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Research Article

EVALUATION OF THE LOCOMOTOR AND DIURETIC ACTIVITIES OF ETHANOLIC EXTRACT OF LEAVES OF *CAPPARIS DIVARICATA* LAM. (CAPPARIDACEAE)

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ABSTRACT

The aim of the present study was to evaluate the locomotor and diuretic activities of ethanolic extract of leaves of *Capparis divaricata* Lam. (EEC) in experimental animals. Locomotor activity was evaluated by actophotometer in mice and diuretic activity was evaluated using in-*vivo* Lipschitz test model in rats. Oral administration of EEC at doses 250 mg/kg and 500 mg/kg produced significant (P< 0.01) CNS depression by reducing locomotor activity while it showed a significant (P< 0.01) increase in the urine volume when compared to control. Thus, in conclusion the EEC has a significant CNS depression action by reducing locomotor in mice and diuretic action in rats. Preliminary phytochemical screening showed the presence of alkaloids, flavonoids, phenolic compounds, glycosides, tannins and saponins.

Keywords: *Capparis divaricata*, Locomotor activity, Diuretic activity, Actophotometer, Lipschitz test.

INTRODUCTION

Capparis divaricata Lam. commonly known as caper bush, belonging to the genus *Capparis* of family Capparidaceae, found throughout the India especially in the Deccan Peninsula from Maharashtra southwards to Tamil Nadu [1]. The caper (*Capparis*) is a native Mediterranean plant and certain species of caper have been cultivated. It has been reported that the genus *Capparis* consists of nearly 80 species [2]. *Capparis* species exhibit different pharmacological activities. The fruits, roots, and seeds of *Capparis* have been used traditionally as antirheumatic, tonic, expectorant, antispasmodic and analgesic agents in Turkey and other countries. *C. zeylenica* L. is commonly known as Indian caper which possesses analgesic and antipyretic properties. *C. spinosa* and *C. decidua* Edgew have also been studied for their analgesic and anti-inflammatory activities, but it has been found that they both possess anti-inflammatory effects.

In general, Capparidaceae family members contain glucosinolates, alkaloids, and flavonoids and have phytochemical differences in plant parts. Various studies have suggested that plant materials which contain tannins, alkaloids, flavonoids, and phenolic acids bring out antinociceptive and anti-inflammatory effects on experimental animals[3].

Anxiety is an extremely dramatic and debilitating multifaceted disorder and it is now becoming clear that without knowledge of clinical and biological aspects of anxiety and depression, it is impossible to offer effective treatment strategies for the patients. Over the past decades, there has been intensive study of a variety of neurobiological aspects of anxiety[4].Currently the most widely prescribed medications for anxiety disorders are benzodiazepines. But the clinical applications of benzodiazepines as anxiolytics are limited by their unwanted side effects. Therefore the development of new pharmacological agents from plant sources is well justified[5]. The use of herbal medications by physicians in Europe and Asia is becoming more common and researchers are exploring the traditional remedies to find a suitable cure for this mind affecting diseases[6].

Diuretics are drugs that increase the rate of urine flow, sodium excretion and are used to adjust the volume and composition of body fluids in a variety of clinical situations. Drug that induced diuresis is beneficial in many life threatening disease conditions such as congestive heart failure, nephritic syndrome, cirrhosis, renal failure, hypertension, and pregnancy toxaemia[7]. Most diuretic drugs have the adverse effect on quality of life including impotence, fatigue and weakness. Naturally occurring diuretics include caffeine in coffee, tea, and cola, which inhibit Na+ reabsorption and alcohol in beer, wine and mixed drinks, which inhibit secretion of ADH[8, 9]. Diuretics relive pulmonary congestion and peripheral edema. These agents are useful in reducing the syndrome of volume overload, decreases cardiac workload, oxygen demand and plasma volume, thus decreasing blood pressure[10].

Herbal medicines derived from the plant extracts are being increasingly utilized to treat a wide variety of clinical diseases, though relatively little knowledge about their mode of action is available. A bibliographic survey showed that there are no systematic studies have been reported for locomotor and diuretic activities of *Capparis divaricata* Lam. This prompted us to investigate the effects of pharmacological activities of *Capparis divaricata* Lam. in experimental model of animals.

MATERIALS AND METHODS

Plant material

The fresh leaves of *Capparis divaricata* Lam. (Capparidaceae) were collected at the flowering stage in August from Sangli District, Maharashtra State, India. It was authenticated and taxonomically identified by Dr. (Mrs.) U.S. Yadav, Head, Department of Botany, Willingdon College, Sangli, India. The selected parts of the plant were then dried in shade at temperature between 21-30° for 15 to 30 days, after which these parts were chopped and ground. Finally extraction was carried out by following procedure.

Preparation of the extract

For the preparation of extract about 200 g of air dried, powdered leaves were charged in to Soxhlet's apparatus and successively extracted with 95% ethanol at room temperature for 7 days. The extract was evaporated to dryness in rotary evaporator. The yield of ethanolic extract was obtained as 4.5 % w/w. Moreover, the extract was subjected to preliminary phytochemical screening for the detection of various plant constituents [11].

Preliminary Phytochemical studies

The EEC was tested for the presence of alkaloids with Dragendorff's reagent, Mayer's reagent and Hager's reagent; flavonoids with the use of lead acetate, Mg and HCl; tannins with ferric chloride and potassium dichromate solutions; glycosides with Baljet's test and Keller-Killiani test and saponins by using standard phytochemical screening procedures[12].

Experimental animals

Swiss Albino mice (20-25 g) and Wister Albino rats (150-180 g) of either sex was used. The animals were maintained on the standard laboratory conditions (light period of 12 h/day and temperature $27^0 \pm 2^0$), with access to food and water *ad libitum*. The experimental

procedures were carried out in strict compliance with the Institutional Animal Ethical Committee Regulations. The experiments were carried out according to CPCSEA guidelines.

Acute toxicity studies

The EEC was employed for the determination of acute oral toxicity and LD_{50} (lethal dose) by using female, non pregnant mice weighing 18-20 g as per revised OECD guidelines No.425. The animals were fasted overnight and then were administered with the ethanolic extract at the following doses; 175, 550, 1750 and 5000 mg/kg by oral route. Animals were observed for their mortality during 48h study period (short term) toxicity and the final LD_{50} values were calculated as per the OECD guidelines 425[13].

Locomotor activity

The locomotor activity can be easily studied with the help of actophotometer. Male Swiss albino mice weighing between 18-25g were divided into four groups, each group comprising of six animals. Each animal was placed individually and the basal activity score of all the animals were recorded after 30, 60 and 120 min of drug treatment. The activity on each mouse was retested for 10 min. The difference in the activity was recorded considering before treatment values and after treatment values. Finally percentage decrease in locomotor activity was calculated[14, 15].

Diuretic activity

The method of Lipschitz was employed for the evaluation of diuretic activity. The male Albino rats weighing about 150 -200 g were divided into four groups of six rats in each and were fasted and deprived of food and water for 18 h prior to the experiment. On the day of experiment, the Group I animals serving as control, received normal saline (25 ml/kg, p.o), the Group II animals received ethanolic extract (250 mg/kg, p.o), Group III animals also received ethanolic extract (500 mg/kg, p.o) and the Group IV animals received Furosemide (20 mg/kg, p.o), in normal saline. Immediately after the administration the animals were kept in metabolic cages (3 per cage) specially designed to separate urine and fecal matter and kept at room temperature (25 ± 0.5ºC) throughout the experiment. The total volume of urine was collected at the end of 5 h after dosing. During this period no water and food was made available to animals[16, 17].

Statistical analysis

All data were expressed as Mean **±** S.E.M. The results were analyzed statistically by one-way ANOVA followed by Dunnett's multiple comparisons test. The results obtained were compared with the vehicle control group. P< 0.01 was considered to be statistically significant.

RESULTS

Preliminary Phytochemical studies

Preliminary phytochemical screening of the EEC revealed the presence of alkaloids, flavonoids, phenolic compounds, glycosides, tannins and saponins.

Acute toxicity studies

There was no mortality amongst the graded dose in groups of animals and they did not show any toxicity or behavioral changes at a dose level of 5000 mg/kg. This finding suggests that EEC were safe in or non-toxic to mice up to 5000 mg/kg. Hence, in our study 250 and 500 mg/kg doses of extract were selected.

Locomotor activity

The average actophotometer reading in the control group was 358 ± 2.77, after administration of EEC 250 and 500 mg/kg after 60 min significantly reduced the locomotor activity to 176.66 ± 1.02 and 142 ± 0.73 respectively. It may be due to the CNS depressant property of the drug (Table 1).

Table 1: Effect of EEC on locomotor activity (actophotometer) in mice at different time intervals (min)

Groups	Treatment	Photocell Counts			
		30 min	% Inhibition	60 min	% Inhibition
I	Control (3% Tween 80) (p.o.)	359.66 ± 1.57	--	358.00 ± 2.77	--
II	Diazepam (3 mg / kg) (p.o.)	104.66 ± 0.33**	70.90	102.66 ± 0.88**	71.32
III	EEC (250 mg / kg) (p.o.)	182.66 ± 0.72**	49.21	176.66 ± 1.02**	50.65
IV	EEC (500 mg / kg) (p.o.)	148.66 ± 1.02**	58.66	142.00 ± 0.73**	60.33

(Observation period: 10 min for all parameters) Values are expressed as mean ± SEM, from 6 mice. Significant at **P< 0.01 as compare to control using One way ANOVA followed by followed by Dunnett's *t*-test.

Diuretic activity

Oral administration of a single dose of EEC significantly (P<0.01) increased the urine output (Table 2). The reference diuretic (Furosemide), increased urine volume up to 3.5±0.04 ml. For the ethanolic extract, the increase in urine volume at the doses of 250 mg/kg body weight and 500 mg/kg body weight was 2.8±0.03 and 3.0±0.09 ml, respectively, compared to the control group which was 1.0±0.06 ml. It shows that the EEC at high doses may have equipotent diuretic activity as that of the standard drug (Furosemide).

Table 2: Effect of EEC on urinary output in rats

Group	Treatment	Volume of urine for 5 h (ml)	Urine volume deviated from control (ml)	% Diuretic activity
I	Control (Normal saline) (25 mg/kg, p.o.)	1.0±0.06	--	--
II	Standard (Furosemide) (20 mg/kg, p.o.)	3.5±0.04**	2.5	100
III	EEC (250mg/kg, p.o.)	2.8±0.03**	1.8	72
IV	EEC (500mg/kg, p.o.)	3.0±0.09**	2.0	80

Values are expressed as mean ± SEM, from 6 mice. Significant at **P< 0.01 as compare to control using One way ANOVA followed by Dunnett's *t*-test.

DISCUSSION

Preliminary phytochemical study indicated the presence of alkaloids, flavonoids, phenolic compounds, glycosides, tannins and saponins, which might be responsible for the locomotor activity and diuretic activity of the EEC.

In the present study, no mortality case was observed up to the dose of 5000 mg/ kg of EEC (p.o.). Therefore, it may suggest that the extract has no lethal toxicity in mice.

Fear and anxiety are defined as the response of a subject to real or particular threats that may impair its homeostasis; this may include physiological and/or behavioral response. Measuring anxiety like behavior in mice has been mostly undertaken using a few classical animal models of anxiety, such as the elevated plus maze, light dark model and locomotor activity by actophotometer. All these procedures are based upon the exposure of subject to unfamiliar aversive place[18].

In the present study, EEC (250 and 500 mg/kg) produced significant depressant effect in mice in actophotometer as compared to control.

GABA appears to play an important role in the pathogenesis of several neuropsychiatric disorders. Many of the traditional agents used to treat psychiatric disorders are known to act, at least in part, by enhancing GABA activity, while some of the newer agents may exert their therapeutic effects exclusively via GABAergic actions.

The effect of EEC on diuresis was accompanied by marked increase in urine volume. Previous studies have demonstrated that there are several compounds which could be responsible for the plants diuretic effects such as flavonoids, saponins or organic acids[19]. The effect may be produced by stimulation of regional blood flow or initial vasodilation[20] or by producing inhibition of tubular reabsorption of water and anions[21]. Preliminary phytochemical investigation of *Capparis divaricata* Lam. has suggested the presence of flavonoids and saponins compounds. It may be suggested that these substances might be responsible, at least in part, for the observed diuretic activity and that they may act individually or synergistically.

From the results of the present study this can be suggest that the EEC possess significant locomotor and diuretic activity. Further study is necessary to determine the mechanism of action and isolation of active principle(s) from ethanol extracts of leaves of *Capparis divaricata* Lam. for locomotor and diuretic activity.

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