SCREENING FOR ANTIDEPRESSANT-LIKE ACTIVITY OF *CONVOLVULUS PLURICAULIS* CHOISY IN MICE

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Summary

The present study was undertaken to investigate the effect of petroleum ether, chloroform and ethyl acetate fractions of total ethanolic extract of *Convolvulus pluricaulis* Choisy (Family: Convolvulaceae) on depression in mice. Petroleum ether fraction (25 and 50 mg/kg), chloroform fraction (25, 50 and 100 mg/kg) and ethyl acetate fraction (25, 50 and 100 mg/kg) were administered orally for 10 successive days in separate groups of Swiss young male albino mice. The effects of extracts on immobility periods of mice were assessed in forced swim test (FST) and tail suspension test (TST). Effects of reserpine (2 mg/kg i.p.), sulpiride (50 mg/kg i.p.), prazosin (62.5 µg/kg i.p.) and p-chlorophenylalanine (100 mg/kg i.p.) on antidepressant-like effect of the extracts in TST was also studied. The antidepressant-like effect of the extracts was compared to that of imipramine (15 mg/kg p.o.) and fluoxetine (20 mg/kg p.o.) administered for 10 successive days. Only chloroform fraction in doses of 50 and 100 mg/kg significantly reduced the immobility time of mice in both FST and TST. This fraction was without any significant effect on locomotor activity of mice. Its efficacy was found to be comparable to that of imipramine and fluoxetine administered for 10 successive days. The chloroform fraction reversed reserpine-induced extension of immobility period of mice in FST and TST. Prazosin, sulpiride and p-chlorophenylalanine significantly attenuated the chloroform fraction-induced antidepressant-like effect in TST. Chloroform fraction of total ethanolic extract of *Convolvulus pluricaulis* elicited a significant antidepressant-like effect in mice by interaction with adrenergic, dopaminergic and serotonergic systems.

Key Words: Antidepressant; *Convolvulus pluricaulis;* Forced Swim Test; Tail Suspension Test; Reserpine.

Running Head: Shankhpushpi - an effective antidepressant

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Introduction

Mood disorders are among the most prevalent forms of mental illness. Depression is a chronic illness that affects a person's mood, thoughts, physical health and behavior. Patients with major depression have changes in brain monoamine neurotransmitters, specifically norepinephrine, serotonin and dopamine [1]. Reserpine, an antihypertensive drug that depleted neuronal storage granules of norepinephrine, serotonin and dopamine, produced clinically significant depression in 15% or more of patients [2].

Convolvulus pluricaulis Choisy (Syn. *Convolvulus microphyllus* Sieb ex. Spreng) (family - Convolvulaceae) is also called as Shankhpushpi in India. It is a herb that has been used in India for hundreds of years for nervous disorders such as stress, anxiety and insomnia. It is mainly used as a rasayana which is mainly advocated for use in rejuvenation therapy. A rasayana is one which promotes longevity and prevents diseases by providing strength and immunity. It produces a feeling of peace and calm, reduces stress, anxiety and mental fatigue. The Ayurvedic system of medicine advocates its use as a brain tonic. The drug exhibited anti-anxiety and memory enhancing effects and relieved the symptoms like nervousness, palpitation, insomnia, weakness, fatigue and dyspepsia [3]. Dietary feeding of this plant increased protein synthesis of the hippocampus, thus enhancing memory and learning in experimental animals [4]. It showed reduction in the level of plasma cortisol and urinary catecholamines [5]. It is astringent, hot, aphrodisiac and a nervine tonic. It improves strength, digestive power, complexion, voice and cures intestinal worms, animal poisoning, skin disease, cough, dyspnoea, diabetes, dysuria and uterine disorder. The drug is also helpful in epilepsy, insomnia, heart disease and haemetemesis [6, 7]. The plant has been found to be effective in reducing different types of stress including psychological, chemical and traumatic [8]. The ethanolic and methanolic extracts of the whole plant reduced spontaneous motor activity, potentiated pentobarbitone hypnosis and morphine analgesia, reduced fighting response, abolished the conditioned avoidance response, antagonized convulsive seizures and tremorine induced tremors in mice [9, 10]. Alcoholic extract of *Convolvulus pluricaulis* possessed antifungal activity [11]. The juice of whole plant prevents excessive menstruation. The fine paste made by grinding the plant is helpful to cure abscess [12]. Ethanolic extract of whole plant when administred to cholesterol fed gerbils, reduced serum cholesterol, LDL cholesterol, triglycerides and phospholipids significantly after 90 days [13]. The root extract of this plant regulated hyperthyroidism in female mice [14]. The juice of fresh

whole plant of *Convolvulus pluricaulis* possessed anti-ulcerogenic effect and is comparable to sucralfate [15]. Ethanolic extract of the entire plant exerted a negative inotropic action on amphibian and mammalian myocardium. It also exerted spasmolytic activity on smooth muscles [16].

The plant contains alkaloid shankhpushpine, volatile oil, flavonoid-kampferol, phytosterol, β-sitosterol, carbohydrates-glucose, rhamnose, starch, ceryl alcohol and scopoletin [17]. The chloroform fraction of this plant contains 20-oxodotriacontanol, tetratriacontanoic acid and 29-oxodotriacontanol. These components were proved to be potent insect antifeedant constituents [18].

The present study was undertaken (i) to study the effect of the petroleum ether, chloroform, ethyl acetate fractions of total ethanolic extract of whole plant of *Convolvulus pluricaulis* (CP) on depression in mice employing forced swim test and tail suspension test and (ii) to explore the possible underlying mechanisms of antidepressant-like activity of these different fractions. Standard antidepressant drugs like fluoxetine, a selective serotonin reuptake inhibitor, and imipramine, a tricyclic antidepressant were employed to standardize the animal models of depression and to compare the antidepressant efficacy of the fractions. (±) Sulpiride (a D_2 - receptor antagonist), prazosin (an α_1-adrenoceptor antagonist), p-chlorophenylalanine (a serotonin synthesis inhibitor) and reserpine were used to evaluate the probable mechanisms of antidepressant-like effect of different fractions in mice.

Materials and Methods

Animals:

Swiss male albino mice (3 months old and weighing around 25 g) were procured from Disease Free Small Animal House, CCS Haryana Agricultural University, Hisar (Haryana, India). There is not any significant effect of sex variation of mice in the induction of depression models [19]. Animals had free access to food and water, and were maintained under standard laboratory conditions with alternating light and dark cycles of 12 h each. The food was withdrawn 1hr before and 2hr after administration of drugs to mice. The animals were acclimatized for at least 5 days before behavioral experiments. Experiments were carried out between 10:00 to 17:00 h.

Institutional Animals Ethics Committee (IAEC) approved the experimental protocol and care of laboratory animals was taken as per the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Govt. of India.

Plant Material, Drugs and Chemicals:

The dried whole plant of Shankhpushpi was purchased from commercial source and authenticated as *Convolvulus pluricaulis* Choisy (Syn. *Convolvulus microphyllus* Sieb ex. Spreng) by Raw materials, Herbarium and Museum, National Institute of Science Communication and Information Resources, New Delhi, India (Voucher specimen no. NISCAIR/RHM/F-3/2005/Consult/534/09). The dried whole plant was crushed to make coarse powder.

Fluoxetine hydrochloride (Fludac®, Cadila Pharmaceuticals, Ahmedabad, India), reserpine (Otto Kemi, Mumbai, India), acetic acid glacial (Central Drug House (P) Ltd., New Delhi, India), sodium hydroxide pellets (Hi-Media, Mumbai, India), Tween 80 (Loba Chemie, Mumbai, India), (±) sulpiride, prazosin hydrochloride, DL-p-chlorophenylalanine, imipramine hydrochloride (Sigma-Aldrich, St. Louis, USA) were used in the present study.

Preparation of Different Fractions of Total Ethanolic Extract of *Convolvulus pluricaulis* (CP):

About 1 kg dried coarse powder of *Convolvulus pluricaulis* Choisy was imbibed in ethanol (95%) for 24 h. This moistened drug was extracted with ethanol (95%) at 70^0C using Soxhlet Apparatus for 48 h. The ethanolic extract was concentrated using vacuum and stored in a refrigerator. The yield of total ethanolic extract was 24.3%. About 30 g of total ethanolic extract was suspended in ethanol (95%) and further fractionated into three fractions using three different solvents. Firstly, the total ethanolic extract was extracted three times with petroleum ether (40:60) using separating funnel. The upper petroleum ether layer was collected and evaporated to dryness using vacuum. The lower layer of remaining ethanolic extract was extracted three times with chloroform. Lower chloroform layer was collected and evaporated to dryness using vacuum. Remaining upper layer was extracted three times with ethyl acetate. The upper ethyl acetate layer was collected and evaporated to dryness using vacuum. The remaining lower layer

was discarded. The yields of petroleum ether, chloroform and ethyl acetate fractions were 17.5%, 17.8% and 15.9% respectively.

Vehicle:

The petroleum ether, chloroform, ethyl acetate fractions of total ethanolic extract were emulsified separately in 10% v/v Tween 80. Fluoxetine, imipramine and prazosin were dissolved separately in normal saline (0.9% sodium chloride). Sulpiride was dissolved in normal saline followed by addition of one drop of glacial acetic acid. p-Chlorophenylalanine (p-CPA) was dissolved in minimum quantity of 0.1 N sodium hydroxide and pH was adjusted to 7 with 0.1 N hydrochloric acid. Reserpine (2 mg) was dissolved in a drop of acetic acid glacial. The volume was made up with distilled water, giving a solution with pH 3.3. The pH was adjusted with 5 drops of 1 N sodium hydroxide, giving a solution with pH 5.4. The volume for oral administration and intraperitoneal injection was 1 ml/100 g of mouse.

Tests for evaluating antidepressant activity:

(i) Forced Swim Test (FST):

Forced swim test was proposed as a model to test for antidepressant activity by Porsolt et al [20]. The procedure was essentially the same as followed earlier (21, 22). Mice were forced to swim individually in a glass jar ($25 \times 12 \times 25$ cm^3) containing fresh water of 15 cm height and maintained at 25°C (± 3°C). After an initial 2 min period of vigorous activity, each animal assumed a typical immobile posture. A mouse was considered to be immobile when it remained floating in the water without struggling, making only minimum movements of its limbs necessary to keep its head above water. The total duration of immobility was recorded during the next 4 min of a total 6 min test. The changes in immobility periods were studied after administering drugs in separate groups of animals. Each animal was used only once.

(ii) Tail Suspension Test (TST):

The total duration of immobility induced by tail suspension was measured according to the method described as a means of evaluating potential antidepressants [21, 22, 23].

Mice were suspended on the edge of a table 50 cm above the floor by the adhesive tape placed approximately 1 cm from the tip of the tail. Immobility time was recorded during a 6 min period [24]. Animal was considered to be immobile when it did not show any movement of body and hanged passively.

Locomotor Activity:

Locomotor function of mice was studied using photoactometer (INCO, Ambala, India) to rule out the increase in locomotor performance of mice due to the extract. Photoactometer operates on photoelectric cells which are connected in circuit with a counter. When the beam of light falling on the photocell is cut off by the animal, a count is recorded. A photoactometer has rectangular arena in which the animal moves. Each animal is placed individually in the activity cage for 10 min. Locomotor activity scores of animals are measured before and after treatment with the extract.

Drug Protocol:

Animals were divided into 34 groups and each group comprised of a minimum of 5 animals.

Animal Groups for Forced Swim Test (FST):

Group I (n = 7): Control group (vehicle treated): 10% v/v Tween 80 was administrated orally for 10 successive days. At 90 min after administration on 10^{th} day, immobility period was recorded.

Group II (n = 6): Control group (vehicle treated): Normal saline was administrated orally for 10 successive days. At 90 min after administration on 10^{th} day, immobility period was recorded.

Group III (n = 6): Fluoxetine (20 mg/kg) was administrated orally for 10 successive days. At 90 min after administration on 10^{th} day, immobility period was recorded.

Group IV (n = 5): Imipramine (15 mg/kg) was administrated orally for 10 successive days. At 90 min after administration on 10^{th} day, immobility period was recorded.

Group V and VI (n = 6 each): Petroleum ether fraction of CP (25 and 50 mg/kg, respectively) was administered orally for 10 successive days. At 90 min after administration on 10^{th} day, immobility period was recorded.

Group VII, VIII and IX (n = 7, 6, 6 respectively): Chloroform fraction of CP (25, 50 and 100 mg/kg, respectively) was administered orally for 10 successive days. At 90 min after administration on 10th day, immobility period was recorded.

Group X, XI and XII (n = 7 each): Ethyl acetate fraction of CP (25, 50 and 100 mg/kg, respectively) was administered orally for 10 successive days. At 90 min after administration on 10th day, immobility period was recorded.

Animal Groups for Tail Suspension Test (TST):

Group I to Group XII are same as for forced swim test (FST) except that immobility period was recorded using TST.

Animal Groups for Studying Probable Mechanism of Action using Tail Suspension Test (TST):

Group I (n = 7): Control group (vehicle treated): same as group I for TST.

Group II (n = 6): Chloroform fraction (100 mg/kg): same as group IX for TST.

Group III (n = 6 each): 10% v/v Tween 80 (vehicle) was administered orally for 10 successive days and then sulpiride (50 mg/kg, i.p.) was injected on 10th day after 45 min of last oral administration of vehicle. The animals were subjected to TST after 45 min of sulpiride injection.

Group IV (n = 6): Chloroform fraction of CP (100 mg/kg) was administered orally for 10 successive days and then sulpiride (50 mg/kg, i.p.) was injected on 10th day after 45 min of last oral administration of chloroform fraction. The animals were subjected to TST after 45 min of sulpiride injection.

Group V (n = 6): 10% v/v Tween 80 (vehicle) was administered orally for 10 successive days and then prazosin (62.5 µg/kg, i.p.) was administered on 10th day after 45 min of last vehicle administration. The animals were subjected to TST after 45 min of prazosin injection.

Group VI (n = 6): Chloroform fraction of CP (100 mg/kg) was administered orally for 10 successive days and then prazosin (62.5 µg/kg, i.p.) was administered on 10th day after 45 min of

last oral administration of chloroform fraction. The animals were subjected to TST after 45 min of prazosin injection.

Group VII (n = 6): 10% v/v Tween 80 (vehicle) was administered orally for 10 consecutive days. p-CPA (100 mg/kg, i.p.) was administered from 7^{th} day to 10^{th} day after 45 min of vehicle administration. The animals were subjected to TST after 45 min of p-CPA injection on 10^{th} day.

Group VIII (n = 6): Chloroform fraction of CP (100 mg/kg) was administered orally for 10 consecutive days. p-CPA (100 mg/kg, i.p.) was administered from 7^{th} day to 10^{th} day after 45 min of each oral administration of chloroform fraction. The animals were subjected to TST after 45 min of p-CPA injection on 10^{th} day.

Group IX (n = 6): Fluoxetine (20 mg/kg): same as group III for TST.

Group X (n = 6): Fluoxetine (20 mg/kg) was administered orally for 10 successive days to mice. From 7^{th} day to 10^{th} day, p-CPA (100 mg/kg, i.p.) was administered after 45 min of injection of fluoxetine. The animals were subjected to TST after 45 min of p-CPA injection on 10^{th} day.

Animal Groups for Studying Effect of chloroform fraction of CP on reserpine-induced extension of immobility period using Tail Suspension Test (TST):

Group I (n = 7): Control group (vehicle treated): same as group I for TST.

Group II (n = 6): 10% v/v Tween 80 (vehicle) was administered orally for 10 successive days. Reserpine (2 mg/kg) was injected i.p. on 10^{th} day. After 3 h of reserpine injection, vehicle was administered orally. After 60 min of vehicle administration, animals were subjected to TST. Mice were again tested in TST after 24 h of reserpine injection.

Group III (n = 6): Chloroform fraction of CP (100 mg/kg) was administered orally for 10 successive days. Reserpine (2 mg/kg) was injected i.p. on 10^{th} day. After 3 h of reserpine injection, chloroform fraction was administered orally. After 60 min of administration of the fraction, animals were subjected to TST. Mice were again tested in TST after 24 h of reserpine injection.

Animal Group for Studying Effect of chloroform fraction of CP on Locomotor Activity of Mice (n = 6):

Effect of chloroform fraction of CP (100 mg/kg, p.o.) on locomotor activity of mice was studied using photoactometer (INCO, Ambala, India). The difference in the locomotor activity scores were noted before and after administration of chloroform fraction.

Statistical analysis

All results are expressed as mean ± standard error of mean (SEM). Data were analyzed by one-way ANOVA followed by Dunnett test. The data for locomotor activity scores was subjected to Student's paired t-test. In all the tests, the criterion for statistical significance was $P < 0.05$.

Results

Effects of different fractions of total ethanolic extract of CP on immobility periods in TST and FST:

Petroleum ether fraction (25 and 50 mg/kg, p.o.) and ethyl acetate fraction (25, 50 and 100 mg/kg, p.o.) administered for 10 successive days to mice did not show any significant effect on the immobility periods in FST and TST. Lower dose of chloroform fraction (25 mg/kg, p.o.) did not show any significant effect on immobility period in FST and TST. On the other hand, the higher doses (50 and 100 mg/kg p.o.) of this fraction decreased the immobility periods significantly as compared to control group in both TST and FST, indicating significant antidepressant-like activity. Fluoxetine (20 mg/kg, p.o.) and imipramine (15 mg/kg, p.o.) administered for 10 successive days significantly reduced the immobility period as compared to saline treated control in both FST and TST (Tables- 1 and 2).

Reserpine (2 mg/kg, i.p.) induced significant increase in immobility period after 4 h and 24 h of its administration in TST. Chloroform fraction (100 mg/kg) administered orally for 10 successive days significantly reversed the reserpine-induced extension of immobility period after 4 h and 24 h as compared to reserpine alone (Fig-1).

Table - 1

Effect of different fractions of total ethanolic extract of *Convolvulus pluricaulis* (CP) on immobility period of mice using Forced Swim Test (FST)

Group No.	Treatment for 10 days p.o.	Number of animals	Dose (kg^{-1})	Immobility Period (sec) ± SEM
I	Vehicle (10% v/v Tween 80)	7	10 ml	176.7 ± 3.9
II	Vehicle (Saline)	6	10 ml	175.5 ± 3.5
III	Fluoxetine	6	20 mg	149.8 ± 2.7*
IV	Imipramine	5	15 mg	138.4 ± 3.3*
V	Pet ether fraction	6	25 mg	174.2 ± 2.4
VI	Pet ether fraction	6	50 mg	171.5 ± 2.6
VII	Chloroform fraction	7	25 mg	168.3 ± 4.4
VIII	Chloroform fraction	6	50 mg	93.7 ± 3.2**
IX	Chloroform fraction	6	100 mg	94.8 ± 5.0**
X	Ethyl acetate fraction	7	25 mg	186.3 ± 4.9
XI	Ethyl acetate fraction	7	50 mg	184.4 ± 6.5
XII	Ethyl acetate fraction	7	100 mg	195.6 ± 4.4

Values are in Mean ± SEM

Statistical analysis of data was carried out by one-way ANOVA followed by Dunnett test.

$F(11, 64) = 62.73$; $p < 0.0001$

* $p < 0.05$ when compared with vehicle group (saline)

** $p < 0.05$ when compared with vehicle group (10% v/v Tween 80)

Table - 2

Effect of different fractions of total ethanolic extract of *Convolvulus pluricaulis* (CP) on immobility period of mice using Tail Suspension Test (TST)

Group No.	Treatment for 10 days p.o.	Number of animals	Dose (kg^{-1})	Immobility Period (sec) ± SEM
I	Vehicle (10% v/v Tween 80)	7	10 ml	195.7 ± 4.3
II	Vehicle (Saline)	6	10 ml	193.3 ± 2.6
III	Fluoxetine	6	20 mg	149.2 ± 6.7*
IV	Imipramine	5	15 mg	148.2 ± 3.3*
V	Pet ether fraction	6	25 mg	212.5 ± 1.8
VI	Pet ether fraction	6	50 mg	211.3 ± 2.8
VII	Chloroform fraction	7	25 mg	181.9 ± 5.4
VIII	Chloroform fraction	6	50 mg	110.8 ± 3.1**
IX	Chloroform fraction	6	100 mg	101.8 ± 5.1**
X	Ethyl acetate fraction	7	25 mg	192.6 ± 8.6
XI	Ethyl acetate fraction	7	50 mg	202.6 ± 6.6
XII	Ethyl acetate fraction	7	100 mg	214.0 ± 4.3

Values are in Mean ± SEM; Statistical analysis of data was carried out by one-way ANOVA followed by Dunnett test. $F(11, 64) = 55.42$; $p < 0.0001$

$p < 0.05$ when compared with vehicle group (saline);

** $p < 0.05$ when compared with vehicle group (10% v/v Tween 80)

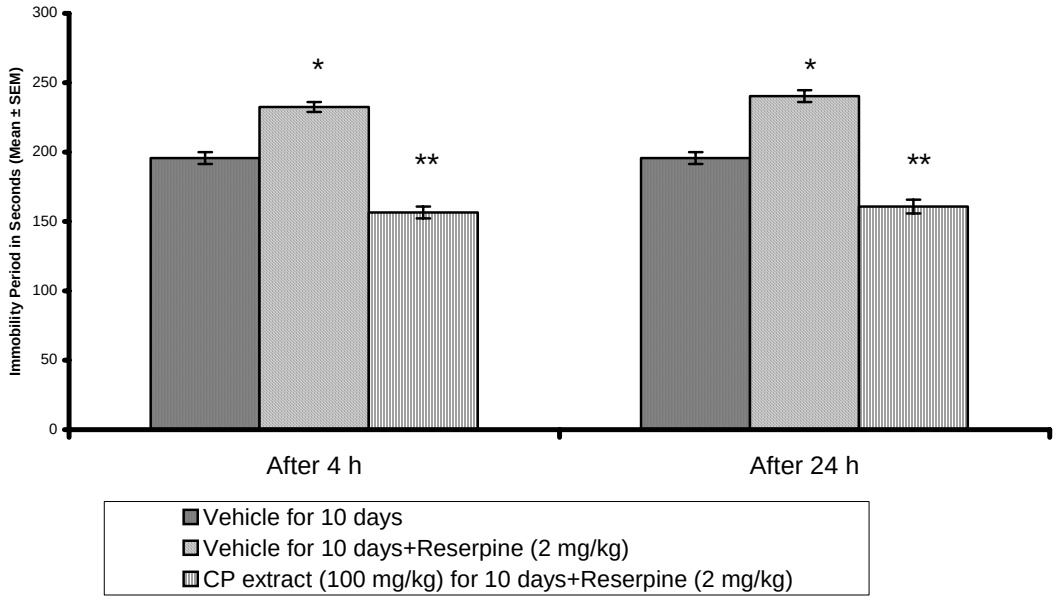

Fig. 1: Effect of chloroform fraction of total ethanolic extract of *Convolvulus pluricaulis* (CP) on reserpine-induced extension of immobility period in TST

Statistical analysis of data was carried out by one-way ANOVA followed by Dunnett test.

After 4 h; $F(2, 16) = 82.88$; $p < 0.0001$

After 24 h; $F(2, 16) = 74.34$; $p < 0.0001$

*$p < 0.01$ when compared with control group (vehicle treated group).
**$p < 0.01$ when compared with respective vehicle + reserpine group.

Effect of combination of chloroform fraction with sulpiride, prazosin and p-CPA on immobility period in TST:

Sulpiride (50 mg/kg, i.p.), prazosin (62.5 mg/kg, i.p.) and p-CPA (100 mg/kg, i.p.) alone significantly increased the immobility period as compared to control group. Pretreatment of

animals with sulpiride or prazosin or p-CPA significantly blocked the decrease of immobility period elicited by chloroform fraction (100 mg/kg, p.o.). Pretreatment of mice with p-CPA significantly blocked the decrease of immobility period by fluoxetine (Table-3).

Table-3

Effect of combination of chloroform fraction of total ethanolic extract of *Convolvulus pluricaulis* (CP) with sulpiride, prazosin and p-CPA on immobility period in TST

Group No.	Treatment for 10 days p.o.	Number of animals	Dose (kg^{-1})	Immobility Period (sec) ± SEM
I	Vehicle (10% v/v Tween 80)	7	10 ml	195.7 ± 4.3
II	Chloroform fraction	6	100 mg	101.8 ± 5.1*
III	Vehicle + Sulpiride	6	10ml 50 mg	235.2 ± 4.6*
IV	Chloroform fraction + Sulpiride	6	100mg 50 mg	225.7 ± 5.0**
V	Vehicle + Prazosin	6	10ml 62.5 µg	231.2 ±3.7*
VI	Chloroform fraction + Prazosin	6	100mg 62.5 µg	129.0 ±3.6**
VII	Vehicle + p-CPA	6	10ml 100 mg	215.7 ± 3.6*
VIII	Chloroform fraction + p-CPA	6	100mg 100 mg	205.7 ± 2.5**
IX	Fluoxetine	6	20 mg	149.2 ± 6.7*
X	Fluoxetine + p-CPA	6	20mg 100 mg	195.0 ± 3.8***

Values are in Mean ± SEM.

Statistical analysis of data was carried out by one-way ANOVA followed by Dunnett test.

$F(9, 51) = 107.76$; $p < 0.0001$

* $p < 0.05$ as compared to vehicle

** $p < 0.05$ as compared to chloroform fraction

*** $p < 0.05$ as compared to fluoxetine alone

Effect on locomotor activity:

Chloroform fraction (100 mg/kg, p.o.) administered for 10 successive days did not show any significant change in the locomotor function of mice (266.8 ± 8.4) as compared to the control (267.5 ± 7.3).

Discussion

In the present study, only chloroform fraction (50 and 100 mg/kg) of total ethanolic extract produced significant antidepressant-like effect in mice in both TST and FST and its efficacy was found to be comparable to fluoxetine and imipramine. Both the models of depression are widely used to screen new antidepressant drugs [20-23]. These tests are quite sensitive and relatively specific to all major classes of antidepressant drugs [20, 23, 25]. In TST, immobility reflects a state of despair which can be reduced by several agents which are therapeutically effective in human depression. Similarly in the FST, mice are forced to swim in restricted space from which they cannot escape. This induces a state of behavioral despair in animals, which is claimed to reproduce a condition similar to human depression [26]. It has been seen that the TST is less stressful and has higher pharmacological sensitivity than FST [27].

The chloroform fraction of CP did not show significant change in locomotor function of mice as compared to control so it did not produce any motor effect. It confirms the assumption that the antidepressant-like effect of the fraction is specific. The precise mechanisms by which chloroform fraction produced antidepressant-like effect are not completely understood. However according to our results, the antidepressant-like effect of the fraction was significantly reversed by the pretreatment of animals with prazosin (an α_1-adrenoceptor antagonist), sulpiride (a selective dopamine D_2-receptor antagonist) and p-CPA (a serotonin synthesis inhibitor) when tested in TST. This suggests that chloroform fraction might produce antidepressant-like effect by increasing the levels of norepinephrine, dopamine and serotonin in brains of mice. p-CPA significantly reversed the antidepressant effect of fluoxetine (a specific serotonin reuptake inhibitor) in TST, suggesting that fluoxetine has antidepressant effect through serotonergic system. Reserpine produces significant depression by depleting biogenic amines

(norepinephrine, 5-hydroxytryptamine and dopamine) in the brains of rodents. Reserpine (2mg/kg, i.p.) produced significant increase in immobility period after 4 h and 24 h of its treatment when tested in TST. Since chloroform fraction reversed reserpine-induced depression, as indicated by decrease in extension of immobility period in TST, therefore, this suggests that antidepressant-like effect of chloroform fraction might be through the restoration of brain monoamines, like norepinephrine, 5-hydroxytryptamine and dopamine levels. *Convolvulus pluricaulis* showed reduction in the level of plasma cortisol [5]. High levels of cortisol are associated with depressed mood in humans [28]. Thus it might be possible that antidepressant-like effect of chloroform fraction might be due to reduction in plasma cortisol levels. Thus, chloroform fraction of total ethanolic extract of *Convolvulus pluricaulis* may have potential therapeutic value for the management of depressive disorders.

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