https://dx.doi.org/10.4314/jpb.v20i2.2
Vol. 20 no. 2, pp. 74-80 (May 2023)
http://ajol.info/index.php/jpb

Journal of
PHARMACY AND
BIORESOURCES

Analgesic and anti-inflammatory studies of the methanol extract of *Globimetula braunii* (Loranthaceae) growing on *Terminalia catappa* Linn (Combretaceae)

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Received 22nd December 2022; Accepted 30th March 2023

Abstract

This study was carried out to investigate the phytochemical constituents, analgesic and anti-inflammatory activity of methanol stem extract of *Globimetula braunii*. Preliminary phytochemical screening was done using standard protocols, median lethal dose (LD_{50}) was determined by Lorke's method. Analgesic and anti-inflammatory activities of extract were determined using acetic acid induced writhing and hot-plate model and the carrageenan-induced rat's paw edema model respectively at graded doses of 1000 mg/kg, 500 mg/kg and 250mg/kg. The phytochemical screening revealed the presence of flavonoids, steroid, triterpenes and saponins. Intraperitoneal median lethal dose of the extract was estimated to be greater than 5000 mg/kg bodyweight in mice. The extract exhibited highest percentage inhibition of writhing (99.24%) at a dose of 1000 mg/kg. However, the extract showed no central analgesic activity. Furthermore, the extract at lowest and highest dose (250 mg/kg and 1000 mg/kg) significantly (p<0.05) reduced paw size when compared to control group. The findings of this study suggest that the methanol stem extract of *Globimetula braunii c*ontains bioactive compounds with peripherally mediated analgesic and anti-inflammatory activity.

Keywords: Analgesic; Anti-inflammatory; *Globimetula braunii*

INTRODUCTION

Pain is a distressing experience associated with potential tissue damage and it has sensory, emotional, cognitive and social components[1].The processing of noxious stimuli (nociception) involves transduction and transmission of pain impulses through Aδ and C fibers, modulation of those impulses and perception (conscious awareness of pain) [2]. Inflammation is a protective process initiated by the body to eliminate injurious stimuli and accelerate the process of healing. It is associated with a cascade of reactions such as release of inflammatory mediators, tissue repair or breakdown and enzyme activation [3,4]. Pain is debilitating and affects the quality of life and productivity of individuals. It has a huge impact on the economy with its total cost estimated to be up to 3% of gross domestic product [5]. Chronic pain affects at least 10% of the world's population and the prevalence is up to 20-25% in some countries or regions [6].

Plants are invaluable sources of compounds for the development of new drugs [7]. It has been reported that, 80% of the population in developing countries depends on

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plant derived medicines for their health management [8]. Some drugs of plant origin used in pain management include morphine from *Papaver somniferum* and salicin from *Salix alba* [9,10].

Globimetula braunii (Loranthaceae) is a medicinal plant that is commonly found in tropical countries including Nigeria. The plant is used traditionally in the treatment of various ailments such as rheumatic pains [11]. Study conducted [12], reported that, the leaves of *Globimetula braunii* possess analgesic and anti-inflammatory activities. In our previous study, extensive literature of the plant was reported [13]. The aim of this study is to investigate the analgesic and anti-inflammatory properties of methanol stem extract of *Globimetula brauni* growing on *Terminalia catappa* in Swiss albino mice and Wistar rats.

EXPERIMENTAL METHODS

Plant collection and identification. The fresh whole plants of *Globimetula braunii* growing on *Terminalia catappa* were collected in July 2021 from Aminu Kano Teaching Hospital premises, Tarauni Local government Area, Kano State. The plant was identified and authenticated by Mallam Namadi Sunusi of the Herbarium Unit, Department of Biological Sciences, Ahmadu Bello University Zaria, by comparing with voucher specimen number (2839) already deposited in the herbarium. The Whole plants were processed by removing the leaves and flowers from the stem.

Extraction of plant materials. The whole stem of the plant was shade dried for fourteen days. The dried plant material was pulverized in a wooden mortar and pestle. The pulverized plant material was extracted using 70% methanol by cold maceration and filtered using Whatman filter paper No. 1. The extract was concentrated using rotary evaporator at 40°C under reduced pressure. The extract was stored in a desiccator for further use.

Phytochemical screening. The extract was tested for the presence of secondary metabolites as described by Evans [14].

Animals. Adult Swiss albino mice (18-22g) and Wistar rats (130-150 mg/kg) of both sex were purchased from the Animal House Facility, Department of Pharmacology, Faculty of Pharmaceutical Sciences, Bayero University Kano. They were maintained in a well-ventilated room in cages with stainless steel wire mesh covers under standard conditions, and fed with standard rodent feed with access to water *ad libitum*. The ethical approval for the experimental procedure was obtained from the Animal Care and Use Research Ethics Committee, Bayero University Kano and all experiments were conducted according to guidelines of the Committee.

Acute toxicity studies. The LD_{50} was determined according to the method described by Lorke [15]. This method was carried out in two phases and the extract was administered intraperitoneally. In the first phase the mice were grouped into three groups of three mice. Group I received 10 mg/kg of the extract, while group II and III received extract at dose of 100 and 1000 mg/kg respectively. The animals were observed for signs of toxicity including death within 24 hours. In the second phase, three mice received extract at doses of 1600, 2900 and 5000 mg/kg and observed for signs of toxicity including death within 24 hours.

Analgesic studies. Acetic acid-induced writhing in mice: Thirty mice were divided into five groups of six mice each. Group 1 was administered 10 ml/kg distilled water, group 2 received piroxicam 20 mg/kg and groups 3, 4 and 5 received graded doses of the extract (250 mg/kg, 500 mg/kg and 1000 mg/kg respectively) intraperitoneally. Thirty minutes later, all animals received 10 ml/kg Acetic acid (0.6% w/v) intraperitoneally. The number of abdominal writhing for each mouse was counted for 10 mins. The percentage inhibition

of abdominal writhing was calculated using the formula below [16].

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

Hot plate method: Thirty mice were divided into five groups of six mice each. Group 1 served as the negative control (distilled water 10 ml/kg) was administered to the group, group 2 was used as the positive control (morphine 10 mg/kg) was administered to the group, while groups 3, 4 and 5 served as the test groups and were treated with graded doses of the extract (250 mg/kg, 500 mg/kg and 1000 mg/kg respectively). Administration of drug and extract was done intraperitoneally and the mice where placed on a hot plate maintained at 55°C ± 1°C. Reaction time of the animals was noted at time 30, 60, 90, 120 and 150 minutes after treatment. The basal reaction time was to be taken by observing hind paw licking or jump response in mice while on the hot plate. A cut off period of 10 seconds was observed to avoid damage to paws [17].

Anti-inflammatory studies. Carrageenan-induced rats' paw edema: Twenty five Wistar rats were divided into five groups each containing five rats. Group 1 was administered distilled water 10 ml/kg (negative control group), group 2 was administered ketoprofen 10 mg/kg (positive control group) while groups 3, 4 and 5 were treated with graded doses of the extract (250 mg/kg, 500 mg/kg and 1000 mg/kg respectively). A 0.1 ml of freshly prepared 1.0 % carrageenan suspension was injected into the sub plantar region of the left hind paw of each rat 30 minutes after intraperitoneal administration of drug and extract. The paw diameter was measured using a digital Vernier caliper at 0, 1, 2, 3 and 4 hours after injection of carrageenan. The difference between readings obtained at 0 hour and the subsequent time intervals was taken as the thickness of edema [18].

$$\text{Inhibition (\%)} = \frac{Mean\ paw\ size\ (CONTROL) - Mean\ paw\ size\ (TEST)}{Mean\ paw\ size\ (CONTROL)} \times 100$$

Statistical analysis. Data were expressed as mean $\pm$ standard error of mean. Mean difference was analysed by one-way ANOVA followed by Dunnett post hoc test for Acetic acid induced writhing model, and repeated measure ANOVA followed by Bonferroni post hoc test for hot-plate and carregeenan-induced rats' paw edema model to regulate family-wise error and determine which group differs. The values were considered to be significantly different at $p < 0.05$.

RESULTS

Phytochemical screening. Preliminary phytochemical screening of methanol stem extract of *Globimetula braunii* growing on *Terminalia catappa* showed the presence of flavonoids, steroids, triterpenes and saponins (Table 1).

Median lethal dose (LD_{50}). The result of the intraperitoneal median lethal dose (LD_{50}) of methanol stem extract of *Globimetula braunii* was estimated to be 5000 mg/kg (Tables 2 and 3).

Analgesic Studies

Acetic acid induced writhing: Results of the acetic acid-induced writhing test are presented on Table 4. The methanol stem extract of *Globimetula braunii* growing on *Terminalia catappa* significantly reduced the mean number of writhing when compared to the negative control group ($p < 0.05$). Similarly, the extract at doses 1000 mg/kg and 500 mg/kg exhibited higher percentage inhibition of writhing when compared to the positive control (piroxicam) group (Figure 1).

Hot-plate method: Results of the hot-plate method are presented on Table 5. Positive control group (Morphine 10 mg/kg) significantly increased the mean reaction time when compared to negative control group (distilled water 10 ml/kg), no significant difference was obtained between the extract

groups and negative control group (distilled water 10 ml/kg).

Anti-Inflammatory Studies

Carrageenan-induced rats' paw edema. Results of the carrageenan-induced rats' paw edema are presented on Table 6 and 7. The extract at lowest and highest dose (250 mg/kg and 1000 mg/kg) significantly reduced the paw size when compared to negative control group (distilled water 10 ml/kg). Similarly, positive control group (ketoprofen 10 mg/kg) significantly reduced the paw size when compared to negative control group (distilled water 10 ml/kg) (Table 5).

Table 1: Preliminary phytochemical constituents of methanol stem extract of *Globimetula braunii* growing on *Terminalia catappa*

Secondary metabolite	Test	Inference
Alkaloids	Mayer's	-
	Dragendoff's	-
	Wagner's	-
Flavonoids	Sodium hydroxide	+
	Ferric chloride	+
Steroids/ Terpenes	Liebermann-Burchard's	+
	Salkwoski's	+
Saponins	Frothing	+

- =absent, + = present

Table 2: Phase 1 acute toxicity studies of methanol stem extract of *G. braunii* in mice

Groups	Dose (mg/kg)	Number of rats (n)	Mortality
A	10	3	0
B	100	3	0
C	1000	3	0

Table 3: Phase 2 acute toxicity studies of methanol stem extract of *G. braunii* in mice

Groups	Dose (mg/kg)	Number of rats (n)	Mortality
D	1600	1	0
E	2900	1	0
F	5000	1	0

Table 4: Acetic acid induced writhing in mice

Groups	Percentage inhibition	Mean number of writhes±SEM
Distilled water 10ml/kg	0	43.5±5.702*
Piroxicam 20mg/kg	58.62	18±2.733*
Extract 1000mg/kg	99.24	0.33±0.211*
Extract 500mg/kg	95.4	2±1.238*
Extract 250mg/kg	55.56	19.33±3.801*

Values are Mean number of writhes ±SEM. Data obtained were analysed using one way analysis of variance (one-way ANOVA) followed by Dunnet's post-hoc analysis, n=5 ***** $p \leq 0.05$ is considered statistically significant. SEM= Standard Error of Mean

Table 5: Effect of methanol stem extract of *G. braunii* on Mean reaction time in hot plate model

Group	Mean reaction time ± SEM				
	0 mins (sec)	30 mins(sec)	60 mins (sec)	90 mins(sec)	120 mins(sec)
Distilled water 10ml/kg	2.47± 0.25	1.48 ± 0.11	1.65 ± 0.05	1.62 ± 0.11	1.39 ± 0.07
Morphine 10 mg/kg	1.53 ±0.09[b]	3.16 ± 0.30[b]	2.25 ± 0.25[b]	2.83 ± 0.19[b]	3.06 ± 0.06[b]
Ex tract 1000 mg/kg	2.19 ± 0.19	2.06 ± 0.14	2.05 ± 0.24	1.93 ± 0.24	2.00 ± 0.09
Extract 500 mg/kg	1.70 ± 0.10	1.99 ± 0.18	2.51 ± 0.57	2.04 ± 0.29	2.77 ± 0.49
Extract 250 mg/kg	1.82 ± 0.18	2.06 ± 0.24	1.85 ± 0.24	2.59 ± 0.23	2.82 ± 0.31

Values are mean ± SEM. The data were analysed using repeated measure ANOVA followed by bonferroni post hoc test, n=5, [b] $p<0.05$ significant difference from negative control group (distilled water 10ml/kg).SEM= Standard Error of Mean

Table 6: Effect of methanol stem extract of *G. braunii* on carrageenan-induced paw oedema in rats

Groups	Paw size (mm) (mean ± SEM)				
	0 hour	1 hour	2 hours	3 hour	4 hours
Distilled water 10ml/kg	2.01 ± 0.04	3.23 ± 0.17	3.25 ± 0.12	3.74 ± 0.26	3.18 ± 0.27
ketoprofen 10 mg/kg	1.96±0.05[b]	2.63± 0.09[b]	2.25 ± 0.05[b]	2.29 ± 0.14[b]	2.298± 0.05[b]
Extract 1000 mg/kg	1.91±0.04[b]	2.40± 0.08[b]	2.56 ± 0.11[b]	2.46 ± 0.14[b]	2.56 ± 0.23[b]
Extract 500 mg/kg	2.14 ± 0.13	2.83 ± 0.24	2.73 ± 0.37	2.53 ± 0.35	2.79 ± 0.35
Extract 250 mg/kg	1.93±0.02[b]	2.48± 0.08[b]	2.57 ± 0.07[b]	2.21 ± 0.09[b]	2.40 ± 0.09[b]

Values are mean ± standard error of mean. The data were analysed using repeated measure ANOVA followed by bonferroni post hoc test, n=5, [b] $p<0.05$ significant difference from negative control group (distilled water 10ml/kg).

Table 7: Percentage Inhibition of methanol stem extract of *G. braunii* on carrageenan-induced paw oedema in rats

Groups	Percentage inhibition				
	0 hour	1 hour	2 hours	3 hours	4 hours
Distilled water 10 ml/kg	0	0	0	0	0
ketoprofen 10 mg/kg	2.49	18.58	30.77	38.77	27.74
Extract 1000 mg/kg	4.98	25.69	21.23	34.22	19.49
Extract 500 mg/kg	0	12.38	16	32.35	12.26
Extract 250 mg/kg	3.98	23.22	20.92	40.9	24.52

Values indicate the extent of inhibition of edema development.

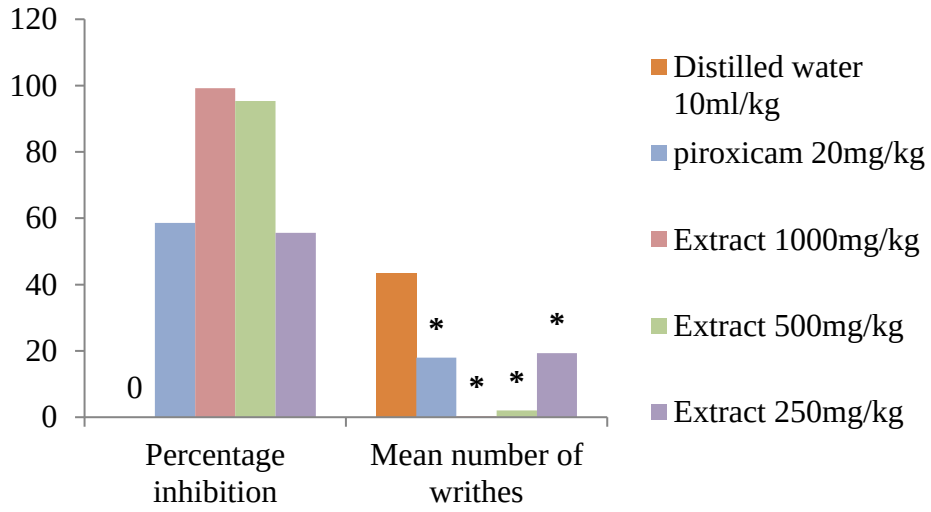

Figure 1: Effect of methanol stem extract of *G.braunii* on acetic acid-induced writhing in mice, Values are Mean number of writhes ±SEM. Data obtained were analysed using one way analysis of variance (one-way ANOVA) followed by Dunnet's post-hoc analysis, n=5 ***** $p<0.05$ is considered statistically significant. SEM= Standard Error of Mean

In addition, the extract (250 mg/kg) at the end of 3rd hour produced highest inhibition of the inflammation (40.9%) (Table 6).

DISCUSSION

Preliminary phytochemical screening of methanol stem extract of *Globimetula braunii* growing on *Terminalia catappa* showed the presence of flavonoids, steroids, triterpenes and saponins (Table 1). The findings of this study is corroborative with previous findings [12,19]. However, in this study alkaloids were found to be absent from the stem part of *Globimetula braunii*, this might be due to the difference in the part of plant used or the season at which plant was collected [20]. In this study, intraperitoneal median lethal dose (LD_{50}) of the methanol stem extract of *Globimetula braunii* growing on *Terminalia catappa* was found to be high (5000 mg/kg). This high median lethal dose (LD_{50}) value suggests that the extract is

relatively non-toxic when administered intraperitoneally [15]. The methanol stem extract of *Globimetula braunii* at all doses tested has shown to possess analgesic activity. This might be due to the presence of some secondary metabolites such as flavonoids and steroids/triterpenes which were reported to possess anti-inflammatory and analgesic activities [21,22]. Therefore, presence of these metabolites in the stem part of *Globimetula braunii* might be responsible for its anti-inflammatory and analgesic activities thereby validating the ethno-medicinal use of the plant in the treatment of headache and rheumatic pains [11].

Hot-plate test was carried out to determine central analgesic activity; the methanol stem extract of *G.braunii* did not produce significant central analgesic activity. However, previous study has shown that the ethanol leaf extract of *G.braunii* possessed central mediated activity [12]. The differences in the activity of the different parts of the plant might be due to the differences in their phytochemical constituents; as it was reported that alkaloid possesses central analgesic activity [23].

In rats, carrageenan injection elicits a biphasic edema, consisting of the early phase (from 30 to 120 minutes) and late phase (3–4 h) [24]. The early phase is mediated by the actions of vasoactive amines: histamine, serotonin, and bradykinin, which result in increase in vascular permeability [25]. The late phase on the other hand is sustained by prostaglandins, leukotrienes, and cytokines such as IL-6 and TNF-α, produced by tissue macrophages and polymorphonuclear cells [26,27]. *G.braunii* extract and ketoprofen (positive control) reduced the inflammation induced by carrageenan through both phases but showed higher inhibition at the late phase. This suggests that the plant inhibits the release and or action of histamine, serotonin, bradykinin, prostaglandins, leukotrienes, and cytokines which are implicated in the inflammatory process.

Conclusion

The findings of this study suggests that methanol stem extract of *G. braunii* growing on *Terminalia catappa* contains bioactive compounds with peripherally mediated analgesic and anti-inflammatory activity. The combination of both leaves and stem of *G. braunii* in the management of peripheral pain might be more beneficial than the use of either part alone.

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