

# In vivo Study on Depressant Effects and Muscle Coordination Activity of *Galphimia glauca* Stem Methanol Extract

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## ABSTRACT

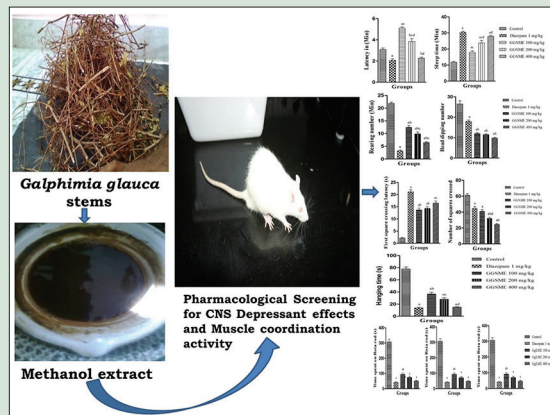
**Background:** *Galphimia glauca* is an evergreen shrub found across peninsular India, belonging to family *Malpighiaceae*. **Objective:** The objective of this study was to assess the *in vivo* depressant effects and muscle coordination activity of *G. glauca* stem methanol extract (GGSME). **Materials and Methods:** The stem methanol extract was administered in Swiss albino mice in 1 day to study the central nervous system (CNS) depressant and muscle coordination activity employing animal models such as sodium pentobarbital-induced sleep test, hole-board test, open field test, pentylenetetrazole (PTZ)-induced convulsions, picrotoxin-induced convulsions, grip strengthening test in mice, and Rota-rod test. **Results:** The LD<sub>50</sub> of GGSME was found to be >2000 mg/kg body weight (b.w.). Mice treated with stem methanol extract at 100, 200, and 400 mg/kg, b.w. doses extended the sleeping time induced by sodium pentobarbital (40 mg/kg, b.w., i.p.). The stem methanol extract at 400 mg/kg dose showed a significant ( $P \leq 0.001$ ) dose-dependent decrease in the number of rears and head dipping number in the hole-board test. The extract exhibited a significant ( $P \leq 0.001$ ) effect on the ambulatory behavior of mice in the open field test and also extended the onset of seizures induced by PTZ (90 mg/kg b.w., i.p.) and picrotoxin (10 mg/kg, b.w., i.p.). The extract also exhibited significant ( $P \leq 0.001$ ) effects on muscle coordination in rota-rod and grip strengthening test in mice. **Conclusion:** The study results conclude that the GGSME has a potential CNS depressant and muscle relaxant effects compared to the standard drugs.

**Key words:** Convulsions, *Galphimia glauca*, grip strength test, sodium pentobarbital, stem methanol extract

## SUMMARY

- Anxiety is implicated in the number of psychiatric disorders
- In vivo* depressant activity is studied employing animal models like Sodium pentobarbital-induced sleep test, Hole-board test, Open field test, Pentylenetetrazole induced convulsions and Picrotoxin-induced convulsions tests.
- Muscle coordination activity is studied employing animal models like Grip strengthening test in mice and Rota-rod test.
- The GABAergic system plays a significant role in CNS depressant and muscle relaxant effects.

- The study proves the traditional claims of the plant used in the treatment of phobia, panic, stress, anxiety and it is as well used in producing a calming effect on the nerves.



**Abbreviations Used:** WHO: World Health Organization; CNS: Central nervous system; GGSME: *Galphimia glauca* stem methanol extract; IAEC: Institutional Animal Ethics Committee; OECD: The Organization for Economic Co-operation and Development; PTZ: Pentylenetetrazole; REM: Rapid eye movement; GABA:  $\gamma$ -aminobutyric acid; AMPA:  $\alpha$ -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor; b.w: Body weight; i.p: Intraperitoneal; p.o: per oral

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## INTRODUCTION

India is the hub of traditional medicines; this is not only due to the long uninterrupted practice of alternative systems of medicines in the country, but also due to the global popularity of this authentic medicine and therapies. India had a long great indigenous system of health care such as Ayurveda, Yoga, and Sidda. Over time, there was an interaction with different civilizations and assimilated other systems of medicines as well.

According to the WHO, health is defined as “a complete state of physical, mental, and social well-being and not merely the absence of disease or infirmity.”<sup>[1]</sup> The alternative systems of medicines primarily using medicinal plants try to create balance and preserve health to keep up friendly practices and sustainable sources of medicinal herbs. Unfortunately, the real potential of these systems of medicines is untapped because of many reasons, most importantly, because of inadequate scientific scrutiny and concerns regarding standards and

quality, if these issues are addressed properly, the alternative systems of medicines will provide solutions to many health problems. Among the natural sources of drugs, plants, in particular, serve as the primary source of lead molecules of therapeutic significance. Till today, there are vast numbers of plants, whose real potential is untapped.

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*Galphimia glauca* is found distributed in peninsular plateau of India consisting of Central highlands and the Deccan Plateau. It is extensively seen in the Eastern parts of Deccan Plateau. The Deccan trap (black soils), tropical climate, drainage systems, and other diverse physical features of this region favor its rich habitat. It belongs to the family of the *Malpighiaceae*. The shrub is commonly known by the name “*Calderona amarilla*” and “*Flor estrella*.”<sup>[2,3]</sup> The ethyl acetate extract of plant's aerial parts is reported for antiasthmatic activity, which acts by inhibiting the LTD<sub>4</sub>-induced airway smooth muscle contraction.<sup>[4]</sup> Nader *et al.*, 2006, reported the production of triterpenoids from the liquid-cultivated hairy roots of *G. glauca*.<sup>[5]</sup> Ruiz *et al.*, 2007, reported the anxiolytic actions of galphimines of the plant and their chemical derivatives in the Institute for Cancer Research mice that are exposed to the elevated plus-maze test.<sup>[6]</sup> Solid-phase extraction method is employed to extract phenolic acids from *G. glauca* using pure zirconium silicate and bismuth citrate powders as sorbents, and their efficacy was determined by employing high performance liquid chromatography photodiode array detection (HPLC-DAD), further the isolated compounds were identified using ultraviolet and mass spectra.<sup>[7]</sup>

Traditionally, the shrub is used in conditions of anxiety,<sup>[8]</sup> stress,<sup>[9]</sup> phobia, panic state,<sup>[10]</sup> and used to produce a calming effect on the nerves.<sup>[11]</sup> Tortoriello and Lozoya 1992, reported the sedative and anticonvulsant activity of a methanolic extract prepared with aerial parts of *G. glauca*.<sup>[12]</sup> Our present study is specifically confined to explore the pharmacological significance in *G. glauca* stem. Based on the traditional uses of this shrub, the current study is planned to explore the central nervous system (CNS) depressant effect and muscle coordination activity of *G. glauca* stem methanol extract (GGSME) using *in vivo* models.

## MATERIALS AND METHODS

### Plant material

The plant *G. glauca* was collected from the lawn existing in the School of Pharmacy, Anurag Group of Institutions. The stems were collected on November, 2014. The plant was identified and authenticated by taxonomist, Dr. E. Narsimha Murthy, Satavahana University, Karimnagar, Telangana State, India. A voucher copy is stored with the reference number No. 333 in the Department of Pharmacognosy and Phytochemistry, School of Pharmacy.

### Chemicals and drugs

All the chemicals were of analytical grade and purchased from SD Fine chemicals, Mumbai, Maharashtra, India. The sodium pentobarbital used in this study was purchased from Sigma Chemicals Co., USA, diazepam was procured from Natco Pharmaceuticals, India Inc., and pentylenetetrazole from Sigma-Aldrich, USA. Picrotoxin is received as a gift sample from Sri Disha biotech, Hyderabad, India Inc.

### Preparation of the extract

*G. glauca* stems were collected, dried in shade, and powdered. Stem powder of 150 g was subjected to soxhlet extraction using 600 ml of solvent methanol. The methanol extract was collected and then concentrated to dryness and stored. The percentage yield obtained for GGSME was 26%.

### Animals

Swiss albino mice of 6–8 weeks of age with  $22.5 \pm 2.5$  g of either sex were employed. Animals were acclimatized for 7 days to the laboratory conditions. The mice were retained in 12 h light/dark cycles at  $22^\circ\text{C} \pm 2^\circ\text{C}$  with 60–70% relative humidity. Complete pharmacological studies were carried out randomly using 6 animals of either sex in each group.

The study protocol was approved by the Institutional Animal Ethics Committee of the institute (IAEC), School of Pharmacy, Anurag Group of Institutions (the approval number: I/IAEC/LCP/032/2014/SM-13).

### Acute toxicity studies

According to the Organization for Economic Co-operation and Development (OECD) guidelines, 423-2d, acute oral toxicity studies were conducted.<sup>[13]</sup>

### Central nervous system depressant activity

#### Test for sodium pentobarbital-induced sleeping time

This method for the sleeping time test was described by Fujimori.<sup>[14]</sup> The hypnotic and sedative effects of GGSME in combination with sodium pentobarbital was evaluated. For this purpose, animals were grouped as mentioned below. Group I was considered as negative control and received distilled water before intraperitoneal injection of the sodium pentobarbital (40 mg/kg, body weight [b.w.]). Group II received diazepam (1 mg/kg, b.w., i.p.), which was considered as positive control while Groups III to V received a methanol extract 60 min before the administration of sodium pentobarbital. Individual animal was placed on a table and recorded for the uncoordinated movements to the sedative phase of the test. Loss of the righting reflex related to the hypnosis and the time span of the sleep were also noticed and recorded. The time elapsed between the loss and recovery of the righting reflex was treated as the sleeping time.<sup>[15]</sup>

Group I: Negative control, received distilled water (10 ml/kg, b.w., per oral [p. o.]

Group II: Positive control, treated with diazepam (1 mg/kg, [b.w.], intraperitoneally [i.p.]

Group III–V: Treated with GGSME (100, 200, and 400 mg/kg, b.w., respectively, [p.o.]).

#### Hole-board test

This test was initially reported by Boissier *et al.* to assess certain components of behavior of mice such as curiosity or exploration.<sup>[16]</sup> For this test, the apparatus used is a wooden box of 50 cm × 50 cm × 30 cm with 4 equidistant holes (3 cm in diameter) on the floor. The Group I animals received only distilled water. The reference standard (Group II) was treated with diazepam (1 mg/kg, b.w., i.p.) 30 min before performing the study. GGSME was given orally to the groups as mentioned in the procedure to test for sodium pentobarbital-induced sleeping time. After 60 min, individual mouse was placed in the middle of the hole-board test apparatus, and total number of head-dips of mice into the holes and the number of rears were observed and recorded over a time span of 5 min. The floor of the apparatus was bathed to clear away the traces of earlier paths after each trial. A decrease in the total number of head dips, dipping time of the head, and the total number of rears compared to the control was considered to indicate a sedative effect.<sup>[17]</sup>

#### Open field test

This test procedure was initially reported by Barros *et al.*<sup>[18]</sup> The open field is a nonconditioned anxiety test to record locomotion, the speed of locomotion, rearing, and general motor activity. The experiment was performed according to the procedure earlier described by López-Rubalcava *et al.* with a few modifications.<sup>[19]</sup> The apparatus was fabricated using plywood measuring 60 cm × 60 cm × 40 cm. Thin transparent glass was used to frame to ensure that the mice under observation was visible. The floor built up of cardboard was divided into 12 equal squares. The mice were grouped as mentioned in the procedure to test for sodium pentobarbital-induced sleeping time. The Group I animals served as a negative control; Group II as a positive control

which received diazepam (1 mg/kg, b.w., i.p.); and Groups III to V were treated with GGSME as mentioned in the procedure to test for sodium pentobarbital-induced sleeping time. After 30 and 60 min post-treatment of standard and GGSME administration, individual mouse was placed in the corner of the apparatus and the animal behavior was observed for 5 min session through video recording. The locomotion (the total number of times the mice entered each square [counts per 5 min]) and rearing (frequency with which the animal stood on its hind legs) was observed and recorded.

#### Effect on pentylenetetrazole-induced convulsions in mice

Pentylenetetrazole (PTZ)-induced convulsions test was carried out to assess the anticonvulsant activity of the GGSME as reported earlier by Löscher *et al.*<sup>[20]</sup> The mice used in this test were grouped as mentioned in the procedure to test for sodium pentobarbital-induced sleeping time. Group I and Groups III–V were treated as mentioned in the procedure to test for sodium pentobarbital-induced sleeping time. Group II was treated with diazepam 1.0 mg/kg b.w., i.p. 30 min after i.p. injection, and 60 min after oral administration, convulsions were induced to all the groups by the administration of PTZ (90 mg/kg b.w., i.p.). The period of time that elapsed between the administration of the pro-convulsive and the first myoclonus and tonic extension was visually assessed for a time span of 40 min. The percentage of mice that died within 40 min was observed and recorded. Mice that did not convulse within 40 min after PTZ administration were considered protected.

#### Picrotoxin-induced convulsions in mice

This test was carried out to assess the anticonvulsant activity of the GGSME as reported earlier by Estrada-Reyes *et al.*<sup>[21]</sup> GGSME was administered to mice of Groups III to V as mentioned in the procedure to test for sodium pentobarbital-induced sleeping time. The Group I animals received only distilled water. Groups II was treated with diazepam (1 mg/kg, b.w., i.p.). Thirty minutes after i.p. injection and 60 min after oral administration, convulsions were induced to all the groups by the administration of picrotoxin (10 mg/kg, b.w., i.p.). The presence or absence of clonic convulsions, as well as the latency to clonic and tonic seizures, was monitored for 40 min following the administration of picrotoxin. The percentage of mice protected from the administration of picrotoxin was observed and recorded.

### Muscle coordination test

#### Grip strength test

This test is performed to evaluate the muscle strength or neuromuscular activity in mice. This test procedure was earlier described by Boissier and Simpon.<sup>[22]</sup> Animals of either sex were placed on a thin steel rod, which is fixed to a stand at a height of 50 cm. All animals which continue hanging to steel rod for about 1 min duration were selected for this test. The mice were divided randomly into groups as mentioned in the procedure to test for sodium pentobarbital-induced sleeping time. Group II received diazepam (1 mg/kg i.p.), at the same time, the remaining groups received treatment as mentioned in the procedure to test for sodium pentobarbital-induced sleeping time. The fall off time of the standard and extract treated groups from thin steel rod was compared with the control group as a measure of muscle relaxant activity.

#### Rota rod test

This test was performed as described previously by Dunham and Miya (1957) to study drugs interfering with motor coordination.<sup>[23]</sup> This experiment is performed using rota rod apparatus, which is of four-compartment model (V. J. Instruments, India Inc.). Mice of either sex were divided randomly into groups ( $n = 6$ , of either sex) and were treated as cited in the procedure for test for sodium pentobarbital-induced

sleeping time. Group I and Groups III to V were treated as cited in the procedure for test for sodium pentobarbital-induced sleeping time, while the positive control group (Group II) received 1 mg/kg i.p. of diazepam. Thirty minutes after i.p., and 60 min after oral administration of standard and test extract, animals were kept on rota rod revolving at a speed of 24 rpm/min. Mice of all the groups were subjected to rota rod test, and mice, which fell off from the revolving rod were recorded at 30 min, 60 min, and 90 min, respectively. The difference in fall of time observed with a negative control group and extract treated group was an index of muscle relaxant activity.

### Statistical analysis

Numerical data were expressed as mean  $\pm$  standard error of mean. Statistical analysis was carried out with one-way analysis of variance, followed by Tukey's multiple comparison test and  $P \leq 0.05$  was considered to be statistically significant. The statistical analysis was carried out with GraphPad Prism 5.0 software (GraphPad Software, Inc. La Jolla, USA).

## RESULTS

### Acute toxicity studies

The study results showed no toxic symptoms/mortality in mice treated with GGSME (2000 mg/kg) until the 14<sup>th</sup> day of the treatment period, according to the OECD 423-2d guidelines. Based on the obtained results, we have selected 100, 200, and 400 mg/kg as low, moderate, and high doses to evaluate the depressant effects and muscle coordination activity.

### Central nervous system depressant activity

#### Test for sodium pentobarbital-induced sleeping time

Sodium pentobarbital-induced sleep in mice is a classic pharmacological method for the screening of sedative-hypnotic drugs. The sedative and hypnotic actions of GGSME administered to animals in combination with sodium pentobarbital are shown in Figure 1. GGSME in combination with sodium pentobarbital exhibited significant ( $P \leq 0.001$ ) synergistic sedative and hypnotic effects, and these effects are in a dose-dependent manner. The duration of the sleeping time produced by sodium pentobarbital was significantly ( $P \leq 0.05$ ) prolonged in a dose-dependent manner with GGSME (400 mg/kg, b.w.).

#### Hole-board test

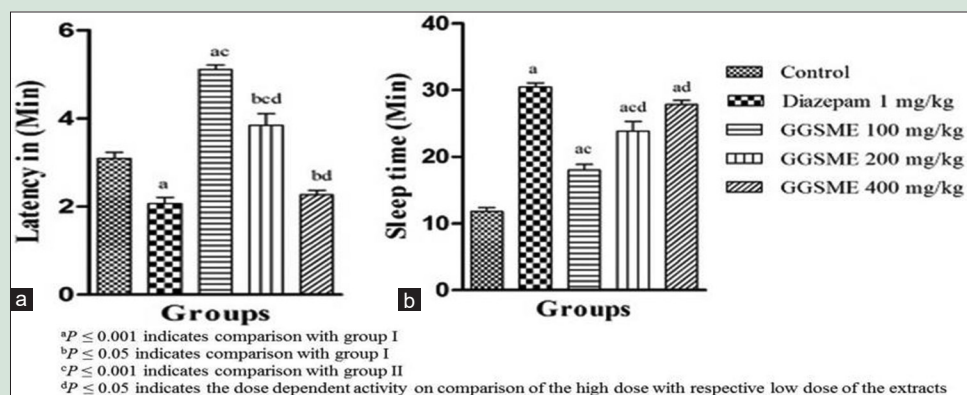
The sedative action of GGSME was proved in the hole-board test. Figure 2 shows the actions of both diazepam (1.0 mg/kg) and GGSME of varying doses on the performance of mice in the hole-board test. Treatment with GGSME (400 mg/kg) significantly ( $P \leq 0.001$ ) decreased the head-dipping number ( $9.8 \pm 0.3$ ) and the total number of rears, when compared to the control group.

#### Open field test

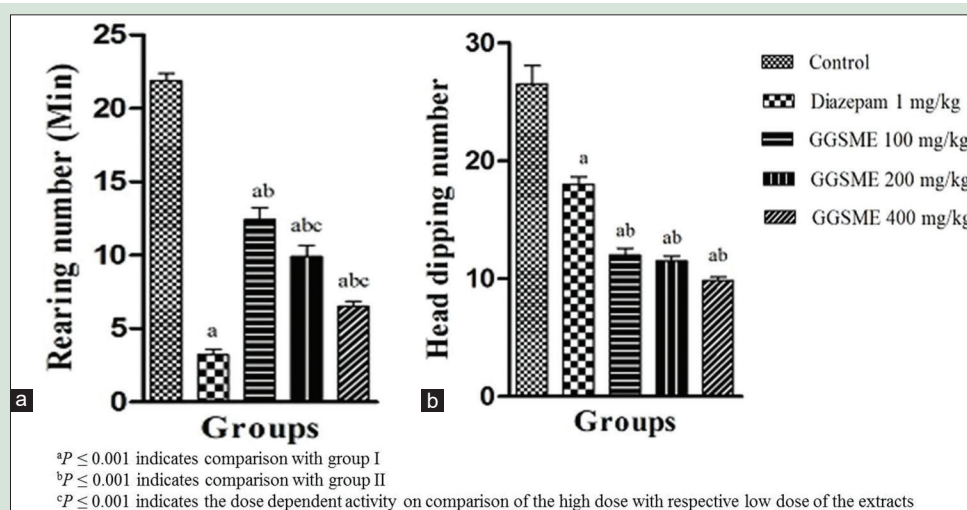
The CNS depressant effect of GGSME was proved by the behavior of mice in open field test. The GGSME significantly ( $P \leq 0.001$ ) decreased the rearing and spontaneous ambulatory activity of the animals. The above study results are shown in Figure 3.

#### Effect on pentylenetetrazole-induced convulsions in mice

In the PTZ model, the latency time of myoclonus was delayed by diazepam (1.0 mg/kg), tonic seizures were kept at a halt, and the occurrence of death was prevented in comparison to the control group, whereas GGSME did not inhibit the appearance of myoclonic seizure, but inhibited the tonic seizures ( $P \leq 0.001$ ). Nevertheless, GGSME at all test doses significantly ( $P \leq 0.001$ ) delayed the onset of PTZ-induced convulsions. The percentage of dead animals was found to be nil when treated with GGSME. The results are shown in Table 1.



**Figure 1:** Effects of *G. glauca* stem methanol extract (GGSM) on sodium pentobarbital induced sleeping time. (a) Effect of GGSM on the latency of sodium pentobarbital induced sleep; (b) Effect of GGSM on the time of sleep induced by sodium pentobarbital



**Figure 2:** Effects of *G. glauca* stem methanol (GGSM) extract on (a) sedative behavior (rearing number); (b) sedative behavior (head dipping number)

**Table 1:** Effect of *Galphimia glauca* stem methanol extract on pentylenetetrazole-induced convulsions in mice

Group	Dose (mg/kg)	Number of animals convulsed/used	Latency of tonic convulsions in mice (min)	Mortality (%)
I. Negative control	Distilled water	10/10	5.5±0.3	100
II. Diazepam	1	0/10	- <sup>a</sup>	0
III. GGSM	100	0/10	19.3±1.5 <sup>b</sup>	0
IV. GGSM	200	0/10	26.8±1.7 <sup>b</sup>	0
V. GGSM	400	0/10	- <sup>a,c,d</sup>	0

<sup>a</sup> $P \leq 0.01$  indicates comparison with Group I; <sup>b</sup> $P \leq 0.001$  indicates comparison with Group I; <sup>c</sup> $P \leq 0.001$  indicates comparison with Group II; <sup>d</sup> $P \leq 0.01$  indicates the dose-dependent activity in comparison of the high dose with respective low doses of the extracts. GGSM: *Galphimia glauca* stem methanol extract

### Picrotoxin-induced convulsions in mice

GGSM significantly ( $P \leq 0.01$ ) delayed the appearance of both clonic and tonic seizures and the GGSM decreased the mortality to 0% induced by picrotoxin. The results are shown in Table 2.

## Muscle coordination test

### Grip strength test

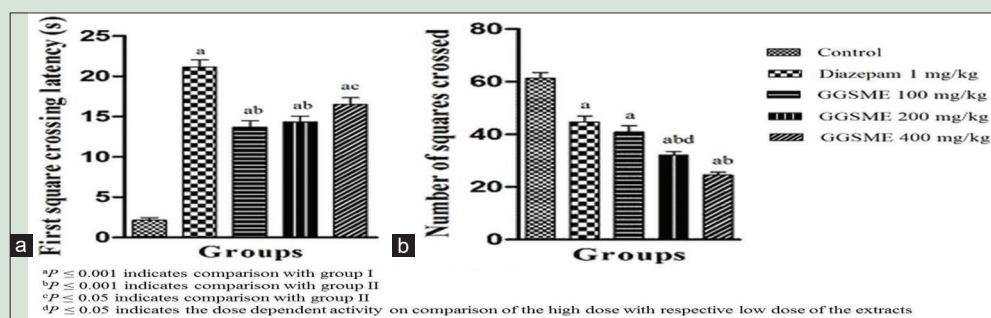
The GGSM exhibited a significant ( $P \leq 0.05$ ) muscle relaxant activity with grip test, which is proved by the poor performance of the mice when subjected to hanging to the thin steel rod. When compared to control group, the percentage of mice losing their catching reflex is found to be significant ( $P \leq 0.001$ ) when treated with the extract. GGSM also showed a significant ( $P \leq 0.05$ ) dose-dependent activity. The results are shown in Figure 4.

### Rota rod test

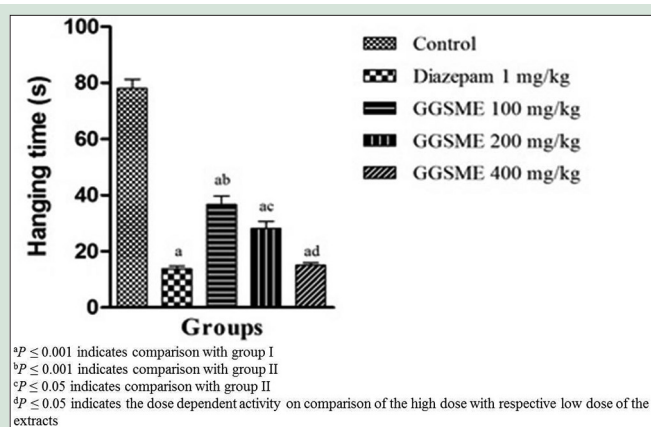
The experimental results were shown in Figure 5. The percentage of mice falling from the rota rod in both diazepam (1 mg/kg) and GGSM-treated groups exhibited a significant ( $P < 0.001$ ) reduction in time spent by the mice in the rota rod test when compared with the control group.

## DISCUSSION

The shrub *G. glauca* was previously reported regarding its usage in the treatment of anxiety.<sup>[8]</sup> Ethnomedical information assumes that the above effects may be mediated through its actions on the CNS. From the above findings in this current study, we made an effort in assessing the presumed



**Figure 3:** Effect of *G. glauca* stem methanol extract (GGSME) on the ambulatory behavior of mice in open field test. (a) First square crossing latency (s); (b) number of squares crossed



**Figure 4:** Effect of *G. glauca* stem methanol extract (GGSME) on grip test in mice

**Table 2:** Effects of *Galphimia glauca* stem methanol extract on picrotoxin-induced convulsions in mice

Group	Dose mg/kg	Number of animals convulsed/ used	Latency of tonic convulsions in mice (min)	Mortality (%)
I. Control	Distilled water	10/10	6.5±0.2	100
II. Diazepam	1	0/10	— <sup>a</sup>	0
III. GGSME	100	0/10	16.1±1.1 <sup>a,b</sup>	0
IV. GGSME	200	0/10	21.1±1.6 <sup>a,b</sup>	0
V. GGSME	400	0/10	— <sup>a,b,c</sup>	0

<sup>a</sup>*P*≤0.001 indicates comparison with Group I, <sup>b</sup>*P*≤0.001 indicates comparison with Group II, <sup>c</sup>*P*≤0.001 indicates the dose-dependent activity in comparison of the high dose with respective low doses of the extracts. GGSME: *Galphimia glauca* stem methanol extract

depressant effects and muscle coordination activity on GGSME.

Anxiety is implicated in the number of psychiatric disorders such as panic attacks, phobia, generalized anxiety disorders, obsessive-compulsive disorders, and stress disorders. In such situations, individuals experience an emotional state of fear felt by persistent feelings of nervousness, tension, or unrealistic worried thoughts, and experience physical changes such as increased blood pressure showing its effect on a person’s physical and mental health. People with such neuropsychiatric disorders experience symptoms such as restlessness, irritability, sleep disturbance, muscle tension, difficulty in concentrating, being easily tired, feeling stressed and panic. Psychiatric disorders can occur any time round-the-clock, and a person with anxiety always tries to avoid places or situations where they have occurred. Depressants are used for relaxation that slows normal brain function, and hence, used in the treatment of various conditions of anxiety, panic attacks, stress, sleep disorders, soothing the nerves, anticonvulsants and as well as in sedatives and hypnotics.

In the present study, GGSME showed depressant actions on the CNS. Although the extract did not cause sleep, the mice treated with the extract were found to be awake, relaxed, and calm. However, administration of stem methanol extract with single doses of 100, 200, and 400 mg/kg, b.w. 60 min before the administration of sodium pentobarbital resulted in decreased sleep latency and increased sleep time. The sedation is a CNS depressant effect, and the sedative actions of drugs are commonly evaluated by checking the sleep time spell, which is induced by sodium pentobarbital in experimental animals.<sup>[24,25]</sup>

The pentobarbital acts by depressing the reticular activating system, which is responsible for regulation of sleep, wakefulness, level of arousal, and coordination of eye movements. Due to this, sleep latency was

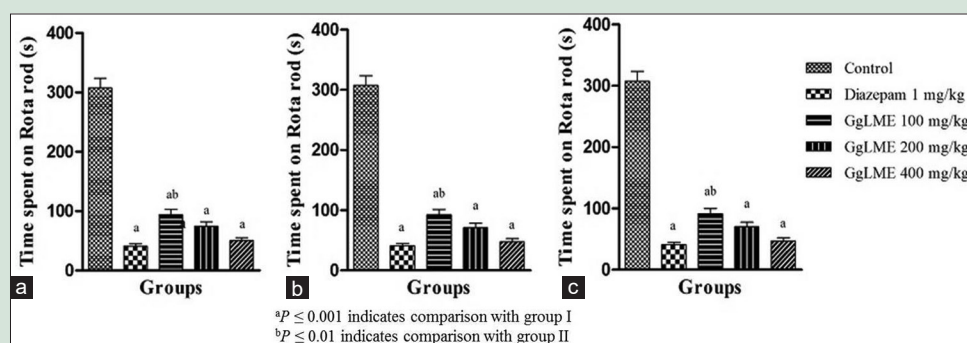
decreased, the number of awakenings was reduced, the time spent in rapid eye movement (REM) sleep was reduced, while non-REM sleep was increased, and the slow wave sleep was reduced. There is an increased reaction time and the individual cannot be easily aroused. These actions are due to their affinity for gamma-aminobutyric acid type A (GABA<sub>A</sub>) receptors. In addition, it also blocks the AMPA receptor, a subtype of glutamate receptor, thereby inhibiting the effects of excitatory transmitter glutamate. The overall effect results in the depression of CNS.<sup>[26,27]</sup>

The GGSME (400 mg/kg, b.w.) showed its capability to reinforce sodium pentobarbital-induced hypnosis in a dose-dependent manner. This effect may be due to its actions on the CNS that are involved in the regulation of sleep,<sup>[28,29]</sup> or perhaps assumed that the GABAergic system participates in the GGSME-induced enhancement effects of pentobarbital.

Diazepam is capable of producing sedative-hypnotic effects. It significantly reduces sleep latency and the number and duration of night-time awakenings, resulting in an increase in total sleep time. It shows its effect by binding to molecular components (α and γ subunits) of GABA<sub>A</sub> receptors present in the neuronal membranes in the CNS and facilitates the opening of chloride ion channel indirectly (GABAergic action) and enhances membrane hyperpolarization. The effects of GGSME are found similar to that of reference standard drug diazepam.<sup>[27]</sup>

The reaction of the mice toward uncommon environment is studied using a hole-board test. This method is generally employed to evaluate the emotional condition, anxiety, and the reaction toward stress.<sup>[30]</sup> The reduced number of head dips is an indication of CNS depressant effects.<sup>[31]</sup> In this evaluation, extract-treated animals showed a significant (*P* ≤ 0.001) reduction in the number of head dips, suggesting the decreased levels of anxiety and increased exploratory behavior in animals.

The open field method provides a concurrent measure of locomotion, exploration, and anxiety.<sup>[32]</sup> Locomotion and rearing is a reaction to



**Figure 5:** Effects of *G. glauca* stem methanol extract (GGSME) on muscle coordination activity in rota rod. (a) Time spent (s) by the mice on rota rod after 30 min; (b) time spent (s) by the mice on rota rod after 60 min; (c) time spent (s) by the mice on rota rod after 90 min

the levels of excitability of CNS. Through this method, the depressant effects of the GGSME are further proved by the locomotion (decreased in the number of times the mice had crossed the squares) and decreased exploration reaction of the animals (object sniffing and rearing).<sup>[27]</sup> The extract significantly ( $P \leq 0.001$ ) decreased the rearing and spontaneous ambulatory activity of the animals. In addition, the CNS depressant actions of extract are confirmed by its dose-dependent reduction in the exploratory behavior and locomotor activity of mice.

The diazepam is used in the treatment of panic disorder, generalized anxiety disorder, phobia disorder, stress disorder, and obsessive-compulsive disorder.<sup>[33,34]</sup> In-hole board test diazepam exhibits its antianxiety effects, producing a state of calmness and quietness, and it also improved the exploratory behavior. In open field test, diazepam is quite effective in rapidly controlling the hyperexcitability of the CNS.

GABA is the major inhibitory neurotransmitter in the CNS, and diazepam potentiates the GABAergic inhibition at different levels of the CNS. GABA<sub>A</sub>  $\alpha_2$ ,  $\alpha_3$ , and  $\alpha_5$  subunits mediate anxiolytic and muscle relaxant effects of benzodiazepines, whereas  $\alpha_1$  receptors mediate the sedative effects.<sup>[33]</sup> The activity of GGSME observed is perhaps due to the above-said mechanisms. In PTZ- and picrotoxin-induced convulsion study in animals, the seizure onset is the time taken from the injection of PTZ/picrotoxin to the first myoclonic jerks of the forelimbs, which is treated as the first sign of the inception of a seizure activity.<sup>[21,35]</sup> The mice used in this investigation were noticed for varied seizure stages which include head twitches, tremor/confusion, individual jumps/jerks, clonic seizure, orofacial seizure, tonic seizure, straub tail, and death.

The generalized seizures consist of both clonic and tonic seizures. The clonic seizures are a stiff extension of the forelimbs/hindlimbs with or without loss of stance, whereas the clonic seizure consisted of rhythmic contractions of forelimbs/hindlimbs. GGSME showed a significant protection against convulsions induced by PTZ. It increased the threshold of clonic seizures induced by PTZ and delayed the progression to tonic convulsion. GGSME was significant ( $P \leq 0.001$  and  $P \leq 0.01$ ) in delaying the onset of seizures induced by PTZ and picrotoxin, respectively. There is a possibility that the increase of the convulsion latency time could be related to a CNS depressant activity.<sup>[21]</sup> Nevertheless, GGSME at all tested doses of 100, 200, and 400 mg/kg b.w. decreased the percentage of deaths to zero in both PTZ- and picrotoxin-induced convulsions.

PTZ/picrotoxin shows its actions by inhibiting GABA activity. The standard diazepam acts by inhibiting the PTZ/picrotoxin-induced seizure. It is effective for stopping continuous activity, especially generalized tonic-clonic status epilepticus and prolonged febrile seizures in children. It suppresses the spread of seizure activity.

The GABA<sub>A</sub> receptor is composed of various combinations of subunits ( $\alpha$ ,  $\beta$ ,  $\gamma$ ,  $\delta$ ,  $\epsilon$ ,  $\pi$ ,  $\rho$ , and  $\theta$ ). Various subunit isoforms have been identified

with  $6\alpha$ ,  $3\beta$ ,  $3\gamma$ , and  $3\rho$  subunits. The binding pocket for benzodiazepines is between  $\alpha_1$  and the  $\gamma_2$  subunits. Diazepam acts on a GABA<sub>A</sub> type of receptors, increases the frequency, but not the duration of the opening of the chloride ion channels.<sup>[34,36]</sup> The activity of GGSME observed is perhaps due to the above-said mechanisms.

The evaluation for muscle coordination was performed employing grip strength test and rota rod test. The muscle strength and neuromuscular activity in mice are evaluated using a grip strength test,<sup>[22]</sup> while the actions of drugs interfering with motor coordination are assessed using a rota rod test. The mice on revolving rod are allowed to spend time on it. The animal which spends less time on revolving rod suggests the muscle relaxant actions of the tested compound.<sup>[23]</sup> Many CNS depressive drugs are effective in this test.<sup>[36]</sup>

The diazepam exhibits skeletal muscle-relaxing effects by binding to GABA<sub>A</sub> receptors containing  $\alpha_2$  and  $\alpha_3$  subunits, enhancing GABA potency to increase the chloride conductance, the result is a neuronal hyperpolarization, probably at both supraspinal and spinal sites for spasmolytic activity.<sup>[37]</sup> GGSME showed a significant ( $P \leq 0.05$ ) dose-dependent activity in grip strength test and exhibited a significant ( $P < 0.001$ ) reduction in time spent by the mice in a rota rod test. Our study results are very much alike to that of diazepam used in the study of grip strength test and rota rod test, which concludes that the GGSME acts similarly to that of standard drug diazepam.

## CONCLUSION

The present study concludes that GGSME has significant CNS depressant effects and muscle relaxant properties against all the tested models. The results support the traditional claims of the plant in the treatment of phobia, panic, stress, anxiety, and used in producing a calming effect on the nerves. This suggests that the biological constituents present in the plant are worthy of further investigation and structure elucidation.

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## Conflicts of interest

There are no conflicts of interest.

## REFERENCES

- WHO. Re-defining "Health"; March, 2016. Available from: <http://www.who.int/bulletinboard/83/ustun11051/en/>. [Last accessed on 2016 Mar 02].
- Tortoriello J, Lozoya X, Gomez E, Brunner M. Mesencephalic effects of galphimine-B, a triterpenoid from *Galphimia glauca*. *Neurophytopharmaceuticals* 1998;3:77-96.
- Herrera-Ruiz M, González-Cortazar M, Jiménez-Ferrer E, Zamilpa A, Alvarez L, Ramirez G, *et al.* Anxiolytic effect of natural galphimines from *Galphimia glauca* and their chemical derivatives. *J Nat Prod* 2006;69:59-61.
- Campos MG, Toxqui E, Tortoriello J, Oropeza MV, Ponce H, Vargas MH, *et al.* *Galphimia glauca* organic fraction antagonizes LTD(4)-induced contraction in Guinea pig airways. *J Ethnopharmacol* 2001;74:7-15.
- Nader BL, Taketa AT, Pereda-Miranda R, Villarreal ML. Production of triterpenoids in liquid-cultivated hairy roots of *Galphimia glauca*. *Planta Med* 2006;72:842-4.
- Ruiz HM, Cortazar GM, Ferrer JE, Zamilpa A, Alvarez L, Ramirez G, *et al.* Anxiolytic effects of natural galphimines from *Galphimia glauca* and their chemical derivatives. *J Nat Prod* 2007;70:2054.
- Hussain S, Schönbichler SA, Güzel Y, Sonderegger H, Abel G, Rainer M, *et al.* Solid-phase extraction of galloyl- and caffeoylquinic acids from natural sources (*Galphimia glauca* and *Arnicae flos*) using pure zirconium silicate and bismuth citrate powders as sorbents inside micro spin columns. *J Pharm Biomed Anal* 2013;84:148-58.
- Herrera-Ruiz M, Jiménez-Ferrer JE, De Lima TC, Avilés-Montes D, Pérez-García D, González-Cortazar M, *et al.* Anxiolytic and antidepressant-like activity of a standardized extract from *Galphimia glauca*. *Phytomedicine* 2006;13:23-8.
- Tortoriello J, Romero O. Plants used by Mexican traditional medicine with presumable sedative properties: An ethnobotanical approach. *Arch Med Res* 1992;23:111-6.
- Estrada E. Jardín Bota'nico de Plantas Medicinales "Maximino Martí'nez". Me'xico Universidad Auto'noma de Cha-pingo, Departamento de Fitotecnia; 1985. p. 15.
- Tortoriello J, Herrera-Arellano A, Herrera-Ruiz ML, Zamilpa A, Gonzalez-Cortazar M, Jimenez-Ferrer JE. New Anxiolytic Phytopharmaceutical Elaborated with the Standardized Extract of *Galphimia glauca*, Anxiety Disorders. InTech; 2011. p. 185-202. Available from: <http://www.intechopen.com/books/anxiety-disorders/new-anxiolytic-phytopharmaceutical-elaborated-with-the-standardized-extract-of-galphimia-glauca>. [Last accessed on 2016 Mar 02].
- Tortoriello J, Lozoya X. Effect of *Galphimia glauca* methanolic extract on neuropharmacological tests. *Planta Med* 1992;58:234-6.
- Acute oral toxicity – Acute toxic class method. OECD Guideline for Testing of Chemicals. OECD Guidelines 423; 2001.
- Fujimori H. Potentiation of barbitol hypnosis as an evaluation method for central nervous system depressants. *Psychopharmacologia* 1965;7:374-8.
- Li T, Xu G, Wu L, Sun C. Pharmacological studies on the sedative and hypnotic effect of solidroside from the Chinese medicinal plant *Rhodiola sachalinensis*. *Phytomedicine* 2007;14:601-4.
- Boissier JR, Simon P, Lwoff JM. Use of a particular mouse reaction (Hole board method) for the study of psychotropic drugs. *Therapie* 1964;19:571-83.
- Viola H, Wasowski C, Levi de Stein M, Wolfman C, Silveira R, Dajas F, *et al.* Apigenin, a component of *Matricaria recutita* flowers, is a central benzodiazepine receptors-ligand with anxiolytic effects. *Planta Med* 1995;61:213-6.
- Barros HM, Tannhauser MA, Tannhauser SL, Tannhauser M. Enhanced detection of hyperactivity after drug withdrawal with a simple modification of the open-field apparatus. *J Pharmacol Methods* 1991;26:269-75.
- López-Rubalcava C, Piña-Medina B, Estrada-Reyes R, Heinze G, Martínez-Vázquez M. Anxiolytic-like actions of the hexane extract from leaves of *Annona cherimola* in two anxiety paradigms: Possible involvement of the GABA/benzodiazepine receptor complex. *Life Sci* 2006;78:730-7.
- Löscher W, Hönack D, Fassbender CP, Nolting B. The role of technical, biological and pharmacological factors in the laboratory evaluation of anticonvulsant drugs. III. pentylenetetrazole seizure models. *Epilepsy Res* 1991;8:171-89.
- Estrada-Reyes R, Martínez-Vázquez M, Gallegos-Solis A, Heinze G, Moreno J. Depressant effects of *Clinopodium mexicanum* Benth. Govaerts (Lamiaceae) on the central nervous system. *J Ethnopharmacol* 2010;130:1-8.
- Boissier JR, Simpon P. L'utilisation du test de la traction, pour l'etude des psycholeptiques. *Therapie* 1960;15:1170-4.
- Dunham NW, Miya TS. A note on a simple apparatus for detecting neurological deficit in rats and mice. *J Am Pharm Assoc Am Pharm Assoc* 1957;46:208-9.
- Carpenedo R, Chiarugi A, Russi P, Lombardi G, Carlà V, Pellicciari R, *et al.* Inhibitors of kynurenine hydroxylase and kynureninase increase cerebral formation of kynurenate and have sedative and anticonvulsant activities. *Neuroscience* 1994;61:237-43.
- Gamaniel K, Amos S, Akah PA, Samuel BB, Kapu S, Olusola A, *et al.* Pharmacological profile of NIPRD 94/002/1-0. A novel herbal antisickling agent. *J Pharm Res Dev* 1998;3:89-94.
- Petty F. GABA and mood disorders: A brief review and hypothesis. *J Affect Disord* 1995;34:275-81.
- Morais LC, Barbosa-Filho JM, Almeida RN. Central depressant effects of reticuline extracted from *Ocotea duckei* in rats and mice. *J Ethnopharmacol* 1998;62:57-61.
- N'gouemo P, Nguemby-Bina C, Baldy-Moulinier M. Some neuropharmacological effects of an ethanolic extract of *Maprounea africana* in rodents. *J Ethnopharmacol* 1994;43:161-6.
- Kaul PN, Kulkarni SK. New drug metabolism inhibitor of marine origin. *J Pharm Sci* 1978;67:1293-6.
- Wei XY, Yang JY, Wang JH, Wu CF. Anxiolytic effect of saponins from *Panax quinquefolium* in mice. *J Ethnopharmacol* 2007;111:613-8.
- Adzu B, Amos S, Dzarma S, Wambebe C, Gamaniel K. Effect of *Ziziphus spina-christi* wild aqueous extract on the central nervous system in mice. *J Ethnopharmacol* 2002;79:13-6.
- Ericson E, Samuelsson J, Ahlenius S. Photocell measurements of rat motor activity. A contribution to sensitivity and variation in behavioral observations. *J Pharmacol Methods* 1991;25:111-22.
- Prasad PJ. *Conceptual Pharmacology*. 1<sup>st</sup> ed. Hyderabad: Universities Press (India) Private Limited; 2010. p. 170-289.
- Katzung BG, Masters SB, Trevor AJ. *Basic and Clinical Pharmacology*. 11<sup>th</sup> ed. Mumbai: Tata McGraw-Hill; 2010. p. 371-86.
- Yemitan OK, Adeyemi OO. CNS depressant activity of *Lecaniodiscus cupanioides*. *Fitoterapia* 2005;76:412-8.
- Vogel HG. *Drug Discovery and Evaluation Pharmacological Assays*. 2<sup>nd</sup> ed. Berlin: Springer; 2002. p. 390-493.
- Lemke TL, Williams DA, Roche VF, Zito SW. *Foye's Principles of Medicinal Chemistry*. 7<sup>th</sup> ed. New Delhi: Wolters Kluwer (India) Pvt. Ltd.; 2013. p. 438-40.

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