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Original Article

ANXIOLYTIC AND ANTI-DEPRESSANT ACTIVITIES OF ETHANOLIC EXTRACTS OF JASMINUM SAMBAC, CHAMOMILLA CAPITULA, LILIUM CANDIDUM, SORGHUM HALPENSE FLOWERS

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ABSTRACT

The objective of the present study to evaluate the anxiolytic and antidepressant activities of ethanolic extract of Jasminum Sambac, Chamomilla Capitula, Lilium Candidum, Sorghum Helpense Flowers using elevated plus maze, actophotometer, Froced swim test and Tail suspension test in mice. Albino mice were treated at a dose of 200 and 400 mg/kg I.P and behavior was observed on these models. Results in elevated plus-maze have showed that both the number of open arm entries and time spent in the open arms were significantly increased in case of the animals treated with combination of flower extracts that is EEJSLC compare to the animals treated individuals i.e. EEJs, EELc, EESh and EECc thereby producing anti-anxiety activity where as Locomotors activity is considered as an index of alertness, and the spontaneous decrease in basal activity score implicates the reduction of anxiety. Such types of effect can be found in the case of sedatives. The anti-depressant activity in forced swim test was observed that EESh1, EESh2 and EECc2 has exhibited significant reduction in immobility time when compared to control in dose dependent manner. Whereas, tail suspension test was significantly increases the immobility time. The magnitude of the antidepressant effects of EEJSLC2 shows same significant effect as that of standard drug Diazepam 10 mg/kg i.p. whereas EEJSLC1 is highly significant.

Key Wards: Jasminum Sambac, Chamomilla Capitula, Lilium Candidum, Sorghum Helpense Flowers, elevated plus maze, actophotometer, Froced swim test and Tail suspension test.

INTRODUCTION

According to the world health report approximately 450 million people suffer from a mental or behavioural disorder, but only a small number them receive even the foremost basic treatment, this accounts for 12.3% of the global burden of disease and will increase to 15% by 2020.1 Herbal drugs are widely used for the treatment of various diseases. Although herbal drugs often contain highly pharmacological compounds but much importance is not given to their safety evaluation, may be due to a popular notion "anything herbal is safe." Lately, with recent increasing interest in traditional or herbal drugs for the prevention and treatment of various disorders. there is also increasing concern about the safety of traditional, herbal product based medicines.2 The current research also focuses on the extraction and CNS activities of peels of citrus fruits which are easily available at zero cost thus decreasing the cost for production. The following fruits have been targeted in the present study.

Jasminum sambac. Linn (Oleaceae) is commonly known as Jasmine. It is a well known glabrous twining shrub widely grown in gardens throughout India. The flower is acrid and bitter taste. It is useful in treating diseases of the mouth and teeth, especially for toothache³. The J. sambac flowers and leaves are largely used in folk medicine to prevent and

treat breast cancer. Flowers of J. sambac are useful to women when brewed as a tonic as it aids in preventing breast cancer and stopping uterine bleeding4. It is widely used in the Ayurveda, as an antiulcerative, anticancer, antileprotic, skin diseases and wound healing. Chamomile capitula L. (family Asteraceae), popularly known as Chamomile is a reputed medicinal and aromatic plant used in both traditional and modern system of medicine. It is an ingredient of several traditional, Unani and Homeopathy preparations^{5, 6}. The capitula of chamomile contain natural oil, known as 'blue oil' (essential oil). The essential oil of chamomile has anti-inflammatory and softening effect and is useful in the treatment of gastric colic enteralagia, gastritis, bloating, inflammation and respiratory tract.

Lilium candidum L. (Liliaceae), the so called "white Madonna lily", is well known in folk medicine for the treatment of burns, ulcers, inflammations and for healing wounds. Lilium candidum L. extract contains various biologically active compounds^{7.8}. As the antimutagenic activity of natural compounds often correlates with antioxidant effects and contents of phytochemical substances from the flavonoids group, our hypothesis is that the LC extract, which is rich in flavonoids and with pronounced antioxidant activity, could possess bioprotective potential⁹.

Sorghum halepense L. (Poaceae) is adapted to a wide variety if habitats including open forests, old fields, ditches and wetlands¹⁰.

It occurs extensively along irrigated canals and at the edges of irrigated fields; its general distribution in these areas is the result of water movement of the seeds, which readily fall from the head when mature¹¹. Select varieties of sorghum have considerably high concentrations of phenolic compounds and antioxidant capacities that are located primarily in the bran fraction of the grain. Flavonoids, phenolic acids and tannins are three phenolic categories found in sorghum¹².

Numerous bioactive compounds such as flavonoids, Saponins, Phenolic and tannins have been isolated from flowers of this four extracts. Some of these bioactive compounds have been worked out for one or the other medicinal attributes. ¹³ But till date, the CNS activities of this flowers extracts have not been scientifically evaluated. Hence, in the present study, the effect of this four flower extracts at a dose of 200 and 400mg/kg body wt on antidepressant and anxiolytic activities has been studied.

MATERIALS AND METHODS

Preparation of plant extracts:

Fresh flowers of Jasminum Sambac, Chamomilla Capitula, Lilium Candidum, Sorghum Helpense were collected and dried under shade. The extracts used were prepared by taking 20gms of finely coarsely powdered was taken in a 250ml beaker containing 200ml of ethanol. The contents were mixed well and then the mixture was boiled up to 50-60°C for 4-5hrs. Further the extract was filtered with whatmann filter paper. The filtrate was boiled until the concentrated residue is formed. The concentrated product was sealed in sample covers and stored under room temperature and used for further experiment to check the activities.

Phytochemical analysis of the extracts

The extracts so obtained were subjected to preliminary phytochemical screening. Phytochemical studies were performed to identify the presence of various phytoconstituents such as alkaloids, terpenoids, saponoins flavonoids, phenols and tannins¹³.

Pharmacological evaluation Preparation of extracts:

The ethanolic extracts of Jasminum Sambac, Chamomilla Capitula, Lilium Candidum, and Sorghum Helpense suspended in water in presence of 3%v/v Tween-80 solution. All the drugs were administered I.P for experimental purpose. Each time preparations of the extracts were prepared when required. The drugs were administered at a constant volume of 10ml/kg for each animal.

Acute Oral Toxicity:

The acute oral toxicity of ethanolic extracts of Lilium Jasminum Sambac, Chamomilla Capitula, Lilium Candidum, Sorghum Helpense and their combinations was determined by using Albino wistar rats (200-250g) which were maintained under standard conditions. The animals were fasted 12 hour prior to the experiment, up and down procedure OECD guideline no. 425 were

adopted for toxicity studies. Animals were administered with single dose of individual extract upto 2000mg/kg and observed for its mortality during 2days and 7days study period (short term) toxicity and observed upto 7days for their mortality, behavioral and neurological profiles.

If mortality was observed in 1 animal, then the same dose was repeated again to confirm the toxic dose. If mortality was not observed, the procedure was repeated for further higher dose such as 2000 mg/kg of body weight.

PROCEDURE FOR ANTIANXIETY ACTIVITY Elevated plus maze (EPM) model

The apparatus comprises of two open arms (35x5cm) and two closed arms (30x5x15cm) that extend from a common central platform (5x5cm). The floor and walls of the closed arms are made of wood and painted black. The entire maze is elevated to a height of 50 cm above the ground level. Rats weighing (150 – 200gms) were housed in a pair of 10 days prior to the test in the apparatus. During this time the rats were handled by the investigator on alternate days to reduce stress. 30 min and 60min after oral administration of the drug treatment, each rat was placed in the center of the maze facing one of the enclosed arms. During five minutes session, number of entries into open arm and time spent in the open arm were noted14, 15. The procedure was conducted preferably in a sound attenuated environment.

Locomotor activity

The locomotor activity can be easily studied with the help of actophotometer, the rats were grouped and treated with drugs. Turn on the equipment (check & make sure that all the photocells are working for accurate recording) and placed individually each rat in the activity cage for 10 minutes. Note the basal activity score of all the animals. Inject the drug diazepam (Dose: 5 mg/kg, i.p; make a stock solution containing 0.5 mg/ml of the drug & inject 1 ml/100 g body wt of mouse), and after 30 mins re-test each mouse for activity scores for 10 mins. Note the difference in the activity, before & after chlorpromazine. Calculate percent decrease in motor activity¹⁶.

PROCEDURE FOR ANTIDEPRESSANT ACTIVITY Despair Swim Test

Apparatus For the determination antidepressant activity, forced swim test (FST) protocol was employed. During the test, animals were individually placed in a glass cylinder (20 cm in height, 14 cm in diameter) filled with water up to a height of 10cm, at 25 ± 2°C. All animals were forced to swim for 5 min and the duration of immobility was observed and measured during the 5 min interval of the test. Immobility period was regarded as the time spent by the rats to float in water with no struggle and making only those movements necessary to keep its head above the water. In order to check the fitness level of each test animal, a pre-test was carried out 24 h before

the FST by subjecting each test animal to a session of 15 min swimming¹⁷.

Tail suspension test

Tail suspension test was performed based on the method prescribed¹⁸. The mice were suspended 58cm above the floor by means of an adhesive tape, placed approximately 1cm from the tip of the tail. The total duration of immobility was quantified during a test period of 5min. Mice were considered immobile when they were completely remain motionless¹⁸.

RESULTS AND DISCUSSION

Preliminary Phytochemical Screenings

Phytochemical investigation of ethanolic extracts of *Lilium Jasminum Sambac, Chamomilla Capitula, Lilium Candidum, and Sorghum Helpense* revealed the presence of alkaloids, tannins, saponins, terpenoids and flavonoids as secondary metabolites.

Acute toxicity testing

Acute toxicity studies revealed that the ethanolic extracts of *Lilium Jasminum Sambac*, *Chamomilla*

Capitula, Lilium Candidum, Sorghum Helpense were safe up to 2000 mg/kg of body weight and approximate LD 50 is more than 2000 mg/kg. No lethality or any toxic reactions was observed up to the end of the study period.

Evaluation of Anti-anxiety Activity ELEVATED PLUS MAZE TEST

Anxiolytic property of ethanolic extract of flowers of *J.Sambac, S.halpense, L.Candidum and C.Capitula and their Combination* were studied at a dose of 200 and 400 mg/Kg by using Elevated plus maze experiment.

In elevated plus-maze test (EPM), the extracts of EEJs1, EELc1, EEJSLC1 and EEJSLC2. Significantly increased the number of entries and time spent into the open arm. The magnitude of the antianxiety effects of ethanolic extracts of *J.Sambac*, *S.halpense*, *L.Candidum and C.Capitula and their Combination* was compared with the standard drug diazepam 10 mg/kg i.p.

Table 1: Effect of Ethanolic extracts of *J.Sambac, S.halpense, L.Candidum and C.Capitula and Combination of four flowers* (200 and 400 mg/kg) on Elevated Plus Maze test in rats.

| C No | Groups | Dose (mg/kg) | No.of entries | | Average time spent (sec) | | No.of |
|------|----------------|--------------|---------------|-----|--------------------------|-----|----------|
| S.No | | | (O) | (C) | (0) | (C) | rearings |
| 1. | Normal control | - | 1 | 7 | 9 | 291 | 7 |
| 2. | Diazepam | 10 | 2 | 4 | 25 | 275 | 9 |
| 3. | EEJs1 | 200 | 3 | 3 | 37 | 263 | 5 |
| 4. | EEJs2 | 400 | 2 | 5 | 19 | 281 | 10 |
| 5. | EESh1 | 200 | 3 | 4 | 28 | 272 | 7 |
| 6. | EESh2 | 400 | 2 | 5 | 18 | 282 | 9 |
| 7. | EELc1 | 200 | 3 | 4 | 22 | 278 | 6 |
| 8. | EELc2 | 400 | 4 | 2 | 47 | 253 | 5 |
| 9. | EECc1 | 200 | 2 | 4 | 22 | 278 | 6 |
| 10. | EECc2 | 400 | 1 | 3 | 18 | 282 | 10 |
| 11. | EEJSLC1 | 200 | 2 | 5 | 38 | 262 | 12 |
| 12. | EEJSLC2 | 400 | 3 | 6 | 44 | 256 | 6 |

Values are expressed as mean ± S.E.M. n=6. Significant values were compared with P<0.05. Normal control Vs all groups. Paranthesis indicates that no.of entries were increased in open arms.

ACTOPHOTOMETER TEST

Anxiolytic property of ethanolic extract of flowers of *J.Sambac, S.halpense, L.Candidum and C.Capitula* and their Combination were studied at a dose of 200 and 400 mg/Kg by using Actophotometer experiment. The percentage of reduction in locomotor activity with diazepam (10 mg/kg i.p) after 1 hour is 91.0 % i.e. there is highly significant (P<0.05) decrease in

locomotor activity compare to control, where as dose of (200 and 400mg/kg i.p) showed dose dependent decrease in locomotor activityis EEJs1, EESh1, EELc1, EELc2, EEJSLC1 and EEJSLC2 that is 78.3%, 77.9%, 76.6%, 72.2%, 82.5% and 79.5% respectively when compared to standard. The values are highly significant (P<0.05).

Table 2: Effect of Ethanolic extracts of *J.Sambac, S.halpense, L.Candidum and C.Capitula and their Combination* on Locomotor activity.

| S.No | Groups | Dose | Locomotor activity (scores) in 10 min | | |
|------|----------|---------|---------------------------------------|-------|------------|
| | | (mg/kg) | Before | After | %change in |
| | | | | | activity |
| 1. | Control | - | 245 | | |
| 2. | Diazepam | 10 | 270 | 85 | 68.5 |
| 3. | EEJs1 | 200 | 365 | 79 | 78.3 |
| 4. | EEJs2 | 400 | 286 | 88 | 69.2 |
| 5. | EESh1 | 200 | 236 | 52 | 77.9 |
| 6. | EESh2 | 400 | 233 | 79 | 66.0 |
| 7. | EELc1 | 200 | 240 | 56 | 76.6 |
| 8. | EELc2 | 400 | 231 | 64 | 72.2 |
| 9. | EECc1 | 200 | 281 | 94 | 66.5 |
| 10. | EECc2 | 400 | 243 | 89 | 63.3 |
| 11. | EEJSLC1 | 200 | 240 | 42 | 82.5 |
| 12. | EEJSLC2 | 400 | 274 | 56 | 79.5 |

Values are expressed as mean ± S.E.M. n=6. Significant values were compared with P<0.05. Normal control Vs all groups. Paranthesis indicates % reduction in locomotar activity.

Evaluation of Antidepressant Activity FORCED SWIM TEST

Antidepresssant activity of ethanolic extract of flowers of *J.Sambac, S.halpense, L.Candidum and C.Capitula and their Combination* were studied at a dose of 200 and 400 mg/Kg by using Forced Swim Test experiment. The anti-depressant activity of various extracts and their combination was assessed using

Forced Swimming Test in Swiss albino rats were illustrated in Table 3. It was observed that EESh1, EESh2 and EECc2 have exhibited significant reduction in immobility time when compared to control in dose dependent manner. Similarly, the animals treated with diazepam (10mg/kg) as expected showed significant decrease in immobility time.

Table 3: Effect of Ethanolic extracts of *J.Sambac, S.halpense, L.Candidum and C.Capitula and Combination of four flowers* (200 and 400 mg/kg) on Forced swim Test in mice.

| S.No | Group | Dose | Immobility period | | % change in activity |
|------|----------|--------------|-------------------|-------|----------------------|
| | | (i.p; mg/kg) | Before | After | |
| 1. | Control | - | 134 | | |
| 2. | Diazepam | 10 | 185 | 62 | 66.48% |
| 3. | EEJs1 | 200 | 179 | 67 | 62.6% |
| 4. | EEJs2 | 400 | 305 | 195 | 36.06% |
| 5. | EESh1 | 200 | 288 | 75 | 73.95% |
| 6. | EESh2 | 400 | 293 | 82 | 72.01% |
| 7. | EELc1 | 200 | 312 | 96 | 69.23% |
| 8. | EELc2 | 400 | 259 | 78 | 69.88% |
| 9. | EECc1 | 200 | 274 | 99 | 63.86% |
| 10. | EECc2 | 400 | 281 | 76 | 72.95% |
| 9. | EEJSLC1 | 200 | 186 | 60 | 67.74% |
| 10. | EEJSLC2 | 400 | 198 | 65 | 66.66% |

Values are expressed as mean ± S.E.M. n=6. Significant values were compared with P<0.005. Normal control Vs all groups. Paranthesis indicates % reduction in motar activity.

TAIL SUSPENSION TEST

Antidepresssant activity of ethanolic extract of flowers of *J.Sambac, S.halpense, L.Candidum and C.Capitula and their Combination* were studied at a dose of 200 and 400 mg/Kg by using Forced Swim Test experiment.In tail suspension test, the ethanolic extracts of flowers of *J.Sambac, S.halpense*,

L.Candidum and C.Capitula and their Combination at a dose of 200 and 400mg/kg i.p. significantly increases the immobility time. The magnitude of the antidepressant effects of EEJSLC2 shows same significant effect as that of standard drug Diazepam 10 mg/kg i.p. whereas EEJSLC1 is highly significant. (Table 4).

Table 4: Effect of Ethanolic extracts of *J.Sambac, S.halpense, L.Candidum and C.Capitula and Combination of four flowers* (200 and 400 mg/kg) on tail suspension in mice.

| S.No | Treatment | Dose (mg/kg) | Duration of immobility | | % change in activity |
|------|-----------|--------------|------------------------|-------|----------------------|
| | | | Before | After | |
| 1. | Control | - | 40 | | |
| 2. | Diazepam | 10 | 20 | 120 | 83.33% |
| 3. | EEJs1 | 200 | 40 | 180 | 77.8% |
| 4. | EEJs2 | 400 | 54 | 167 | 67.7% |
| 5. | EESh1 | 200 | 64 | 196 | 67.3% |
| 6. | EESh2 | 400 | 25 | 173 | 85.5% |
| 7. | EELc1 | 200 | 32 | 148 | 78.3% |
| 8. | EELc2 | 400 | 54 | 152 | 64.4% |
| 9. | EECc1 | 200 | 67 | 146 | 54.1% |
| 10. | EECc2 | 400 | 48 | 137 | 64.9% |
| 11. | EEJSL1 | 200 | 20 | 158 | 87.3% |
| 12. | EEJSL2 | 400 | 28 | 162 | 82.7% |

Values are expressed as mean ± S.E.M. n=6. Significant values were compared with P<0.05. Normal control Vs all groups. Paranthesis indicates % reduction in motar activity.

CONCLUSION

The study was performed to find out the beneficial effects of different extracts of flowers of *J.Sambac, S.halpense, L.Candidum and C.Capitula and their Combination* were studied for different activities such as anti-depressant and anti-anxiety activities. The results reveal that the plant has beneficial effects on these activities.

Preliminary Phytochemical Screening:

In current scenario, flowers are the potent sources of medicines used in the treatment of various disease and disorders. Since, flowers are used as medicine there is prompt need of evaluation of plant species, therefore, the present work was conceived to evaluate the phytochemical and pharmacological screening of flowers of *J.Sambac, S.halpense, L.Candidum and C.Capitula and their Combination*. The Phytochemical evaluation has revealed the presence of alkaloids, terpenoids, saponins, flavonoids, phenols and tannins.

Anti-Anxiety Activity

Complete manifestation of anxiety in mice of the control group is evident from the minimum mean time spent in the open arms of elevated plus-maze by these animals. Among the extracts tested, maximum anxiolytic activity was observed in the ethanol at the dose of 200 mg/kg which was at par with that of

diazepam as is evident from statistical equivalence between the results of this dose and that manifested by diazepam. However, the activity decreased at higher doses, which might be due to sedation. Phytochemical screening showed presence of alkaloids in ethanol extract of *J.Sambac, S.halpense, L.Candidum and C.Capitula*. Alkaloids were subjected to biological evaluation for anti-anxiety activity in rats using EPM apparatus and Actophotometer.

Therefore, this plant merits further attention. Search on the most active principle as well as elucidation of the exact mechanism of its action is needed. Thus, we conclude that ethanol extract of *J.Sambac, S.halpense, L.Candidum and C.Capitula* flowers possess anti-anxiety activity and studies are mandatory to establish the precise nature of active constituents as well as their mechanism of action.

Anti-Depressant Activity

Many researchers showed that plant containing flavonoids, saponins and tannins are useful in many CNS disorders. Earlier investigation on phytoconstituents and plants suggests that many flavonoids and steroids were found to ligands for the GABAA receptors in the central nervous system; which led to the assumption that they can act as benzodiazepine like molecules. The tests are quite sensitive and relatively specific to all major classes of

antidepressant drugs including TCAs, SSRIs, MAOI, Atypical antidepressants. The forced swimming test is the most widely used tool for assessing antidepressant activity pre-clinically. The widespread use of this simple model is mainly due to its ability to detect a broad spectrum of antidepressant agents. It has been argued that TST (Tail Suspension Test) is less stressful than (Forced swim test) and has pharmacological sensitivity. Flavonoids present in this be facilitating monoaminergic extracts may transmission there by producing antidepressant effects. However, the activity decreased at higher doses, which might be due to sedation. Phytochemical screening showed presence of saponins in ethanol extract of J.Sambac, S.halepense, L.Candidum and C.Capitula. Saponins were subjected to biological evaluation for anti-depressant activity in mice using FST and TST.

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CONFLICT OF INTEREST

Authors declare no Conflict of Interest.

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