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Analgesic and Anti-Inflammatory Studies on the Methanol Leaf Extract of *Tacazzea apiculata* Oliv. (Periplocaceae)

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ABSTRACT

Tacazzea apiculata (Periplocaceae) is a widely distributed medicinal plant in Africa, used traditionally for the treatment of pains, skin diseases, worms, hemorrhoids. The aim of the study was to evaluate the analgesic and anti-inflammatory activity of methanol leaf extract of *Tacazzea apiculata* in male Wistar rats and Swiss Albino mice. The *Tacazzea apiculata* leaf was extracted by cold maceration using methanol. Analgesic activity was investigated using acetic acid induced writhing and hot plate models by intraperitoneal administration of the plant extract at the doses of 100, 300, and 500 mg/kg body weight. Anti-inflammatory activity was evaluated by Carrageenan induced paw edema model. The extract of *Tacazzea apiculata* significantly and dose-dependently reduced the number of acetic acid-induced writhing and significantly increased the latency of paw licking in hot plate method. In acute studies, the extract showed anti-inflammatory activity by significant reduction in the paw edema volume in a dose-dependent manner when compared with the control and standard drug. The methanol leaf extract of *Tacazzea apiculata* possesses both analgesic and anti-inflammatory activity in a dose-dependent manner. Thus, provides some scientific basis for the ethnomedicinal use of the plant in the management of pain and inflammation conditions.

Keywords: Analgesic, Anti-inflammatory, Carrageenan, *Tacazzea apiculata*

INTRODUCTION

Pain is defined as an unpleasant sensation and an emotional experience associated with a real or potential damage to tissue, or equivalent of such damage according to International Association for the Study of Pain [1].

Analgesics are medicines that are used to relieve pain. They are also known as painkillers or pain relievers. Technically, the term analgesic refers to a medication that provides relief from pain without putting you to sleep or making you lose consciousness [2].

Inflammation is a defensive response which causes the different physiological adaptions which limits the tissue damage and removes pathogenic insult. This type of mechanism involves a complex series of events which includes dilation of arterioles, venules and capillaries with increased vascular permeability, exudation of fluids which includes plasma proteins and the migration of leukocyte into the inflammatory area [3]. Inflammation is a process which arises due to tissue damage causing dilation of venules, increase in vascular permeability, and infiltration of histamine, cytokines and other inflammatory components. Inflammation occurs due to the stress responses and is an integral part of it [1].

Nonsteroidal anti-inflammatory drugs are generally used to treat inflammation but these drugs are associated with harmful side effects like GI irritation, ulceration, bleeding etc. [2]. In the same manner opioids which are used as powerful analgesics are accompanied with side effects such as addiction and dependence [4]. As a result, researcher's interest has been increased towards herbal medicines which can be safer and more efficacious then the conventional analgesics and NSAIDS. The identification of such natural products which possess lesser side effects and no addictive potential like opioids could play an important role in treatment of pain disorders and inflammation.

Tacazzea apiculata possesses medicinal properties and used in folk medicines as tonic, stomachic, anti-microbial, diuretic, and antitumor [5]. However, to the best of our knowledge there is yet no published data regarding analgesic and anti-inflammatory activities on the aerial part of the plant. Based on the above evidences, the present study was carried out to scientifically validate the analgesic and anti-inflammatory activities of methanol leaf extract of *Tacazzea apiculata.* The specific objectives of this study were: (1) To determine the analgesic and anti-inflammatory activity of the methanol leaf extract of *Tacazzea apiculata* using animal models (2) To determine the median lethal dose (LD_{50})of the methanol leaf extract of *Tacazzea apiculata* in mice.

MATERIALS AND METHODS

Collection and Preparation of Plant Material

The leaves of *Tacazzea apiculata* were collected in October, 2019 from Sakaru village old Jos road, Zaria-Nigeria. It was identified and authenticated at the Herbarium Unit of the Department of Biological Sciences, Ahmadu Bello University, Zaria by comparing with existing specimen (Voucher number 066633). The leaves were air dried under shade and pulverized manually using mortar and pestle.

Drugs and Chemicals

The drugs and chemicals used in the study were Carrageenan (Sigma-Aldrich, USA), acetic acid (Ranbaxy laboratory Ltd., Punjab), Ibuprofen, Pentazocine and Piroxicam (Cipla Limited, India). Other chemicals used in this experiment were of analytical grade from commercial sources.

Extraction of Plant Material

The dried and powdered plant material was extracted with methanol (70%) using cold maceration method for 5 days with shaking at regular interval. The solvent was removed using rotary evaporator. The extract was suspended in distilled water and filtered using a Whatman filter paper to obtain water soluble and water insoluble portions.

Animals

Swiss Albino mice and Wister rats of either sex (18-25g) for the mice and (150-180g) for the rats were obtained from Animal house facility of Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria. They were kept in standard animal cages at room temperature and provided with standard laboratory diet and water *ad libitum*. The studies were conducted in accordance with the rules governing the use of laboratory animals.

Acute Toxicity Study

The method described by Lorke [6] was employed and the route of administration was intra-peritoneal. In the first phase, nine mice of either sex were divided into three groups containing three mice each. The first, second and third groups received 10 mg/kg, 100 mg/kg and 1000 mg/kg body weight of the plant extract respectively and observed for signs of toxicity and death after 24 hours. In the second phase, four (4) groups each containing one mouse were injected

with 4 more specific dose; 1200, 1600, 2900, and 5000 mg/kg respectively based on the result of the first phase. The median lethal dose (LD_{50}) was calculated using the following formula: -

$$LD_{50} = \sqrt{minimum\ lethal\ dose \times maximum\ tolerated\ dose}$$

Analgesic Activity

Acetic acid-induced writhing test

Thirty mice of either sex weighing between 18 to 25 g were used for this experiment. The mice were divided into 5 groups each containing 6 mice. The control group received normal saline (10 ml/kg *i.p.*), the test groups were treated with 100, 300 and 500 mg/kg *i.p.* of the plant extract, while the fifth group received piroxicam at the dose of 10mg/kg *i.p*; After 30 minutes of drug administration, the mice were treated with 0.6% acetic acid at 10mg/kg weight *i.p* [7]. Five minutes after acetic acid injection, mice were placed in individual cage and number of writhes was counted for each mouse for a period of 10 minutes after 5 minutes latency and the percentage inhibition of writhing was calculated using the following formula.

$$\% \text{ inhibition} = \frac{\text{Mean number of writhes (control)} \times \text{Mean number of writhes (test)} \times 100}{\text{Mean number of writhes (control)}}$$

Thermally induced pain method (Hot plate)

This is one of the most commonly used methods for evaluating central analgesic activity of a drug. In this method heat was used as a source of pain. Mice were divided into 5 groups of six each.

Thirty mice of either sex weighing between 18 to 25 g were used for this experiment. The paws of the mice are sensitive to temperature of 50 ^{0}C which are not damaging to the skin. The animals were placed on hot plate for 15 seconds (cut off time) and kept at a temperature of 55 ± 0.5 $^\circ$C to avoid damage to the paw. The time taken to flick the hind paw or lick or jump from the hot plate was considered as the reaction time of the particular animal. The reaction time was recorded at 30, 60, and 90 min interval after *i.p.* administration of the test drug. The animals of the test group received extract at the dose of 100, 200, and 500 mg/kg respectively. The positive control group was treated with pentazocine 10 mg/kg. Prolongation of the latency times was taken as an analgesic response i.e. percent maximum possible effect (%MPE).

$$\%MPE = \frac{\text{Test Baseline}}{\text{Cut off Baseline}} \times 100$$

Where; Test = latency to respond after treatment; Baseline = latency to response prior to treatment and cut-off (15 sec) = preset time at which the test was ended in the absence of response.

Anti-inflammatory Activity

Carrageenan-Induced Paw Oedema Test in Rats

Thirty rats of either sex weighing 150 to 180 g were used for this experiment. The rats were divided into five groups each containing 6 rats, acute inflammation was induced by injecting 0.1ml of freshly prepared 1% (w/v) Carrageenan into sub plantar surface of rat hind paw [8]. The methanol leaf extract (100, 300, and 500 mg/kg), normal saline (1 ml/kg) and ibuprofen (10 mg/kg) as positive control were administered 30 minutes before carrageenan injection. The paw diameter was measured at 1, 2, and 3 hours, using Vernier caliper to determine the diameter of oedema. The difference between the readings at time 0 hour and different time interval was taken as the thickness of oedema. The percentage inhibition of oedema was calculated using the following formula:

$$\% \text{ Inhibition of Oedema} = (1 - V_t / V_c) \times 100$$

Where, V_t is the edema volume in the drug treated group, V_c is the edema volume in the control group.

In practice, carrageenan activity is maximum at 3 hour and the effect of the extract at that time is accepted as the optimum inhibitory effect.

Statistical analysis

The results were expressed as mean ± SEM and the mean values of the control groups were compared with mean values of the treated groups using One-Way ANOVA (for Acetic acid Writhing test) and repeated measure ANOVA (for Hot Plate and Carrageenan induced paw edema) by using SPSS Software package version 20. The results obtained were considered statistically significant at $p < 0.05$.

RESULTS AND DISCUSSION

The median lethal dose (LD_{50}) of the extract in mice was found to be greater than 5000 mg/kg. The methanolic extract of the plant *Tacazzea apiculata* leaf at the doses of 100, 300 and 500 mg/kg body weight and piroxicam (10 mg/kg body weight) induced significant decrease (35.99, 55.07, 60.63 and 69.57%) in the number of writhes when compared to control group. The three doses tested (100, 300 and 500 mg/kg body weight) produced significant analgesic activity. Results of acetic acid-induced writhing is shown in Table 1.

Table 1: Effect of CMLE on Acetic acid induced writhing test in mice

Treatment	Dose (mg/kg)	Number of Abdominal Writhes Mean ± SEM	% Inhibition of Writhes
N/Saline	10	41.4 ± 0.78	-
Extract	100	26.5 ± 0.78*	35.99
Extract	300	18.6 ± 1.2*	55.07
Extract	500	16.3 ± 0.84*	60.63
Piroxicam	10	12.6 ± 1.65*	69.57

The observations (n=5) are mean ± SEM. *p< 0.05, compared with control (One-Way ANOVA, followed by Dunnett's test).

In the hot plate model, comparing the latency pre and post treatment, the extract at the doses of 100 and 300 mg/kg significantly increased the pain reaction time and the extract at the dose of 500 mg/kg had a better analgesic effect than other groups which was expected in this model. The result showed that the increase in reaction time in the extract treated group was found to be significant after 30, 60 and 90min ($p < 0.05$) as compared to control group. The result is shown in Table 2.

The result shows that there was no significant difference in the latency during the pre-drug testing time. After drug and extract administration, comparing the pre and post drug latency using ANOVA showed that the reference drug pentazocine (10 mg/kg body wt.) and the methanolic extract of the plant at the doses of 100, 300 and 500 mg/kg body weight significantly increased the latency.

Table 3: Effect of CMLE on Thermally-Induced Pain Test in Mice (Hot Plate)

Treatment	Dose mg/kg	Pain Latency (seconds) Mean ± SEM		
		30 min	60 min	90 min
N/Saline (ml/kg)	10	0.94 ± 0.02	1.07 ± 0.03	0.94 ± 0.05
Pentazocine	10	1.67 ± 0.02*	2.68 ± 0.03	5.37 ± 0.12*
Extract	100	1.54 ± 0.33	1.68 ± 0.07	3.13 ± 0.10*
Extract	300	1.71 ± 0.02	2.34 ± 0.04	4.63 ± 0.10
Extract	500	2.64 ± 0.04	4.78 ± 0.06*	4.67 ± 0.16*

The observations (n=5) are mean ± SEM. *p< 0.05, compared with control (Repeated Measure ANOVA).

The anti-inflammatory effects of the methanol leaf extract of *Tacazzea apiculata* on carrageenan-induced edema in rats' hind paws are presented in Table 3. There was a gradual increase in edema paw volume of rats in the control group. However, in the test groups, the extract showed a significant reduction in the edema paw volume. At doses of 100, 300, and 500 mg/kg *i.p*. 30 min before carrageenan, a dose-related inhibition of hind paw edema between 1 and 3 h was exhibited. The inhibitory effect was highest with 500 mg/kg. Significant effects were demonstrated by the extract. Ibuprofen as standard drug (10 mg/kg *i.p.*) produced a significant inhibitory effect comparable with the extract. Extract and ibuprofen exhibited 47.41% and 61.48% inhibition of edema respectively at 3 h after carrageenan administration.

The acetic acid induced writhing reflex is a model of visceral pain which is highly useful for screening analgesic drugs and could induce writhing reflex in laboratory animals. Intraperitoneal injection of 0.6% glacial acetic acid produced abdominal writhing in this experiment. Acetic acid produces writhing reflex in animals by activating the chemo sensitive nociceptors [9]. In this experiment, the reference drug and *Tacazzea apiculata* extract at 100, 300 and 500 mg/kg body weight significantly decreased the mean number of abdominal writhes which was dose dependent. The extract showed no inhibition against the acetic acid induced writhing in the control group, where the inhibition increased to 60.63% at the dose of 500 mg/kg.

Table 3: Effect of CMLE on Carrageenan Induced Inflammation in Rats

Treatment (Dose mg/kg)	Mean Diameter (cm) Mean ± SEM				
	1 h	2 h	3 h	Average Reading	Average % Inhibition
Control 1 ml/kg	1.32 ± 0.06	1.40 ± 0.06	1.33 ± 0.02	1.35	-
Ibuprofen (10)	0.12 ± 0.04*	0.16 ± 0.05*	0.22 ± 0.02*	0.17*	61.48
Extract (100)	0.49 ± 0.10*	0.63 ± 0.13*	0.69 ± 0.09*	0.60	29.63
Extract (300)	0.56 ± 0.15	0.60 ± 0.07	0.84 ± 0.10	0.67	24.44
Extract (500)	0.32 ± 0.07*	0.39 ± 0.08*	0.36 ± 0.10*	0.36*	47.41

The observations (n=5) are mean ± SEM. *p< 0.05, compared with control (Repeated Measure ANOVA).

The analgesic effect of *Tacazzea apiculata* leaf extract seen in this experiment may be mediated through peripheral pain mechanism and or through suppression of prostaglandin pathway since it has been observed that any agent that decreases the number of writhing will demonstrate analgesia preferably by inhibition of prostaglandin synthesis, a peripheral mechanism of pain inhibition [10].

The hot plate method has been used to study centrally acting analgesic activity. In this model, sensory nerves sensitize the nociceptors and the involvement of endogenous substances such as prostaglandins are minimized [11]. From the results, though the extract showed analgesic action in hot plate model, it was more pronounced as seen in the acetic acid-induced model and this may suggest that the analgesic activity of *Tacazzea apiculata* may be fully mediated through central mechanism.

Furthermore, the hot plate model has been employed extensively for the screening of compounds exhibiting analgesia by central mechanism, which is thought to be mediated by

modulation of descending pain inhibition pathways [12]. It is well known fact that, the response (paw licking, jumping) by mice to noxious thermal stimuli in hot plate method is supra-spinally mediated response [2].

Carrageenan-induced inflammation is the majorly applied experimental model for determining the anti-inflammatory effectiveness of compounds or natural products [4].

The paw edema induced by carrageenan is a biphasic event. The release of histamine, serotonin, and kinins form the first phase, while the release of prostaglandins (PGEs), protease, and lysosome result in the second phase of edema. This phase is responsive to majority of clinically efficient anti-inflammatory drugs [13]. The methanol leaf extract at the dose of 100 and 300 mg/kg suppressed only the second phase of carrageenan-induced inflammation but, at the dose of 500 mg/kg, significantly suppressed both first and second phases of carrageenan-induced inflammation. The standard (ibuprofen, 10 mg/kg) significantly suppresses the biphasic response of carrageenan-induced inflammation. So, the anti-inflammatory effect of the extract at the dose of 500 mg/kg may be owing to its suppression action on prostaglandins, protease, or lysosome synthesis or activity. Extract and ibuprofen exhibited 47.41% and 61.48% inhibition of edema respectively at 3 h after carrageenan administration, suggesting that the extracts could possibly have an inhibitory effect on the release of prostaglandins at the second phase. In practice, carrageenan activity is maximum at 3 hours and the effect of the extract at that time is accepted as the optimum inhibitory effect.

CONCLUSION

In this study, the methanol leaf extract of *Tacazzea apiculata* (500 mg/kg) significantly reduced edema induced by carrageenan in all the phases. Also, the extract significantly and dose-dependently reduced the number of acetic acid-induced writhing and significantly increased the latency of paw licking in hot plate method.

Thus, therefore provides some scientific basis for the use of the leaf of the plant in the management of pain and inflammatory conditions. Further research work is needed to segregate the active constituents from the active extract exhibiting significant analgesic and anti-inflammatory activities. In addition to this, research regarding the mechanism responsible for these activities is also required which will guarantee its clinical worth.

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