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CNS depressant activity of extracts from *Flaveria trinervia* Spring C. Mohr.

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Abstract

The methanolic and aqueous extracts of *Flaveria trinervia* were screened for CNS depressant activity by forced swim, actophotometer and rotarod methods using mice. Imipramine and chloropromazine hydrochloride were used as the standard references. Forced swim test revealed that the animals treated with the extracts showed decrease in their immobility times, which was significant when compared with the control. Locomotor activity test by actophotometer revealed that among both the extracts, methanol extract showed more significant effect. Motor coordination test revealed that the methanol and aqueous extracts exhibited marked reduction in motor coordination in mice after an oral administration. But aqueous extracts showed significant effect. However, the standard treated group revealed a statistically significant decrease in the immobility time and motor coordination activity as compared with the control. *F. trinervia* extracts showed a significant depression pattern revealing their neuropharmacological effect.

Keywords: *Flaveria trinervia*, forced swim, actophotometer, rotarod, CNS depressant activity.

Introduction

CNS depression is considered as an affective disorder characterized by change in mood, lack of interest in the surroundings, apathy, loss of energy, psychomotor retardation, melancholia as well as profound feelings of gloominess, despair and suicidal ideation. The prevalence of CNS depression in general population is estimated to be around 5% and is recognized to be symptomatically, psychologically and biologically heterogeneous (Thase et al., 1995). This disorder was characterized by retardation of thinking and activity. In spite of the availability of CNS depressant and antidepressant drugs, depression or anxiety continue to be a major medical problem (Yu et al., 2002). At present 121 million people are estimated

to suffer from depression (WHO 1998, Stahl et al., 1998, Richelson et al., 2001). Despite the development of new molecules for pharmacotherapy of depression, it is unfortunate that this disorder goes undiagnosed and untreated in many patients. Interestingly, angiotensin converting enzyme inhibitors like captopril (Giardina et al., 1989), perindopril (Martin et al., 1990) and ceronapril (Gard, 2002) have been reported to possess antidepressant activity in experimental animals. Although the currently prescribed molecules provide some improvement in the clinical condition of patients, it is at a cost of having to bear the burden of their adverse effects (Tripathi, 2008, Hardman et al., 2007).

Basic neuroscience offers the promise of improving our understanding of disease pathophysiology, identifying novel mechanisms that can be targeted by more effective pharmacotherapies and screening of herbal sources of drugs. These considerations implicate the search for new CNS depressant and antidepressant agents that have a fast onset of action, with less side effects and a wider safety margin. Various plants are being used in complementary and alternative medicines for management of mood disorders (Santosh et al., 2011).

Ayurveda, the Indian traditional system of medicine, mentions a number of single and compound drug formulations of plant origin that are used in the treatment of psychiatric disorders (Sembulingam et al., 1997; Tripathi, 2008). Several herbal products are available all over the world with an acclaimed CNS depressant and antidepressant activity, which are considered to be less toxic and free from side effects. *Flaveria trinervia* Spring C. Mohr (Asterecae) population grows only in alkaline soil [pH 7.2-8.2], mainly in marshy land near Chitradurga Dist, Karnataka State, India. This plant is locally referred as Bellary halabu or katthe kivi gida. Traditionally it is used as a promising analgesic agent in Karnataka state, India and it has also shown its action on central nervous system in mice model. In the present investigation, methanolic and aqueous extracts of *F. trinervia* were screened for CNS depressant activity by actophotometer, rotarod and forced swim methods.

Material and Methods

Plant resource and extract

F. trinervia herb was collected from the agricultural fields near Chitradurga city of Karnataka, India. Plant was authenticated by Dr. Manjunatha by comparing with the voucher specimen deposited at Kuvempu University herbarium specimen FDD-No. 53 (Manjunatha et al., 2004). The fresh whole plant material was shade dried, powdered mechanically and was subjected for soxhlet extraction using methanol as solvent system for about 48 h followed by distilled water with 5% ethanol successively. The extract was filtered and concentrated in vacuum under reduced pressure using rotary flash evaporator (Buchi, Flawil, Switzerland). The extracts were allowed for complete evaporation of the solvent. Methanolic and aqueous extracts were vacuum dried.

Animals

Male Swiss albino mice weighing between 20 – 25 g were used for the present study. The animals were maintained under standard environmental conditions (25 + 2° C and

relative humidity of 45 to 55%) and were fed with standard pellet diet and water *ad libitum.* The study was approved by Institutional Animal Ethics Committee (Reg.No.SETCP/IAE-C/2010-11/165). CPCSEA guidelines were adhered during the maintenance and experiment.

Drugs & chemicals

Chloropromazine hydrochloride (FLUDAC®, Cadila Pharmaceuticals, Ahmedabad, India) and imipramine (M/s.Alkem Ltd.Mumbai) were used reference standards for CNS depressant activity.

Experimental protocols

Overnight fasted animals were selected randomly for the experimentation. The animals were acclimatized one hour before for behavioral tests. 1 h time interval between drug administration and behavioral tests were maintained during oral administrations. The animals were divided into four groups of six mice each. Group I was maintained as control group and received 2% DMSO in distilled water, orally. Group II maintained as standard and received imipramine commercial drug for forced swim test at the dose of 10 mg/kg and chloropromazine hydrochloride (10 mg/kg) for rotarod and actophotometer test, orally. Group III animals were administered with methanol extract in 2% DMSO at the dose of 50 mg/kg, orally. Group IV animals received aqueous extract in distilled water 100 mg/kg, orally.

Behavioral test

Forced swim test (FST)

Forced swim test, the most frequently used behavioral model for screening CNS depressant like activity in rodents as proposed by Porsolt et al. (1977). The procedure was same as followed previously. Mice were individually forced to swim in open glass chamber (25 × 15 × 25cm) containing fresh water to a height of 15 cm and maintained at 26 ± 1°C. At this height of water, animals were not able to support themselves by touching the bottom or the side walls of the chamber with their hind paws or tail. Water in the chamber was changed after subjecting each animal. Each animal showed vigorous movement during initial 2 min period of the test. The duration of immobility was manually recorded during the next 4 min of the total 6 minutes testing period. Mice were considered to be immobile when they ceased struggling and remained floating motionless in water, making only those movements necessary to keep their head above water. Following swimming session, mice were towel dried and returned to their housing conditions (Dunham et al., 1957; Dhingra et al., 2006).

Test for locomotor activity

The spontaneous locomotor activity of each mouse was recorded individually for 10 min using actophotometer. The methanol and aqueous extracts of *Flaveria trinervia* whole plant was administered 60 minutes before the test and the standard drug chloropromazine hydrochloride was given 60 min before the test. The control group was treated with 2% DMSO orally, 60 min before test (Dhingra et al., 2006).

Muscle co-ordination test

This test was carried out using rotarod apparatus. The rotarod apparatus consists of a metal rod (3 cm diameter) coated with rubber attached to a motor with the speed adjusted to 20 rotations per minutes. The rod was 45 cm in length and is divided into 3 sections by metallic discs, allowing the simultaneous test of 3 mice. The rod is in a height of about 50 cm above the table top in order to discourage the animals from jumping off the roller. Cages below the section serve to restrict the movements of the animals when they fall from the roller Swiss albino mice underwent a pretest on the apparatus. Only those animals, which had demonstrated their ability to remain on the revolving rod (20 rpm) for 5 min, were used for the test. The animals were treated in the same way as mentioned under inclined plane test (Porsolt et al., 1977).

Statistical analysis

All the data of antidepressant activity was expressed as mean ± S.E.M of six animals in each group. The statistical analysis was carried out using one way ANOVA followed by Tukey's *t*-test. All analyses were performed using the ezANOVA statistical software. The difference in the values at $p \leq 0.01$ was considered as statistically significant.

Results

General pharmacological observation

Mice orally treated with the *Flaveria trinervia* whole plant methanol and aqueous extract (50 and 100 mg/kg) and were submitted to the general observations, which did not show any difference in their behavioral patterns as determined during the observation periods. They were alert with normal grooming, touch response and pain response. Alertness, limb tone and grip strength were normal and the animals did not show staggering gait or contractions.

Forced swim test (FST)

The possible CNS depressant effect of methanol and aqueous extract of *F. trinervia* after oral administration was studied by the forced swimming test. In this test (Figure 1), animals treated with the extracts showed decrease in their immobility times, which was significant (109.5 ± 5.36 and 129.33 ± 3.97, respectively) when compared with the control group (168.33 ± 1.84). Similarly, animals treated with the standard drug imipramine (10 mg/kg), as expected showed a significant decrease in their immobility time (51.5 ± 2.79).

Test for locomotor activity

The methanol and aqueous extracts of *F. trinervia* (50 and 100 mg/kg) showed a significant effect on the locomotor activity as determined by the actophotometer performance. But among them, methanol extract showed significant effect (33.17%) when

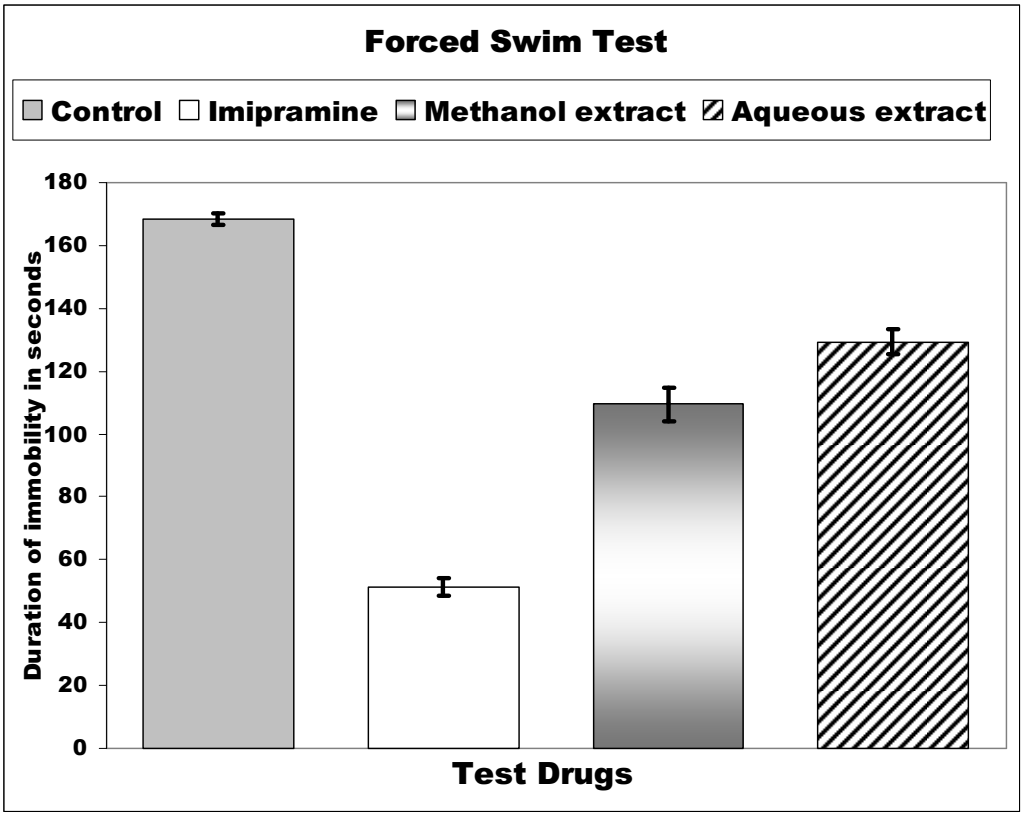

Figure 1. Effects of methanol and aqueous extracts of *F. trinervia* on the duration of immobility in the forced swim test.

compared with the aqueous extract (18.06%). These extracts also showed the onset and duration of reduction of locomotor activity. However, the chloropromazine hydrochloride revealed a statistically significant decrease in motor coordination activity (93.69) as compared with the control group (1.02%), this negligible percentage observed in the control group may be due to the effect of 2% DMSO treatment (Table 1).

Muscle co-ordination test

Both the extracts of *Flaveria trinervia* showed significant effect on the motor coordination as determined by the rotarod performance, which revealed a significant decrease in the spontaneous motor activity in mice. This effect was observed after 1h of drug administration. Results of motor coordination test revealed that the methanol and aqueous extracts also exhibited marked reduction in motor coordination in mice after an oral administration. But among them, aqueous extracts (51.71%) showed significant effect than methanol extract (49.03%). However, the chloropromazine hydrochloride treated group revealed a statistically significant decrease in motor coordination activity (97.56%) as compared with the control (2.11%) this negligible percentage observed in the control group may be due to the effect of 2% DMSO treatment (Table 2).

Discussion

There is high rate of incidence due to anxiety and depression in the community and this is directly or indirectly associated with morbidity and to some extent mortality. Hence, a-

Table 1. Effects of methanol and aqueous extracts of *F. trinervia* on locomotor activity by actophotometer.

Groups	Test Drug	Dose	Mean reaction time before drug administration (sec)	Mean reaction time after drug administration (sec)	% Decrease in time
Group I	Control	2% DMSO	765.5 ± 12.75	757.67 ± 17.38	1.02%
Group II	Standard	10 mg/kg	719.17 ± 17.67	45.33 ± 6.37 **	93.69%
Group III	Methanol extract	50 mg/kg	868 ± 40.10	580 ± 17.10 **	33.17%
Group IV	Aqueous extract	100 mg/kg	846.17 ± 42.81	693.33 ± 65.5 *	18.06%

Immediate attention towards addressing these problems and find effective remedies is important. Though various pharmaceutical industries have come up with several drugs for these disorders, these synthetic drugs are usually associated with some limitations and there is an urgent need for alternative medications to combat with these disorders. The most widely used animal models for CNS depressant screening are the forced swimming test, actophotometer based locomotion test and rotarod based muscle co-ordination tests. These tests are quite sensitive and relatively specific to all major classes of CNS depressants (Porsolt et al., 1977).

The CNS depressant activity of the methanol and aqueous extracts of *F. trinervia* revealed significant depression pattern in forced swim test, test for locomotor activity and muscle co-ordination test in mice. The reduced locomotor activity assessed by actophotometer and the decrease in grip by rotarod was found to be extract-dependent. Decrease on locomotion reveals depression effect on CNS (Leewanich et al., 1996). The CNS depressant activity may be due to the increase in the concentration of GABA in brains (Nagarjun et al., 2003). In the present study, methanolic and aqueous extracts (50 mg/kg and 100 mg/kg p.o.) administered to mice, produced significant CNS depressant activity. Results

Table 2. Effects of methanol and aqueous extracts of *F. trinervia* on muscle coordination activity by rotarod.

Groups	Test Drug	Dose	Mean reaction time before drug administration (sec)	Mean reaction time after drug administration (sec)	% Decrease in time
Group I	Control	2% DMSO	605.50 ± 11.15	592.67 ± 16.88	2.11%
Group II	Standard	10 mg/kg	644.17 ± 18.13	15.67 ± 1.76 **	97.56%
Group III	Methanol extract	50 mg/kg	635.67 ± 26.65	324 ± 29.88 **	49.03%
Group IV	Aqueous extract	100 mg/kg	799 ± 16.10	385.83 ± 12.34 **	51.71%

showed that the administration of the methanol and aqueous extracts produced a diminution of immobility time of mice exposed to the forced swimming test. However both the extracts could spontaneously depress the animals in locomotor and muscle coordination tests, but their efficacies were found to be less as compared to standard drug imipramine (10 mg/kg, p.o.) and chloropromazine (10 mg/kg, p.o.). The extracts of *F. trinervia* showed a significant decreased in the immobility, locomotor activity and muscle coordination activity in mice indicates central nervous system depressant effect (Morais et al., 1998). On the basis of results obtained from this investigation, we can conclude that the extracts of *F. trinervia* have neuropharmacological activity as evident by significant reduction in immobility time, motor activity and muscle coordination. It is logical to suggest that it may be useful as CNS depressant agent in clinical conditions. Present work was a preliminary effort which will require further detailed investigation including characterization of active compounds and requires preformulation studies for development of a potential dosage form

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