraperitoneally (*i.p).* After 30 min, acute inflammation was produced by sub planter administration of 0.1 ml of carrageenan suspension (1 % w/v in 0.9 % normal saline) into the left hind paw of each rat. The paw diameter was measured using Vernier caliper at 0 hour, 1^{rst} hour, 2nd hour, 3rd and 4th hour after carrageenan injection. The difference between readings at 0 hour and the different time intervals were taken as thicknesses of oedema.

Statistical analysis. Results were expressed as mean ± standard error of mean (mean ± SEM). The data were then subjected to one-way Analysis of Variance (ANOVA), Repeated measure ANOVA for carrageenan induced oedema and hot plate tests. Where a statistically significant difference was obtained, a post hoc Dunnett's t-test for multiple comparisons was employed. Differences were considered significant at $p < 0.05$.

RESULTS

Phytochemical screening. The extractive value for the CEE of *Cadaba farinosa* from

1,500 g plant material was found to be 12.63 % w/w (189.37 g; dark gummy mass). The preliminary phytochemical examinations of the crude and the solvents partitioned portions revealed the presence of alkaloids, anthraquinones, carbohydrates, cardiac glycosides, flavonoids, glycosides, saponins and tannins.

Acute toxicity studies. The intraperitoneal (*i.p.*) median lethal dose (LD_{50}) value of CEE of *Cadaba farinosa* was found to be 2154.1 mg/kg body weight in mice.

Acetic acid-induced writhing test. CEE of *Cadaba farinosa* at all the doses tested (i.e. 75 mg/kg, 150 mg/kg, 300 mg/kg) and piroxicam (10 mg/kg) produced significant decrease in number of writhes at $p \leq 0.001$, $p \leq 0.05$, $p \leq 0.001$ for extract and $p \leq 0.01$ for standard drug respectively as compared to normal saline (control) group (Table 1).

Hot plate test. There was a significant increase ($p \leq 0.01$) in the pain-reaction time for thermally-induced pain at 30 min of both 300 mg/kg CEE and 20 mg/kg pentazocine (standard drug). At 60 min, the significant increase ($p \leq 0.05$) was at 75 mg/kg CEE and 20 mg/kg pentazocine when compared to the control. The highest significant increase in mean pain reaction time ($p \leq 0.05$ for 75 mg/kg CEE and 20 mg/kg Pentazocine; $p \leq 0.01$ for 150 mg/kg CEE) was observed at 90 min when compared to the control (Normal saline). There was no significant difference in the mean pain latency time for thermally-induced pain between CEE and pentazocine for doses tested at 30 min (75 mg/kg and 150 mg/kg), 60 min (150 mg/kg and 300 mg/kg), 90 min (300 mg/kg) and 120 min (except 20 mg/kg pentazocine with significant increase of $p \leq 0.05$) when compared to the control (Table 2).

Carrageenan-induced rat paw oedema. There was generally a significant reduction ($p \leq 0.01$) in mean paw diameter for both the extract and piroxicam treated groups as compared to control (normal saline) group observed throughout the 1^{rst} hour (for all doses tested), 2nd hour (150 mg/kg CEE; 10 mg/kg piroxicam), 3rd hour (75 mg/kg and 300 mg/kg CEE; 10 mg/kg piroxicam) and 4th hour (75 mg/kg and 300 mg/kg CEE; 10 mg/kg piroxicam) study periods. Another significant reduction of $p \leq 0.001$ in mean paw diameter for 150 mg/kg CEE treated group was observed in the 3rd hour (Table 3).

Table 1: Effect of CEE of *Cadaba farinosa* on acetic acid-induced writhing in mice

Treatment	Dose (mg/kg)	% Protection	No. of writhes (Mean±SEM)
N/Saline	10 (ml/kg)		13 ± 3.0
CEE	75	78.5	2.8 ± 1.4^{b}
CEE	150	53.9	6. 0 ± 1.6^{a}
CEE	300	78.5	2.8 ± 1.0^{b}
Piroxicam	10	47.7	6.8 ± 1.0^{a}
One way ANOVA			
Df	4, 20		
F	8.313		
A	0.000		

N/Saline = normal saline; CEE = crude ethanol leaf extract; df = degree of freedom, F = F-factor; α = confidence level; $^{a}p \leq 0.05$ and $^{b}p \leq 0.01$ (compared with control. Dunnett test for multiple comparison); n = 6.

Table 2: Effect of CEE of *Cadaba farinosa* on thermally-induced pain of hot plate in mice

Treatment	Dose (mg/kg)	Mean pain reaction time (min)			
		30	60	90	120
N/Saline	10(ml/kg)	1.0 ± 0.2	0.9 ± 0.1	1.0 ± 0.1	1.5 ± 0.2
CEE	75	1.2 ± 0.1	1.4 ± 0.2[a]	2.7 ± 0.9[a]	2.4 ± 0.8
CEE	150	1.8 ± 0.5	2.3 ± 0.9	2.3 ± 0.4[b]	1.8 ± 0.2
CEE	300	2.0 ± 0.3[b]	1.6 ± 0.5	1.7 ± 0.4	1. 9 ± 0.3
Pentazocine	20	2.0 ± 0.2[b]	2.6 ± 0.8[a]	4.4 ± 1.6[a]	4.4 ± 1.7[a]
Repeated measure ANOVA					
Df		4, 25	4, 25	4, 25	4, 25
F		1.940	1.437	2.172	1.829
A		0.135	0.251	0.101	0.155

N/Saline = normal saline; CEE = crude ethanol leaf extract; df = degree of freedom, F = F-factor; α = confidence level; [a]$p \leq 0.05$ and [b]$p \leq 0.01$ (compared with control. Dunnett test for multiple comparison); n = 6.

Table 3: Effect of CEE of *Cadaba farinosa* on carrageenan-induced inflammation in rats

Treatment	Dose (mg/kg)	Paw diameter (mm)			
		1hr	2hr	3hr	4hr
N/Saline	1 (ml/kg)	1.2 ± 0.1	1.8 ± 0.3	2.4 ± 0.3	2.1± 0.3
CEE	75	0.8 ± 0.1[b]	1.2 ± 0.2	1.3 ± 0.1[b]	1.0 ± 0.1[b]
CEE	150	0.7 ± 0.1[b]	0.9 ± 0.0[b]	1.0 ± 0.1[c]	1.0 ± 0.2
CEE	300	0.8 ± 0.1[b]	0.8 ± 0.1	1.2 ± 0.0[b]	0.9 ± 0.1[b]
Piroxicam	10	0.7 ± 0.1[b]	0.7 ± 0.1[b]	0.8 ± 0.1[b]	0.7 ± 0.1[b]
Repeated measure ANOVA					
Df		4, 25	4, 25	4, 25	4, 25
F		8.420	7.414	14.344	11.753
A		0.000	0.000	0.000	0.000

N/Saline = normal saline; CEE = crude ethanol leaf extract; df = degree of freedom, F = F-factor; α = confidence level; [b]$p \leq 0.01$ and [c]$p \leq 0.001$ (compared with control. Dunnett test for multiple comparison); n = 6.

DISCUSSION

The leaf of *Cadaba farinosa* contain substances with potential values in the treatment of general body pains, dysentery, rheumatism, cough, fever, poisoning, amenorrhea, dysmenorrhea, liver damage, cancer and uterine obstruction amongst others.

In acetic acid-induced writhing test, the behavioural reaction (writhing) is sensitive to drugs with analgesic activity similar to aspirin, antagonists of kinin receptors and the centrally and peripherally acting opioid analgesics [22-23]. The ability of CEE of *Cadaba farinosa* to reduce the number of writhes suggested it possesses analgesic activity. Intraperitoneal (*i.p*) administration of acetic acid releases prostaglandins and sympathomimetic system mediators like PGE_2, $PGF_{2\alpha}$ and their levels were increased in the peritoneum fluid of induced mice [24]. Similarly, lipo-oxygenase products [25-26] has been found in peritoneal fluid after intraperitoneal injection. The observed abdominal constrictions is said to involve local peritoneal receptors [27-28] that sensitize nociceptive receptors to prostaglandins. This model is used to evaluate mild analgesics and non-steroidal anti-inflammatory compounds [29-30]. The writhing response of the mouse to *i.p*-injected noxious chemicals such as acetic acid is used to screen for both central and peripheral analgesic activity [31]. The acetic acid - induced writhing test is very sensitive and able to detect anti-nociceptive effects of compounds at dose levels that may appear inactive in other methods like tail flick test [32-33]. The phytochemicals (alkaloids, flavonoids, saponins, tannins, etc) in the leaf extract of the plant may be responsible for the analgesic activity [20].

Thermally-induced pain stimulus like hot plate test is used to selectively evaluate centrally acting analgesics [34] and predicts opioid-like analgesic compounds [35]. Since the CEE of the plant produced a moderate activity at the lowest dose tested. It can be suggested that the extract possesses moderate CNS analgesic activity.

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