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Research Article

Pharmacological Evaluation of the Methanolic Extract of *Tribulus bimucronatus* Growing in Saudi Arabia in Rats

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ABSTRACT
The methanolic extract of *Tribulus bimucronatus* (Zygophyllaceae) growing in Saudi Arabia was screened using albino rats in a dose of 100, 200 & 400 mg/kg of body weight.The tail cuff for hypotensive, the hot plate for the antinociceptive and the carrageenan-induced rat paw edema method for the anti-inflammatory activity were adopted. The results revealed a significant dose-dependent anti-inflammatory activity. Furthermore marked hypotensive and antinociceptive effect were observed at the higher dose (400 mg/kg).*Tribulus bimucronatus* is shown to be a promising plant and may be comparable to the famous Ayurvedic *Tribulus teresteris* from which commercial products are available.

Keywords: *Tribulus bimucronatus*, hypotensive activity, antinociceptive activity, anti-inflammatory activity.

INTRODUCTION

The genus *Tribulus* of the Zygophyllaceae comprises about 20 species which grow in subtropical areas around the world[1]. Several *Tribulus* species are found among the Saudi flora[2]. *Tribulus terrestris* has been used for a long time in both the Indian and Chinese systems of medicine for treatment of various kinds of diseases. In Saudi Arabia it is used in renal colic[3]. This species has received comprehensive studies; that proved stimulant, diuretic, aphrodisiac, immunomodulatory, antidiabetic, absorption enhancing, hypolipidemic, cardiotonic, hepatoprotective, anti-inflammatory, analgesic, antispasmodic, anticancer, antibacterial, anthelmintic and larvicidal, activities[4]. *Tribulus alatus* was also found to have antioxidant[5], diuretic[6]and testosterone-increasing effects[7]. *Tribulus bimucronatus* is of common occurrence in the Saudi flora[2]. Till date few studies were reported in the current literature describing the pharmacological activity of this species. Therefore, the main goal of this study is the pharmacological evaluation of the methanolic extract of *Tribulus bimucronatus* growing in Saudi Arabia using rats. Anti-inflammatory, antinociceptive, and hypotensive activities were investigated.

MATERIALS AND METHODS

Plant material: The fresh aerial parts of *Tribulus bimucronatus* were collected from Makkah district, Saudi Arabia, in January 2014 and authenticated by Dr. Kadry Abdel Khalik, Botany Department, Faculty of Applied Science, Umm Al-Qura University. A voucher specimen has been deposited in the herbarium of the Department of Pharmacognosy, Faculty of Pharmacy, Umm Al-Qura University(UQU-2014-1). Dried aerial parts were reduced to a fine powder with a mechanical grinder and stored in a non-toxic polyethylene bag in the fridge.

Preparation of plant extract: The fine powder (500 g) was extracted on cold with methanol till exhaustion, concentrated in a rotary evaporator, (Percentage yield (w/ w) of 10% was obtained), the residue was used for preparation of water suspension intween 80 (2% solution).

Phytochemical screening: The screening of the methanolic extract was performed using standard procedures[8]to identify the presence of chemical constituents.

Animals: Experiments were performed using albino rats of either sex (200-300g). The animals were obtained from the laboratory animal house, Faculty of Pharmacy, King Abdulaziz University. The animals were housed under controlled environmental conditions: temperature of 22 ± 2 ◦C, 55 ± 5% relative humidity and a constant light/dark cycle. The animals were fed on rodent diet and had free access to drinking water. The experimental protocol was designed according to institutional regulation of the ethics committee of Umm Al-Qura University.

Hypotensive activity: The following experimental protocol of measurement of blood pressure and heart rate using tail cuff was performed.

Adult albino rats were divided into five groups each composed of six animals.

Group I: Control animals received tween 80

Group II: Standard group received irbesartan 100 mg/kg, i.p.

Group III: Animals received methanolic extract at the dose of 100 mg/kg, i.p.

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Table1: Effect of four days treatment of methanolic extract of *Tribulus bimucronatus* on blood pressure and heart rate of normotensive rats.

Treatment	Systolic Blood Pressure		Diastolic Blood Pressure		Heart Rate	
	mm Hg (X±S.E.)	% Change	mm Hg (X±S.E.)	% Change	Beat/minute (X±S.E.)	% Change
Control (2 % tween 80)	150.00 ± 0.58	-	119.00 ± 1.16	-	357.33 ± 1.98	-
Irbesartan (100 mg/kg)	123*abc ± 0.86	18	95*abc ± 0.73	20.17	392.17*ab ± 3.20	-9.75
Methanolic extract (100 mg/kg)	150.50 ± 2.62	0.33	105.17*± 1.92	11.62	298.50* ± 0.76	16.46
Methanolic extract (200 mg/kg)	146.17 ± 0.60	2.55	118.50a± 1.23	0.42	372.83*a ± 1.2	-4.34
Methanolic extract (400 mg/kg)	137.67*ab± 1.20	8.22	116.00b± 0.45	2.52	387.00*ab ± 0.93	-8.30

*X=6, *: Significantly different from normal control value at p< 0.05, a: Significantly different from 100 mg value at p< 0.05, b: Significantly different from 200 mg value at p< 0.05, c: Significantly different from400 mg value at p< 0.05.*

Table 2 :Antinociceptive activity of four days treatment with methanolic extract of *Tribulus bimucronatus* using hot plate method in rats

Treatment	Reaction time (seconds) X ±S.E.	% Protection
Control (2 % tween 80)	5.62±0.15	-
Diclofenac sodium (10 mg/kg)	8.50*abc±0.23	51.25
Methanolic extract (100 mg/kg)	5.03±0.08	-10.50
Methanolic extract (200 mg/kg)	5.72a±0.08	1.78
Methanolic extract (400 mg/kg)	7.17*ab±0.11	27.58

*X=6, *Significantly different from normal control value at p< 0.05, a Significantly different from 100 mg value at p< 0.05, b Significantly different from 200 mg value at p< 0.05, c: Significantly different from 400 mg value at p< 0.05*

Group IV: Animals received methanolic extract at the dose of 200 mg/kg, i.p.

Group V: Animals received methanolic extract at the dose of 400 mg/kg, i.p.

Animals were treated with single dose of methanolic extract for four days before experiment.

After 1h from the last dose, systolic and diastolic blood pressure as well as heart rate were measured. Rat blood pressure and heart rate were assessed by CODA, a computerized non-invasive blood pressure system (Kent Scientific, Torrington, CT, USA) which measures tail blood pressure by means of volume pressure[9]. Rats were held in a restrainer on a preheated platform with the tail exposed, and both an occlusion cuff and a volume pressure-recording cuff were placed close to the base of the tail. The digital value for the systolic, diastolic blood pressure and heart rate were recorded. Readings were taken for 20 cycles from each rat, and the highest and the lowest values were excluded. To minimize stress-induced variations in blood pressure, all measurements were taken by the same person in the same peaceful environment at the same time of day.

Determination of the Antinociceptive activity using hot plate method: The same protocol was adopted as that for the hypotensive activity except group II received standard diclofenac sodium 20 mg/kg, i.p. After 1h from the last dose rats were placed on a hot plate maintained at a temperature of 55±0.5°C[10]. The animals were individually placed on the heated surface. The time in seconds between placement and shaking, paw licking and jumping off the plate was recorded as response latency in seconds.The percentage variation was calculated using the following ratio[11]:

% Protection =100 {(drug latency − baseline latency)/ baseline latency}

Determination of the anti-inflammatory activity using carrageenan-induced rat right hind paw edema method: The anti-inflammatory activity was examined in rats according to the method of Winter et al.(1962)[12] with slight modification. The experimental protocol of

Table 3: Effect of four days treatment with methanolic extract of *Tribulus bimucronatus*on carrageenan–induced rat right hind paw edema method.

Treatment	Edema size (mm) X ± S.E.	% inhibition
Control (2 % tween 80)	2.45± 0.03	-
Diclofenac sodium (10 mg/kg)	0.50*a ± 0.01	79.59
Methanolic extract (100 mg/kg)	0.78* ± 0.07	68.16
Methanolic extract (200 mg/kg)	0.53*a ± 0.01	78.37
Methanolic extract (400 mg/kg)	0.31*ab ± 0.02	87.35

*X=6, *Significantly different from normal control value at p< 0.05, a Significantly different from 100 mg value at p< 0.05, b Significantly different from 200 mg value at p< 0.05*

antinociceptive activity was repeated for the anti-inflammatory activity. After 1h from the last dose, rats were injected with 0.1 ml of 1% carrageenan solution in sub-planter tissue of right hind paw. After 3hours of carrageenan the thickness of both left and right paws was measured using micrometer. The thickness of the left paw was subtracted from the right one to calculate edema thickness.

The percent inhibition in edema thickness was calculated as described below[13]

% Inhibition =100{(Tc−Ts)/Tc}

Where Tc represents mean edema in control and Ts mean edema in group treated with standard/extract.

Statistical analysis: Results were expressed as mean ± SEM. Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Tukey Kramer multiple comparison test. P< 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

Phytochemical screening**:** Phytochemical analysis of the methanolic extract of *Tribulus bimucronatus* revealed the presence of sterols/triterpenes, carbohydrates, tannins, saponins and flavonoids while anthraquinones and alkaloids were absent. Measurement of blood pressure and heart rate using tail cuff: The methanolic extract in a dose of 400 mg/kg significantly decreased systolic blood pressure indicating hypotensive activity. Furthermore frequent urination was observed which may lead to the hypotensive effect of this extract. This hypotensive effect was associated with a significant increase in the heart rate. It is well known that following hypotension, reflex stimulation of sympathetic outflow and renin release occur leading to reflex tachycardia (Table 1).

Antinociceptive activity: A significant prolongation in the reaction time on hot plate was observed at the higher dose (400 mg/kg) of the methanolic extract (Table 2).

Anti-inflammatory activity: The edema was measured after 3 hours to enable a full picture of the release of inflammatory mediators (histamine, serotonin, bradikinin, prostaglandins). All the doses tested of the methanolic extract (100, 200 and 400 mg/kg) showed a significant reduction in the paw thickness as compared to control group indicating marked anti-inflammatory activity (Table-3). It has been reported that the anti-inflammatory activity of many plants has been attributed to their content of sterol/triterpene[14]. Several flavonoids such as rutin, quercetin, luteolin produced significant antinociceptive and/or anti-inflammatory activities[15,16]. The phytochemical screening of the methanolic extract of *Tribulus bimucronatus* revealed the presence of these active compounds.

CONCLUSION

The present results showed a marked dose dependent anti-inflammatory activity, in addition to hypotensive and antinociceptive activities. These effects were prominent at the higher dose (400 mg/kg). Frequent urination was also observed. Further studies are needed to identify the active compounds and to elucidate the exact mechanisms of action.

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