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Evaluation of Acute Toxicity, Anti-Arthritic and Analgesic Effects of *Haloxylon salicornicum* Extract on Animal Models

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ABSTRACT

Haloxylon salicornicum is a traditional medicinal plant used for treatment of various diseases in Balochistan Pakistan. Present study was carried out to evaluate acute toxicity, anti-arthritic and analgesic effects of *Haloxylon salicornicum* (whole Plant) methanol extract. Acute toxicity test was performed on rats and extract was administered at the dose 150, 500, 1000, and 2000mg/kg oral dose. Anti-arthritic activity was carried out by formaldehyde induced arthritis on rats. Acetic acid induced abdominal constriction method was used for analgesic activity. In Acute toxicity test *Haloxylon salicornicum* did not produced any toxicity. In anti-anthric activity *Haloxylon salicornicum* methanol extract significantly ($p<0.05$) reduced that rat paw edema as compared with standard drug. In analgesic activity *Haloxylon salicornicum* extract significantly ($p<0.05$) decreased the number writhes as compared with standard drug. It is concluded that *Haloxylon salicornicum* possess anti-arthritic and analgesic effects with safety profile.

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INTRODUCTION

Plant sources have remained an important part of society. After the basic needs which include food and shelter are met, man has searched among flora for a suitable remedy to treat numerous sicknesses. Ethnonpharmacognosy drug treatments are defined as indigenous remedy used to preserve health and to diagnose, prevent and treat sicknesses. Traditional medicinal drug primarily based on specific experiences, ideals and theories. Ethnopharmacological treatment has been used for millennia with exceptional contributions by way of physicians to human fitness, especially as number one health care carriers on the societal degree, and has retained its popularity around the arena. Around 60-eighty five% of the population in cutting-edge global is dependent on Crude drugs [1].

Approximately 6000 species of flowering plants exist in Pakistan and about 600 (600) species of flora have been identified as having medicinal values. Approximately 80% of Pakistan's outlying areas are still making use of crude drugs. Plant based medicines account for about 25% of pharmacopoeial drugs with many greater compounds isolated from herbs [2].

Extracts of these crude drugs contain many different beneficial bioactive molecules such as minerals, terpenoids, flavonoids, polyphenols and alkaloids. These elements have vital cytotoxic, antioxidant, anti-inflammatory and antimicrobial potentials. It provides cure against various diseases. Crude drugs containing these phytochemical components (plant secondary metabolites) are used as substitute for allopathic and synthetic drugs, which are believed to be less hazardous to humans and the environment compared to their counterparts [3].

Haloxylon salicornicum belongs to Chenopodiaceae family, it is found in Afghanistan, India and Pakistan. In Balochistan It is found in Mund (Turbat) locally it is known as “Lana” or “Khar Traht, (Balochi). Previously Alkaloids have been reported from the plant and main alkaloids are Haloxylon, haloxynine, halosaline, haloxine, anabasine and smipine. As traditional remedy it is used (in decoction form) as anti-inflammatory and antiseptic. Also used for the treatment of ulcer by the local people [4]. Current study is carried out to evaluate the acute toxicity, anti-arthritic and analgesic effects of *H. salicornicum* methanolic extract.

MATERIAL AND METHODS

Plant material

H. salicornicum whole plant was collected from trubat district of Balochistan, Pakistan. Plant was identified and Voucher (specimen number PCOG-I, 112) was deposited in the Pharmacognosy Department. Plant was soaked in methanol for 15 days and solvent was evaporated by using rotary evaporator and dark brown semisolid residue was obtained.

Animals

Male wistar rats (n=5) weighing between 230 and 270 g and Swiss albino mice weighing 25-30 g were used to carry out the pharmacological activities. The animals were kept in the animal facility of the Faculty of Pharmacy. The animals were housed in polycarbonate cages and maintained at a controlled room temperature (22 ± 2 °C; relative humidity 45 ± 10%) with a light/dark cycle of 12/12 h. They were fed a standard diet of pellets and had access to water *ad libitum*. Before any experiments, the animals were fasted for 12 hours.

Phytochemical Tests

The presence of diverse phytochemicals in methanolic extractof *H. salicornicum* was assessed by following standard methods [5]. Tests were performed for the presence of alkaloids, coumarin, flavonoids, glycosides, phytosterols, polyphenols, saponins, tannins, and terpenoids.

Acute toxicity Test

In acute toxicity tests, male wistar rats were randomly assigned to 4 groups consisting of 6 rats per group. Each group of rats was administered a fixed single dose of the respective tested extracts in a stepwise procedure (125, 500, 1000 and 2000 mg/kg body weight). Group I rats received distilled water (vehicle) until the end of the experiment. Any signs of clinical toxicity were closely monitored within 4 hours of the treatment period up to 24 hours. Thereafter, observations for toxic manifestations (upfur, wrinkling, tremors, excitability, twitching, salivation, and mortality) were made through the end of the 14-day period [6].

Analgesic Activity

Acetic acid-induced writhing test

Mice were randomly grouped (n=5) and treated with either 10 mL kg^{-1} of 0.9% wv/normal saline, i.p., *H. salicornicum* extract (250 and 50 mg kg^{-1}, p.o.), diclofenac (100 mg kg^{-1}, i.p.) Each animal was injected intraperitoneally with 0.6% acetic acid and placed in a glass chamber. Abdominal contractions along with hindlimb extension were recorded for 30 minutes. Total number of abdominal contractions and stretches were quantified for 30 minutes [6].

Anti-arthritic activity

Formaldehyde induced arthritis in rats

The rats were distributed into 04 groups (n=5). Group I: Arthritic control (administered 5 ml/kg saline). Group II: (administered 50 mg/kg diclofenac sodium). Group III and IV, *H. salicornicum* extract treated groups (administered 250 and 50 mg kg-1, p.o., respectively). On day 1 of experiment, 30 minutes after drug administration, arthritis was induced by subplantar injection of 2% formaldehyde solution (0.1 ml) and was repeated on day 3. Drug treatment was continued for 10 days. Arthritis was assessed by checking the average increase in paw diameter over 10 days using a digital caliper [8].

Statistical analysis

Statistical analysis

Results are presented as the mean ± standard error of the mean of 05 animals. Statistical comparison of the data was performed by a one-way analysis of variance followed by a student's t-test. $P<0.05$ or less was considered significant [9].

RESULT

Phytochemical tests

In phytochemical tests results were positive for the presence of alkaloids, flavonoids, glycosides, saponins, tannins, and terpenoids.

Acute toxicity test

In acute toxicity test methanolic extract of the plant did not produced any sign of toxicity at the dose of 2000mg/kg.

Analgesic Activity

Acetic acid induced writhing test

In writhing test mean number of writhes for control group was 101.68±0.52, for *H. intermedia* 250mg/kg treated group 88.41±0.27, for 500mg/kg treated group it was 74.78±0.48 and for standard drug treated group it was 52.58±0.34. In this test plant produced significant effects ($p<0.05$), number of writhes were decreased at both doses i.e. 250 and 500mg/kg oral doses.

Anti-arthritic activity

Formaldehyde induced arthritis in rats

The sub plantar injection of 2% formaldehyde solution induced inflammation of the hind paw with a maximum volume observed on day 10. The pretreatment of animals with *H. Salicornicum* 250, and 500 mg/kg) and diclofenac (50 mg/kg) significantly inhibited the formaldehyde induced inflammation. With the dose of 500 mg/kg, a significant ($p<0.05$), inhibition effect was observed.

DISCUSSION

Arthritis is a persistent disease that similarly influences adults and the elderly, women are more often than mans. The high arthritis incidence rate in low to center-earnings countries results in an incapacity to fulfill the everyday non-public requirements. Essential endeavors toward prevention and management of arthritis have to be prioritzed. Most people uses drugs like NSAIDs, Glucocorticoids and steroids and these drugs relieves the sign and symptoms but cover the exact cause of the disease and also produces adverse drug effects, otherwise only treatment is the surgical treatments [10]. Phytotherapeutic constituents derived from herbal flora are comprehensively employed in particular in growing international locations for disorder treatment [6]. Balochistan is native home of many medicinal flora and are used in traditional system of medication. *H. Salicornicum* is a medicinal plant used for control of pain and other sickness. No clinical data is to be had on pharmacological activities of the

plant consequently the present study is performed to evaluate the acute toxicity, anti-arthritic, and analgesic activities of *H. Salicornicum* methanolic extract.

In phytochemical tests results were positive for the presence of alkaloids, flavonoids, glycosides, saponins, tannins, and terpenoids. In acute toxicity test *H. Salicornicum* methanolic extract did not showed any sign of toxicity and mortaility, so it is concluded that the plant extract safe up to dose of 2000mg/kg oral dose.

In acetic acid induced writhing test *H. Salicornicum* methanolic extract showed significant analgesic results as the number of writhes had been significantly reduced in comparison with standard drug. The analgesic activity of acetic acid-induced writhing test may characteristic to the release of TNF-α, interleukin-1 (IL-1), and interleukin-eight (IL-eight) with the aid of resident peritoneal macrophages and mast cellular in mice [11]. This model has also been associated with elevated degree of PGE and PGF2a. Enhanced PG stages inside the peritoneal cavity and triggers the inflammatory mediators, with the aid of increasing capillary permeability and activating number one afferent nociceptors [12]. It is hypothesized that *H. Salicornicum* methanolic extract produced analgesic results with the aid of inhibiting these inflammatory mediators.

Anti-arthritic activity was carried out by formalin induced arthritis in rat paw. Changes in the paw volume of the formalin triggered arthritic rats were measured using digital Vernier caliper. From the results obtained, it become clear that *H. Salicornicum* methanolic extract produced significant effects ($p<0.05$) as compared with standard drug Diclofenac sodium on decreasing the paw volume. Best of our knowledge current study was the first study on the arthritic activities of *H. Salicornicum.* In phytochemical studies results were positive for the presence of Flavoinoids, alkaloids and glycosides. A previous study shows that the flavonoids are responsible for anti-arthritic activity [13, 14], therefore it is assumed that the falvonoids present in the plant are responsible for anti-arthritic activity.

CONCLUSION

It is concluded that of *H. Salicornicum* produced significant ant-arthritic and analgesic activities. However further studies are required to isolate the chemical constituents responsible for pharmacological activities.

Table No. 1: Phytochemical tests of *H. Salicornicum* methanolic extract

Sr. No.	Test	Result
1	Alkaloids	Positive
2	Coumarin	Negative
3	Flavonoids	Positive
4	Glycosides	Positive
5	Phytosterols,	Negative
5	Polypheols	Negative
6	Saponins	Positive
7	Tannin	Positive
8	Terpinoids	Negative

Table No. 2: Acute toxicity of *H. Salicornicum* methanolic extract on rats

Sr. No.	Treatment	No of deaths
1	Control	Nil
2	*H. Salicornicum* methanolic extract 125mg/kg	Nil
3	*H. Salicornicum* methanolic extract 500mg/kg	Nil
4	*H. Salicornicum* methanolic extract 1000mg/kg	Nil
5	*H. Salicornicum* methanolic extract 2000mg/kg	Nil

N=06,

Table No. 3: Effect of *H. Salicornicum* methanolic extract on Formaldehyde -induced rat paw edema

Treatment	2days	4days	6days	8days	10days
Control	9.27±0.16	11.39±0.09	13.88±0.10	15.76±0.21	17.61±0.18
***H. Salicornicum* 250mg/kg**	7.70±0.11	7.37±0.33	6.34±0.17	6.04±0.07	5.58±0.19
***H. Salicornicum* 500mg/kg**	6.77±0.18	6.11±0.20	5.80±0.31	5.51±0.23	4.93 ±0.17
Standard Drug Diclofenac Sodium 50mg/kg	5.748 ±0.17	6.57±0.21	5.49±0.06	4.46±0.05	4.09±0.06

N=06, values are expressed as Mean±SEM, *= p<0.05, **= p<0.01

Table No. 4: Effect of *H. Salicornicum* methanolic extract on acetic acid induced writhing test on mice

Sr. No.	Treatments	Number of writhes
1	Control	**101.68**±0.52
2	*H. Salicornicum* 250mg/kg	**88.41**±0.27
3	*H. Salicornicum* 500mg/kg	**74.78**±0.48
4	Standard Drug Diclofenac Sodium 50mg/kg	**52.58**±0.34

N=05, values are expressed as Mean±SEM, *= p<0.05, **= p<0.01

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