ANALGESIC AND ANTI-INFLAMMATORY ACTIVITIES OF METHANOL LEAF EXTRACT OF *BOSCIA SALICIFOLIA* IN RATS AND MICE

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ABSTRACT

Boscia salicifolia (Capparacea) is used commonly in Africa as a medicinal plant due to its therapeutic properties such as healing of wounds due to its antioxidant potentials, treatment of tuberculosis and deworming ability in certain individuals because of its antibacterial and anthelminthic activities. The aim of this investigation is to determine the analgesic and anti-inflammatory activities of *B. salicifolia* in rats and mice using thermal-induced pain and carrageenan-induced inflammation respectively. A preliminary acute toxicity study using Lorke's method which involves a total of 13 mice was performed to determine the LD_{50} of methanol stem bark extract of *B. salicifolia*. Phytochemical screening of the plant extract was conducted to elucidate the bioactive constituents present in the extract. Hot plate method was used to induce pain in five (5) groups of six (6) rats each. The treatment and doses administered were normal saline 1 mL/kg for group 1, *B. salicifolia* at doses of 120, 240, 360 mg/kg, for groups 2, 3, and 4 respectively. While group 5 was given 20 mg/kg piroxicam. In the anti-inflammatory study, Carrageenan (0.6%) was administered intraperitoneally to rats to induce inflammation in five (5) groups of six (6) rats each. Group 1 rats were administered 10 mL/kg normal saline and groups 2-5 were given similar treatment at doses similar to those for the analgesic studies. Results obtained from the studies have shown an LD_{50} of the extract of 3800 mg/kg. The latency period of response to thermal-induced pain increased significantly ($p<0.05$) at 120, 240, 360 of the extract and 20 mg/kg of piroxicam after 60 minutes of administration. The latency period was prolonged at 60 minutes after a dose of 240 mg/kg *B. salicifolia*. Reduction in carrageenan-induced paw edema by *B. salicifolia* was not observed in all the tested doses and piroxicam groups. In conclusion, the investigation has shown that *B. salicifolia* produced a time-dependent maximal analgesic effect after 60 minutes of administration of 240 mg/kg in mice. However, it lacks anti-inflammatory property in rats at the tested doses.

Keywords: Analgesic activity, anti-inflammatory activity, *Boscia salicifolia*, carrageenan, hot plate, piroxicam.
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INTRODUCTION

Natural products such as plants and animals have been used by herbalists locally to treat human illnesses for a long period of time [1]. Traditional medicine in Africa is used by 80% of the population for the purpose of primary health care [2]. Some investigators have reported that traditional medicines have minor side effects and hence their common use [3]. *Boscia salicifolia* (Capparacea) olive is called *Anza* or *Zure* in Hausa Language. It is also commonly referred to as Willow-leaved shepherd tree. The plant is also common to Ghana, Cameroon, Uganda, Kenya, Tanzania, Malawi, Zambia, Botswana, Zimbabwe and Mozambique [4]. The plant is used to treat cramping from diarrhea; as an abortifacient in goats and as an anthelminthic. It is also used as analgesic and antipyretic agent [5]. The bark and leaf of the plant was reported to have been effective in treatment of malaria [6]. A report from a study conducted in South Africa by Alfred *et al*. [7] has shown that *B. salicifolia* contains alkaloids, tannins, anthraquinones, saponins and flavonoids. Current available drugs for pain and inflammation are known to have numerous side effects which include gastrointestinal disturbances, respiratory depression, possible dependence, constipation, renal dysfunction, peptic ulcer and bleeding [2]. Natural products derived from medicinal plants are becoming preferred alternative remedies [2].

MATERIALS AND METHODS

Materials

Drug

Piroxicam ampoules manufactured by Shandong Xier Kangtai Pharmaceutical, China. Batch number: 181204. Manufactured on 1/06/2017, expires on 7/05/2020 and stored in a refrigerator.

Chemicals and reagents

Analytical grade chemicals and reagents used for the study are: Methanol (BDH Pool, London), Formaldehyde 0.39% (BDH Pool, London), Normal saline (Dana, Nigeria Limited).

Equipment

They include hot plate (Ugo Basile, England Cat. No. 35150-002), Vanier caliper (Draper, England), weighing balance (Vickers limited, England), water bath (Ugo Basile, England).

Experimental animals
A total of forty-three (43) adult albino mice of both sexes weighing 20-30 g were used for analgesic studies and acute toxicity studies respectively. Thirty (30) adult albino rats weighing 100-200 g were used for anti-inflammatory studies. All animals were purchased from the Animal House of Department of Pharmacology and Therapeutics, Gombe State University. They were fed standard diet (*Vital* Feed®) and water *ad libitum*.

Plant material
The fresh leaves of *B. salicifolia* were collected from Akko Local Government Area of Gombe State. The leaves were identified in the Department of Biological Sciences of the Faculty of Science and allocated a voucher number of 166 in the month of September, 2019.

Methodology

Preparation of plant extract
The fresh leaves of *B. salicifolia* were cut and dried until there is no moisture. The dried leaves were pulverized into powder. Consequently, 1620 g of the powder was measured and macerated in 1.5 L of methanol for 72 hours with occasional shaking. This was filtered using Whatman No. 1 filter paper and the filtrate was concentrated to semi-solid residue in a water bath at a temperature of 30˚C.

Acute toxicity study
To determine symptoms of toxicity due to acute exposure to methanolic leaf extract of *B. salicifolia* and to establish oral lethal dose (LD_{50}), Lorke's method [8] was conducted using albino mice.

Preliminary phytochemical screening
To determine the bioactive constituents, present in *B. salicifolia* the methods of Harbone [9] and that of Evans [10] were adopted.

Study design
A total of 30 mice and 30 rats were bred until they achieved a body weight of 18-20 g and 180-200 g respectively. For both analgesic and anti-inflammatory studies, the animals were divided into five (5) groups of six (6) rats or six (6) mice each. The mice were given 1 mL/kg and rats 10 mL/kg normal saline, 120, 240, 360 mg/kg *B. salicifolia* respectively and 20 mg/kg piroxicam as standard via the *intraperitoneal* route.

Analgesic study
The analgesic effect of *B. salicifolia* was determined using hot plate by the method of Eddy and Leimbach [11]. The temperature of the hot-plate was maintained at 55 ± 0.5˚C. To evaluate the responses of control or test groups the indices are shaking or licking of paw, jumping off the hot plate. The latency period for such responses were recorded at 0, 30, 60, 90 and 120 minutes post-treatment.

Anti-inflammatory study
The method of Winter et al. [12] was adopted for the carrageenan-induced anti-inflammatory study in which 0.2 mL 1% carrageenan was administered into the sub-plantar region of the left hind paw of each rat, one (1) hour after administration of normal saline, *B. salicifolia* or piroxicam. The thickness of the rat paw was measured using a Vernier caliper at 0, 1, 2, 3, 4 and 24 hours.

Statistical analysis
Data were presented as Mean ± Standard Error of Mean and analyzed using one-way repeated measure ANOVA followed by Bonferroni post hoc test. P values less than 0.05 were considered to be statistically significant.

RESULTS

Extraction of plant materials
The extract was kept in an oven at 50˚C for 7 days before commencement of the study. The calculated % yield of methanol leaf extract of *B. salicifolia* from the crude was only 1.23%.

Acute toxicity study
The oral LD_{50} of methanol extract of *B. salicifolia* in mice was found to be 3800 mg/kg. The result for the study is presented in Table 1 below where mortality was observed only at 5000 mg/kg.

Table 1: Determination of oral LD_{50} of methanol leaf extract of *Boscia salicifolia* in mice

Phase/Group	Dose (mg/kg)	Mortality
I		
1	10	0/3
2	100	0/3
3	1000	0/3
II		
1	1600	0/1
2	2900	0/1
3	5000	1/1

LD_{50} = √Highest non-lethal dose - Lowest lethal dose

= √2900 x 5000

= √14500000

= 3807.89

≈ <u>3800 mg/kg</u>

Preliminary phytochemical study

On completion of the phytochemical analysis, the bioactive constituents present in methanol leaf extract of *B. salicifolia* were; alkaloids, flavonoids, steroids, saponins, glycosides, terpenoids and tannins.

Analgesic study

The latency period of response to thermal-induced pain steadily increased from 0 minutes through 60 minutes in all test groups and piroxicam group. The latency periods were statistically significant ($p<0.05$) at all-time points compared to distill water control group (Figure 1).

DISCUSSION

Acute exposure of rats to doses of *B. salicifolia* up to 2900 mg/kg resulted in no mortality within 24 hours and beyond due to little or no effect on respiration and other toxic effects. Some of the acute symptoms of toxicity which can cause death are dyspnea, diarrhea, restlessness, hyperactivity and respiratory depression. The presence of phytochemical constituents in an extract is a distinguishing feature of a plant that confer peculiar bioactivity attributed to plant species. The presence of flavonoids among other bioactive constituents reported is responsible for the analgesic and anti-inflammatory activities of methanol extract of *B. salicifolia*. Ishola *et al.* [13] after bioactivity guided isolation from *Cnestis ferruginea* vahl ex DC (Connaraceae) root extract, concluded that amentoflavone was responsible for the analgesic and anti-inflammatory activities observed. Plant secondary metabolites are rich sources of bioactive compounds eliciting many beneficial health effects in man and animals. Recent report revealed the possibility of phytochemicals as important source of therapeutics and preventive agents against diseases [14]. The synthesis of prostaglandins, leukotrienes and thromboxanes occur consequent to stimulation of peripheral nociceptors by heat or chemicals such as acetic acid, carrageenan, formalin, etc. Consequently, pain and inflammation occur which can be reduced by conventional drugs or medicinal plants such as *B. salicifolia* with analgesic and anti-inflammatory properties. The methanolic leaf extract of *B. salicifolia* produced a time-dependent increase in latency period to hot plate-induced pain up to 60 minutes in 120, 240, 360 and 20 mg/kg piroxicam because the extract possesses analgesic property capable of inhibiting cyclooxygenase enzyme that is implicated in generation of mediators of pain and inflammation. The extract at all tested doses has no anti-inflammatory effect probably because it lacks the ability to inhibit the release of leukocytes into the blood and the leakage of some fluids into the tissues resulting in swelling [15].

CONCLUSION

In conclusion, the investigation has shown that *B. salicifolia* produced a time-dependent maximal analgesic effect after 60 minutes of administration of 240 mg/kg in mice. However, it lacks anti-inflammatory property in rats at the tested doses.

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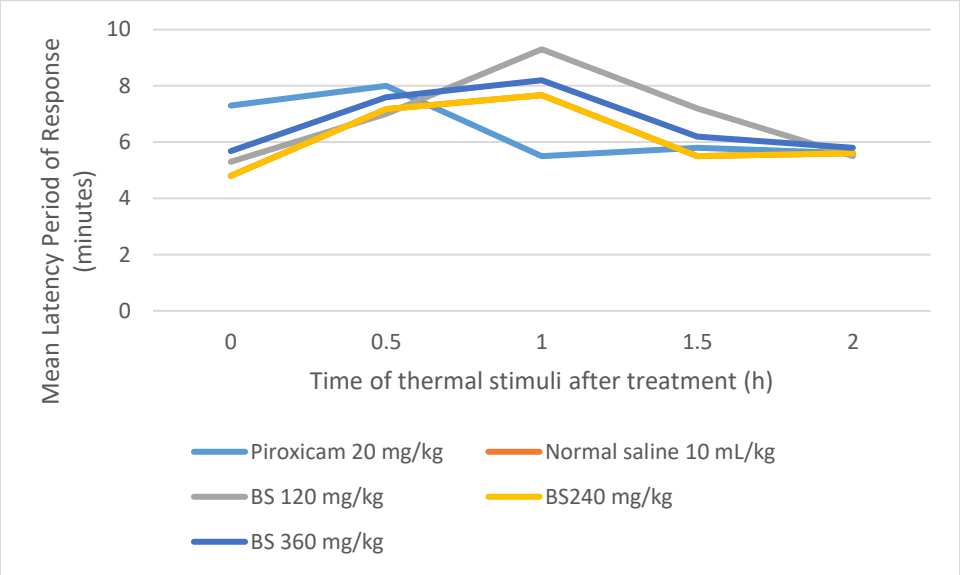

Figure 1: Effect of methanol stem bark extract of *Boscia salicifolia* on hot plate-induced pain in rats

Values are presented as Mean ± SEM and analyzed using one-way repeated measure ANOVA, p-values < 0.05 were considered statistically significant, n = 6; BS = *Boscia salicifolia*

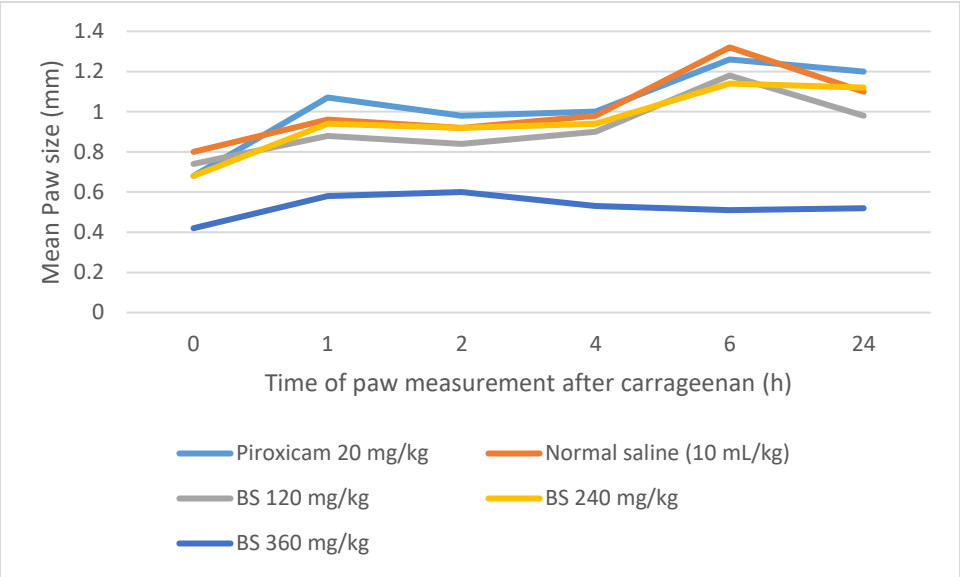

Figure 2: Effect of methanol stem bark extract of *Boscia salicifolia* administration on carrageenan-induced paw edema in rats

Values are presented as Mean ± SEM and analyzed using one-way repeated measure ANOVA, p values< 0.05 were considered statistically significant, n = 6; BS = *Boscia salicifolia*