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Original Research Article

Phytochemical investigation and exploration of the central nervous system depressant activity of ethanolic extract of the flowers of *Jasminum grandiflorum* L. as folklore medicine in Swiss albino mice

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ABSTRACT

Background: In Indian traditional medicine, the flowers of *Jasminum grandiflorum* L. (*Oleaceae*) are claimed to possess powerful central nervous system (CNS) depressant activity. Despite these traditional claims, no in-depth scientific study has been performed on the CNS depressant activity of the flowers of *J. grandiflorum*. Therefore, the present study was aimed at evaluating the CNS depressant activity of an ethanolic extract of the flowers of *J. grandiflorum* in Swiss albino mice.
Methods: Acute oral toxicity tests were done at doses of 550, 1750, and 5000 mg/kg. The extract was also subjected to phytochemical tests and TLC tests. The CNS depressant activity of the EEJG was evaluated by various models, such as the forced swimming test, tail suspension test, thiopental sodium-induced sleeping time test, locomotor activity test, and muscle co-ordination test, at two different doses (500 and 1000 mg/kg) in Swiss albino mice. Diazepam (1 mg/kg) was used as a standard drug.
Results: In acute oral toxicity studies, the extract was found to be safe up to a dose level of 5000 mg/kg body weight. EEJG at both doses (500 and 1000 mg/kg) showed significantly ($p<0.05$, $p<0.01$) increase in immobility time in the forced swimming test and tail suspension test. In the thiopental-induced sleeping time test, EEJG at 1000 mg/kg showed a significant ($p<0.05$) effect on the onset of action time and also significantly ($p<0.01$) increase the duration of sleeping time. EEJG at 1000 mg/kg showed a significant ($p<0.01$) decrease in locomotor activity, and the EEJG at both doses (500 and 1000 mg/kg) showed a significantly ($p<0.05$, $p<0.01$) decreased in muscle co-ordination activity when compared to control group.
Conclusions: The present study confirms the significant CNS depressant activity of the ethanolic extract of the flowers of *J. grandiflorum*, which may be due to flavonoids and steroids present in the extract as phytoconstituents confirmed by TLC. This study supports the plant's traditional use as a CNS depressant.

Keywords: *Jasminum grandiflorum* L., CNS depressant activity, Diazepam, TLC, Muscle coordination, Locomotor activity

INTRODUCTION

Psychological stress, anxiety, and sleep disorders can lead to short-term and long-term disability and account for the symptoms of major neuro-psychiatric illnesses worldwide. Insomnia has a high prevalence rate across the globe and is associated with severe psychological and cardio-metabolic health issues. Approximately 20% of the population suffers major psychiatric episodes and manifestations at some point in their lives.[1] These symptoms become more prevalent in elderly patients, and they have a wide range of negative implications in their personal, social, and professional lives. Loss of mental wellbeing and psychological distress are considered the most frequent neuropsychiatric conditions, similar to stroke.[1]

Depressants are pharmacological substances that decrease neuronal or physiological activity. If a person suffers from insomnia, anxiety, panic attacks, or seizures, the doctor may prescribe a class of drugs called CNS depressants. CNS depressants are the medicines that include sedatives, hypnotics, and tranquilizers. These drugs slow down the brain's activity, which produces relaxation, calms the person, and increases the duration of sleep. They work by increasing the production of gamma-aminobutyric acid (GABA). GABA neurons "turn off" arousal systems in the brain at the level of the cell bodies and therefore promote sleep. By blocking the target neurons of the arousal system, GABA receptors in the cortex can also promote sedation and sleep.[2] Thus, present demand has greatly increased the use of these drugs to treat different psychiatric disorders. However, continuous use of this sedative-hypnotic therapy may cause some serious side effects ranging from respiratory and immune system disorders to damage to the cognitive nerve function and can also cause physical dependency and addiction.[3] The development of a new CNS depressant drug is therefore needed with fewer side effects and as a promising approach to prevent different psychiatric disorders.

Jasminum grandiflorum Linn. (*Oleaceae*) is commonly known as Jasmine. It is a well-known glabrous twining shrub with a high medicinal value. It is a native of Asia, including Kashmir, Afghanistan, Persia, the Nilgiris, France, Italy, China, Japan, India, Morocco, and Egypt.[4] The flower is acrid and bitter, with a sharp taste. It is effective in treating dental and oral conditions, particularly toothaches. Also are useful to women when brewed as a tonic as it aids in preventing breast cancer and stopping uterine bleeding.[5] It is widely used in Ayurveda for the treatment of various ailments, including chronic constipation, flatulence, dysmenorrhea, amenorrhea, ringworm, skin diseases, ulcers, giddiness, and diabetes.[6] The plant is reported to possess spasmolytic, anti-inflammatory, anti-microbial, antioxidant, anti-ulcer, cytoprotective, chemopreventive, wound healing, and anti-acne activities.[7] In Indian traditional medicine, the flowers of *J. grandiflorum* are claimed to possess powerful CNS depressant activity. Despite these traditional claims, no in-depth scientific study has been performed on the CNS depressant activity of flowers of *J. grandiflorum*, so the present study was undertaken to investigate the CNS depressant activity of an ethanolic flower extract of *J. grandiflorum*.

METHODS

Preparation of plant extract

Flowers powder *of J. grandiflorum* (100 g) was defatted with 150 ml of petroleum ether and extracted with ethanol by hot percolation method using a Soxhlet apparatus at 40°C to obtain the ethanolic extract of the plant. The filtrate of the extract was concentrated and dried at a temperature of 30°C. The percentage yield was calculated and reported.

Animals

Swiss albino mice (150-250 g) of either sex were obtained from Mahaveer enterprises, Hyderabad, Telangana, India (1656/PO/Bt/S/12/CPCSEA). They were maintained in an HKES MTRIPS animal house at a temperature of 25±1°C and a relative humidity of 45% to 55% under a 12-hour light and 12-hour dark cycle. The animals had free access to food pellets, and water was available ad libitum. Prior permission from the IAEC was obtained for the conduct of the experiments (IAEC approval no. HKES/MTRIPS/IAEC/122/2021-22).

Chemicals

Ethanol LR and petroleum ether 60-80°C LR (SDFCL, Lower Parel, Mumbai, India). Diazepam injection I.P. Lori 5 mg/2 ml (Neon laboratories, Andheri, Mumbai, India). Thiopental sodium injection I.P. Thiotone 1 gm (Flagship Biotech International Pvt. Ltd, Navi Mumbai, India).

Acute oral toxicity test

Acute oral toxicity studies were conducted as per OECD (Organization for economic co-operation and development) guideline 425.[8] Healthy adult Swiss mice of either sex, weighing between 20 and 25 g, were used for the study. The food, but not the water, was withheld for 4 hours before the extract was administered orally. All the extracts were given in a progressive dose manner, initiating at a dose of 175 mg/kg, p.o. When no abnormality or death was observed, the next doses of 550, 1750, and 5000 mg/kg were chosen. At the dose of 5000 mg/kg, an additional four mice were dosed. All the animals were observed for initially 30 m and then 24 h for behavioral, neurological, and autonomic profiles and for any lethality or death over the next 48 h.

Preliminary phytochemical screening

An ethanolic extract of the flowers of *J. grandiflorum* was qualitatively tested for the detection of alkaloids, saponins, terpenoids, tannins, flavonoids and steroids.[9]

Thin layer chromatography identification of bioactive components

One gram of ethanolic extract of *J. grandiflorum* was dissolved in respective solvent and a few drops of distilled water were added for complete solubility, then each extract was subjected to different phytochemical tests.[10] The extract was analyzed qualitatively by thin layer chromatography (TLC) to detect the bioactive components (flavonoid and steroid). The Silica gel GS 254 percolated aluminum plates were used as TLC plates. The solvent system used for flavonoid was ethyl acetate, formic acid, glacial acetic acid and water at a ratio of 10:1.1:1.1:2.6. The fluorescent spots obtained were detected using UV at 365 nm. The solvent system used for steroid was petroleum ether and acetone at a ratio of 7:3. The spots

obtained were detected using anisaldehyde-sulfuric acid spraying reagent.

Neuropharmacological test

Forced swimming test

In this test mice were randomly divided into four groups containing six mice in each group. The control group received distilled water (0.1 ml/mouse, p.o.), whereas the test group received *J. grandiflorum* flowers extract (at the doses of 500 and 1000 mg/kg, p.o.) and standard group received diazepam (1 mg/kg, i.p.). After 30 minutes of treatment administration, all the mice were placed one by one in 45 cm glass cylinder of 20 cm diameter containing water at the temperature of 25±1°C. The immobility time was recorded for a period of 5 minutes in each mouse (They were considered in immobile when floated motionless in water and producing small movements of forepaws to keep their head on top of water). Following swimming session, mice were towel dried and returned to their housing conditions.[11]

Tail suspension test

Swiss albino mice were randomly divided into four groups containing six mice in each group. The control group received distilled water (0.1 ml/mouse, p.o.), whereas the test group received *J. grandiflorum* flowers extract (at the doses of 500 and 1000 mg/kg, p.o.) and standard group received diazepam (1mg/kg, i.p.). After 30 minutes of treatment administration, all the mice were suspended one by one on the table 50 cm above the floor with an adhesive tape placed 1cm from the tip of the tail. Immobility time was calculated for the period of 6 minutes in each mouse (Mice were considered immobile when they were totally static and hanged passively).[11]

Thiopental sodium-induced sleeping time test

In this test, the animals were assigned to four groups, with six mice in each group. The test groups expected the extract at doses of 500 and 1000 mg/kg, while the control group received distilled water (0.1 ml/mouse, p.o.) and standard group received diazepam (1mg/kg, i.p.). After thirty minutes of treatment administration, each mouse was treated with thiopental sodium (fourty mg/kg, i.p.) to induce sleep. The rodents were monitored by placing them in different chambers for the latent period (time between thiopental sodium administrations and loss of righting reflex) as well as duration of sleeping time (time between the loss and recovery of the righting reflex) was recorded.[12]

Locomotor activity test

Locomotor activity was evaluated using an actophotometer (Inco-Ambala, India). The mice were randomly divided into four groups, with six mice in each group. The control group received distilled water (0.1 ml/mouse, p.o.), whereas the test group received *J. grandiflorum* flowers extract (at the doses of 500 and 1000 mg/kg, p.o.), and the standard group received diazepam (1 mg/kg, i.p.). Each mouse was placed individually in the actophotometer for 10 minutes. The locomotor activity was measured in terms of photocell counts before and 30 minutes after the administration of respective treatments. The change in locomotor activity was derived by considering photocell counts recorded before and after the assigned treatments. Finally, the percentage decrease in locomotor activity was calculated.[13]

Muscle coordination test

This test was carried out using a digital rotarod apparatus (Inco-Ambala, India). The animals were trained to remain for 3 minutes on the rod rotating at a speed of 25 rpm. Only animals performing up to the required parameter were included in the test and divided into four groups, each containing six mice. The control group received distilled water (0.1 ml/mouse, p.o.), whereas the test group received *J. grandiflorum* flowers extract (at the doses of 500 and 1000 mg/kg, p.o.), and the standard group received diazepam (1 mg/kg, i.p.). All animals were subsequently assessed for their performance on the rotarod before as well as after thirty minutes of respective treatment. The fall off time from the rod was noted for each animal.[13]

Statistical analysis

Data were expressed as Mean ± SEM and statistical analysis was carried out by one-way analysis of variance (ANOVA) followed by Dunnett's test.

RESULTS

Acute toxicity test

In acute oral toxicity studies, no behavioural or autonomic abnormalities, as well as no mortality, were observed in any of the mice treated with the ethanolic extract of *J. grandiflorum* up to the dose of 5000 mg/kg. The extract was found to be safe up to the maximum dose level of 5000 mg/kg of body weight.

Preliminary phytochemical screening

The percentage yield of ethanolic extract of flowers of *J. grandiflorum* was 8%. The preliminary phytochemical assessment of EEJG showed the presence of alkaloids, saponins, terpenoids, flavonoids and steroids (Table 1). Two steroidal compounds were detected by TLC of EEJG at R_f values of 0.5 and 0.44, and a flavonoidal compound of EEJG was detected by TLC at R_f value of 0.9.

Forced swimming test

EEJG at both doses (500 and 1000 mg/kg) significantly (*$p<0.05$, **$p<0.01$, respectively) increased the duration of immobility time in mice compared to control. Similarly,

the diazepam also significantly (***p<0.001) increased the duration of immobility time (Table 2).

Table 1: Chemical constituents present in ethanolic extract of *Jasminum grandiflorum* Linn flowers.

Test	Ethanolic extract
Alkaloids	+
Saponins	+
Terpenoids	+
Tannin	-
Flavonoids	+
Steroids	+

(+) - Positive, (–) - Negative

Table 2: Effect of ethanolic extract of the flowers of *J. grandiflorum* on forced swimming test.

Treatment	Dose (mg/kg)	Immobility time (sec)
Control	0.1 ml/mouse	61.8±7.39
Diazepam	1	161.6±11.57***
EEJG	500	121.2±19.42*
EEJG	1000	128.4±10.96**

N=6, values are expressed as mean ± S.E.M; *p<0.05, **p<0.01, ***p<0.001 compared to control group. EEJG-Ethanolic extract of *Jasminum grandiflorum* Linn.

Tail suspension test

EEJG at both doses (500 and 1000 mg/kg) significantly (*p<0.05, **p<0.01, respectively) increased the duration of immobility time in mice compared to control. Similarly, the diazepam also significantly (***p<0.001) increased the duration of immobility time (Table 3).

Table 3: Effect of ethanolic extract of flowers of *J. grandiflorum* on tail suspension test.

Treatment	Dose (mg/kg)	Immobility time (sec)
Control	0.5 ml/mouse	85.2±2.89
Diazepam	1	224±4.85***
EEJG	500	156.2±34.74*
EEJG	1000	190.6±5.75**

N=6, values are expressed as mean ± S.E.M; *p<0.05, **p<0.01, ***p<0.001 compared to control group. EEJG-Ethanolic extract of *Jasminum grandiflorum* Linn.

Thiopental sodium-induced sleeping time test

EEJG at 1000 mg/kg and the diazepam (1 mg/kg) significantly (*p<0.05, **p<0.01, respectively) decreased the onset of action time when compared to control. It had a fast onset of action (Table 4). EEJG at 1000 mg/kg and the diazepam (1 mg/kg) showed significantly (**p<0.01, ***p<0.001, respectively) increased the duration of sleep when compared to the control group (Table 4).

Table 4: Effect of ethanolic extract of flowers of *J. grandiflorum* on thiopental sodium-induced sleeping time test.

Treatments	Onset of action (min)	Duration of sleeping time (min)
Control (0.1 ml/mouse)	9.72±1.26	33.04±7.32
Diazepam (1 mg/kg)	4.40±0.76**	95.73±15.18***
EEJG (500 mg/kg)	6.84±0.92	54.01±5.90
EEJG (1000 mg/kg)	5.69±0.61*	77.98±5.56**

N=6, values are expressed as mean ± S.E.M; *p<0.05, **p<0.01, ***p<0.001 compared to control group. EEJG-Ethanolic extract of *Jasminum grandiflorum* Linn.

Locomotor activity test

After 30 minutes of treatment, the EEJG at 1000 mg/kg and the diazepam (1 mg/kg) showed a significantly (**p<0.01, ***p<0.001, respectively) decreased in locomotor activity compared to the control group. EEJG at both the doses (500 and 1000 mg/kg) and the diazepam (1 mg/kg) showed 23.71%, 33.27%, and 64.01% reductions respectively, in locomotor activity compared to values before treatment (Table 5 and Figure 1).

Table 5: Effect of ethanolic extract of flowers of *J. grandiflorum* on locomotor activity test.

Treatments	Locomotor activity (score) in 10 min		Percent decrease in activity (%)
	Before treatment	**After treatment**	
Control (0.1 ml/mouse)	444.8± 22.98	416.6± 30.50	06.34
Diazepam (1 mg/kg)	477.4± 23.83	171.8± 30.53***	64.01
EEJG (500 mg/kg)	502.0± 18.40	383.0± 20.27	23.71
EEJG (1000 mg/kg)	398.0± 19.37	265.6± 40.17**	33.27

N=6, values are expressed as mean ± S.E.M; **p<0.01, ***p<0.001 compared to control group. EEJG-Ethanolic extract of *Jasminum grandiflorum* Linn.

Muscle coordination test

After 30 minutes of treatment, the EEJG at both the doses (500 and 1000 mg/kg) and the diazepam (1 mg/kg) showed a significantly (*p<0.05, **p<0.01, ***p<0.001, respectively) decreased in fall off time compared to the control group. EEJG at both the doses (500 and 1000 mg/kg) and diazepam (1 mg/kg) showed 28.71%, 50.06%, and 75.31% caused reduction in fall-off time compared to values before treatment (Table 6 and Figure 2).

Table 6: Effect of ethanolic extract of flowers of *J. grandiflorum* on muscle coordination activity test.

Treatments	Fall off time (sec)		Percent decrease in time (%)
	Before treatment	After treatment	
Control (0.1 ml/mouse)	308.6± 28.93	337.2± 42.84	-09.27
Diazepam (1 mg/kg)	357.2± 25.58	88.20± 21.08***	75.31
EEJG (500 mg/kg)	282.8± 50.01	201.6± 46.32*	28.71
EEJG (1000 mg/kg)	330.4± 40.42	165.0± 16.71**	50.06

N=6, values are expressed as mean ± S.E.M; *p<0.05, **p<0.01, ***p<0.001 compared to control group. EEJG-Ethanolic extract of *Jasminum grandiflorum* Linn.

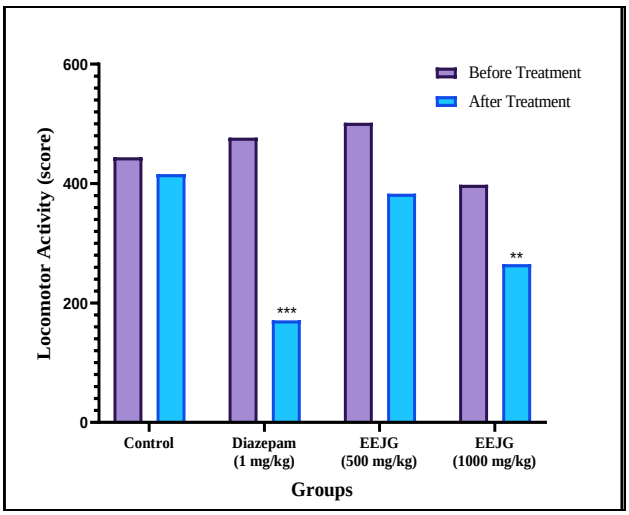

Figure 1: Effect of EEJG and diazepam on locomotor activity.

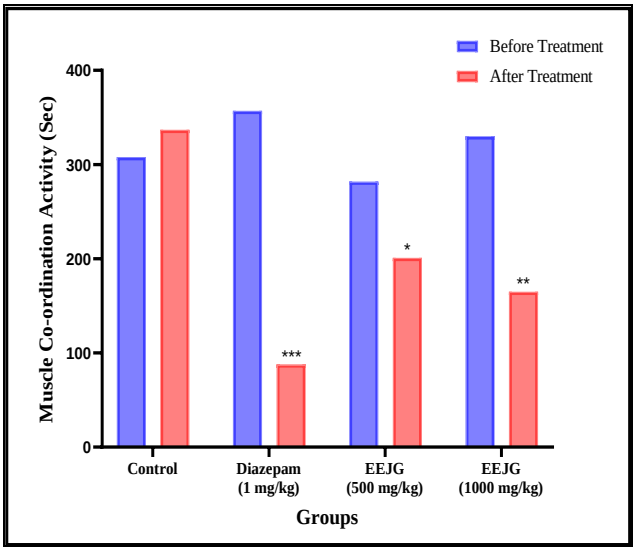

Figure 2: Effect of EEJG and diazepam on muscle coordination activity.

DISCUSSION

The present study was conducted to evaluate the CNS depressant activity of an ethanolic extract of the flowers of *J. grandiflorum* in mice. The CNS depressant effect of EEJG was studied using five neuropharmacological models, namely forced swimming, tail suspension, thiopental sodium-induced sleeping time, locomotor activity, and the muscle coordination test. These models are widely used classical models for screening neuropharmacological activity.

The shortening of immobility duration indicates antidepressant activity, while prolonged immobility time reflects CNS depressant activity.[12] The present finding showed a significant (Table 2) increase in duration of immobility time in the forced swimming test, which reflects a CNS depressant-like effect of EEJG. Additionally, the tail suspension test was carried out by observing the energy developed by mice trying to escape from their suspension. In this test, the EEJG also significantly (Table 3) increased the duration of immobility time, which indicated CNS depressant effects in mice. The diazepam also indicated CNS depressant effects in mice models. The tail suspension test results are in concurrence with the forced swimming test results.

The EEJG at 1000 mg/kg showed a fast onset of action and a significant (Table 4) increase in the duration of sleep in the thiopental sodium-induced sleeping time test. Thiopental sodium belongs to the barbiturate family and induces sleep in both humans and rodents. It binds with the GABA receptor complex and shows GABA-mediated hyperpolarization of postsynaptic neurons.[14] It potentiates GABA activity by entering chloride into the neuron, which decreases neuronal activity and supports the following reference substances that possess CNS depressant action.

A significant (Table 5) reduction in locomotor activity and a significant (Table 6) decrease in muscle coordination (in the actophotometer and rotarod models, respectively) confirm the CNS depressant effect of the extract. Locomotor activity is considered an index of alertness, and a decrease in it indicates the sedative effect. Gamma aminobutyric acid is the major inhibitory neurotransmitter in the CNS which is involved in the physiological functions related to such psychological and neurological disorders as epilepsy, depression, Parkinson syndrome, and Alzheimer's disease.[15,16] Therefore, it is predictable that the extract may act by potentiating GABAergic inhibition in the CNS via membrane hyperpolarization, resulting in a decreased in the firing rate of censorial neurons in the brain, or it may be caused by the direct activation of GABA receptors.[15]

Earlier researchers reported that many flavonoids and neuroactive steroids were found to be ligands for the GABA receptors in the CNS and that they can act as benzodiazepine-like molecules.[11] In the present study TLC results showed the presence of flavonoids and steroids in

the extract, which may be responsible for the CNS depressant activity of the ethanolic extract of flowers of *J. grandiflorum*.

CONCLUSION

The present study confirms the significant CNS depressant activity of the ethanolic extract of the flowers of *J. grandiflorum*, which may be due to flavonoids and steroids present in the extract as phytoconstituents. This study supports the plant's traditional use as a CNS depressant. However, further research is required to fractionate and isolate the molecule from the extract, and studies are also required to be carried out to know the exact mechanism of the fraction for the CNS depressant activity.

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Ethical approval: The study was approved by the Institutional Ethics Committee

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