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in vivo Study of Central Nervous System Depressant Effects and Muscle Relaxant Activity of Methanolic Extract of *Galphimia gracilis* Leaf on Swiss Albino Mice

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This study assesses the central nervous system depressant effects and muscle relaxant activity of methanolic extract of *Galphimia gracilis* leaf in Swiss albino mice employing models like open field test, hole-board test, sodium pentobarbital-induced sleep test, pentylenetetrazole induced convulsions, picrotoxin-induced convulsions, rota rod test and grip strengthening test. The LD_{50} results of the extract was found to be > 2000 mg/kg. The extract exhibited significant ($P \leq 0.001$) effects in both the open field test and hole-board test. The extract at 100, 200 and 400 mg/kg doses extended the duration of sleep induced by sodium pentobarbital (40 mg/kg) and also delayed the onset of seizures induced by pentylenetetrazole (90 mg/kg) and picrotoxin (10 mg/kg) respectively. The extract also exhibited significant ($P \leq 0.001$) effects in Grip strengthening test and Rota rod test. The study concludes that the methanolic extract of *Galphimia gracilis* leaf has significant depressant activity and muscle relaxant effects.

Keywords: Central nervous system, Convulsion, Depression, *Galphimia gracilis*, Grip strength test, Muscle relaxant activity.

INTRODUCTION

The importance of medicinal plants has been recognized and documented well by research scholars since age-old [1]. Apart from the social assistance, much interest has been given to the plants of medicinal significance [2]. Today, many people in the developing countries trust in the alternative system of medicine where medicinal plants are used for their primary health care [3]. Due to the continuously increasing need for medicinal plants, many herbal research centers/institutes across the globe are engaged in scientific investigations, developments, documentation and developing databases on the medicinal plants helping the scientific community [4]. Still, there is a need to identify, document, verify and make use of the tribal based knowledge on medicinal plants. Hence, the search for the medicinal plants for potential therapeutic benefits is always a continuous process.

Government of India at the national level established various independent bodies and research councils to coordinate all matters relating to medicinal plants and support policies and programmes for growth of cultivation, trade, export, conservation, processing technologies and Research and development in areas related to medicinal plants through CCRAS (Central council for Research in Ayurveda Sciences), CCRUM (Central council for Research in Unani Medicine), CCRH (Central council for Research in Homoeopathy), CCRYN (Central council for Research in Yoga and Naturopathy), AYUSH (Department of Ayurveda, Yoga, Unani, Sidda and Homeopathy), NMPB (National Medicinal Plants Board), CIMAP (Central Institute of Medicinal and Aromatic Plants), NEIST (North East Institute of Science and Technology), NRCMAP (National Research Centre for Medicinal and Aromatic Plants) and Ministry of Health & Family welfare.

Galphimia gracilis (Gg) is an evergreen plant measuring about 5-15 feet in height with yellow flowers that belongs to the family of Malpighiaceae [5]. The plant *Galphimia gracilis* is indigenous to Mexico, which has been introduced in mega biodiversity nation India. It is found extensively spread in Eastern Deccan Plateau regions of India. It is commonly known as "Slender goldshower and Thyrallis." *G. gracilis* has been an ever blooming plant with yellow flowers. It has reddish brown hairs on stems, shoots and leaf petioles. The leaves are elliptical in shape measuring about 2.5-7 cm long and 2-3 cm wide [6]. It is identified from other species of galphimia by its clawed yellow petals turning red on ageing. The inflorescence is a terminal raceme with bi-laterally symmetrical [5].

Galphimia glauca and *Galphimia gracilis* both belong to the genius Galphimia. Traditionally *Galphimia glauca* is used

to treat anxiety, fear, phobia, stress and was used to produce a calming effect on the nerves [7-10]. *Galphimia gracilis* belongs to the same genus Galphimia, which has not been investigated for its pharmacological actions to any great extent [5]. Based on the traditional uses of the plants belonging to the genus Galphimia, the current study is planned to explore the central nervous system depressant effects and muscle relaxant activity of methanol extract of *Galphimia gracilis* leaf (GgLME) using animal models.

EXPERIMENTAL

The *Galphimia gracilis* was collected from the lawn in Osmania University campus, Hyderabad, Telangana State, India. The leaves were collected in November, 2014. The plant was identified and authenticated by well-known taxonomist, Dr. E. Narsimha Murthy, Satavahana University, Karimnagar, Telangana State, India. A voucher copy is deposited with the reference number No. 437 in the Department of Pharmacognosy and Phytochemistry, School of Pharmacy, Anurag Group of Institutions, Hyderabad, India.

Chemicals used were purchased from SD Fine chemicals, Mumbai, India. Sodium pentobarbital was purchased from Sigma Chemicals Co., USA; diazepam was procured from Natco Pharmaceuticals, India Ltd, pentylenetetrazole from Sigma-Aldrich, USA. Picrotoxin was received as a gift sample from Sri Disha biotech, Hyderabad, India Ltd.

Preparation of the methanolic extract of *Galphimia gracilis* leaf: *Galphiia gracilis* leaf powder (150 g) was subjected to Soxhlet extraction using 500 mL of methanol. The extract obtained was concentrated to dryness and stored. The yield obtained from *Galphimia gracilis* leaf methanol extract (GgLME) was 12 %.

Swiss albino mice of 6 to 8 weeks of age with 22 ± 2 g of either sex were acclimatized for week days to the laboratory conditions. The animals were maintained in 12 h light/dark cycles at 22 ± 2 °C with 60 to 70 % relative humidity. Complete studies were performed randomly using 6 mice 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 [11].

Phytochemical screening: Qualitative phytochemical screening of the *G. gracilis* leaf methanol extract (GgLME) was performed using various chemical tests *i.e.* alkaloids (Wagner's test, Mayer's test, Hager's test and Dragendorff's test), anthraquinone glycosides (Borntrager's test and Modified Borntrager's test), cardiac glycosides (Keller-killiani test, Legal's test and Balget's test), saponin glycosides (Foam test), tannins and phenolic compounds (ferric chloride test, bromine water test, lead acetate test and iodine test), steroids and terpenoids (Salkowski test and Libermann-Burchard test), volatile oils (1 % osmic acid test and Sudan red-III test), carbohydrates (Fehling's test, Molish's test, Barfoed's test and Benedict's test) and proteins & amino acids (Biuret's test, Millon's test and Ninhydrin test) [12,13].

Central nervous system depressant activity

Open field test: The open field test procedure was first described by Barros *et al.* [14]. It is a non-conditioned anxiety test to evaluate rearing, general motor activity, locomotion and the speed of locomotion. The test was conducted according to the procedure previously stated by Rubalcava *et al*. [15] with some modifications. The apparatus used in this study was made up of plywood measuring 60 cm × 60 cm × 40 cm. Glass of suitable size was used in the making to make sure that the mice under observation were visible. The floor of the apparatus made of cardboard was divided equally into 12 squares. The mice were grouped as cited below Group I served as negative control; Group II served as a positive control received diazepam (1 mg/kg, b.w, i.p.) and groups III to V received the *G. gracilis* extract. After 30 and 60 min post treatment of diazepam and GgLME administration, individual mice were placed in the corner side of the apparatus and the mouse behaviour was monitored for 5 min through video recording. The locomotion (The number of times the mice entered an individual square (counts per 5 min); rearing (frequency with which the mice stood on its hind legs) was observed and recorded [14,15].

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

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

Group III-V was treated with GgLME [100, 200 and 400 mg/kg, b.w., respectively, (p.o)].

Hole-board test: This method was described in the past by Boissier *et al*. [16] to evaluate specific elements of the behaviour of mice like exploration and curiosity. In 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 treated as a negative control, received only distilled water. The group II treated intraperitoneal (i.p) with standard drug diazepam (1 mg/kg, b.w) 30 min before performing the test. Methanol extract of *Galphimia gracilis* leaf was administered to groups as mentioned above. After 60 min, each animal was placed in the central space of the hole-board test apparatus and number of head-dips of animal into the holes and the numbers of rears was observed and recorded for a time interval of 5 min. The floor of the apparatus was maintained clean after each trial. A decrease in the number of head dips and rears compared to the control was treated to indicate a sedative effect [16,17].

Sodium pentobarbital induced sleeping time test: This method was reported by Fujimori [18]. The sedative and hypnotic effects of GgLME in combination with drug sodium pentobarbital were evaluated. Mice employed in this study were grouped as mentioned earlier. The group I treated as a negative control, received distilled water before the intraperitoneal administration of the sodium pentobarbital (40 mg/kg, b.w). Group II treated as standard, received diazepam through intraperitoneal route (1 mg/kg, b.w), while Groups III to V received leaf methanol extract 1h before the administration of sodium pentobarbital. Individual mice were placed on the platform and monitored for the uncoordinated movements to the sedative phase of the study. Loss of the righting reflex related to the hypnosis and the time period of the sleep was

also noticed and recorded. The duration of time elapsed between the loss and recovery of the righting reflex was treated as the sleeping time [18,19].

Pentylenetetrazole (PTZ) induced convulsions in mice: This test was carried out to investigate the anticonvulsant activity of the GgLME as stated earlier by Loscher *et al*. [20]. The animals used in this study were divided into groups as mentioned earlier. Groups I, III, IV and V were treated as mentioned before. Group II treated with diazepam (1.0 mg/kg b.w; i.p.). 30 min after intraperitoneal injection and 60 min after oral treatment, the convulsions were induced to all the groups by the intraperitoneal administration of pentylenetetrazole (90 mg/kg b.w). The time span that elapsed between the treatment of the pro-convulsive and the first myoclonus and tonic extension were assessed over a period of 40 min. The mice that died within 40 min were recorded and their percentage was calculated. Mice that did not convulse within 40 min after pentylenetetrazole treatment were considered safe [20].

Effect on picrotoxin induced convulsions in mice: This study was carried out to assess the anticonvulsant activity of the GgLME as reported by Reyes *et al*. [21]. The group I treated only with distilled water. Groups II was treated intraperitoneal with diazepam (1 mg/kg, b.w). Groups III to V was treated with GgLME as mentioned before. 30 min after intraperitoneal injection and 60 min after oral treatment, the convulsions were induced to all the groups by intraperitoneal administration of picrotoxin (10 mg/kg, b.w). The absence or presence of colonic convulsions, as well as the time period between the stimulus and response (latency) to clonus and tonic seizures, were monitored for a time span of 40 min after the treatment of picrotoxin. The mice protected following treatment of picrotoxin were recorded and their percentage was calculated [21].

Muscle relaxant activity

Rota rod test: This study was conducted as explained previously by Dunham and Miya [22] to examine drugs interfering with motor coordination. It is performed using Rota rod apparatus which is of four compartment model (V.J, Instruments, India Ltd.). Groups I, III, IV and V were treated as mentioned earlier, while the Group II received 1 mg/kg i.p injection of diazepam. 30 min after i.p and 60 min after oral treatment of standard and GgLME, animals were placed on rota rod revolving at an acceleration of 24 rpm. Animals of the groups I to V were subjected to this test and the time span (s) for the mice which spend on the revolving rod before falling down from the rod were recorded at 30, 60 and 90 min, respectively. The difference in fall off time noticed with a negative control group and GgLME treated group was considered as an index of muscle relaxant activity [22].

Grip strength test: This study is used to test muscular strength and neuromuscular functions in rodents. It was described previously by Boissier and Simpson [23]. The mice used in this study of either sex were placed on a metal rod, which is set up to stand at a height of 50 cm. The mice, which remained hanging to a metal rod for a time span of 1 min, were selected for this study. Animals in group II were treated intraperitoneal with diazepam (1 mg/kg) while the other groups received treatment as before. The fall off time of the standard and test groups from the thin metal rod was compared with the control group as a measure of relaxant activity [23].

Statistical analysis: Numerical data were expressed as mean ± SEM (standard error of mean). Statistical analysis was performed with one-way analysis of variance (ANOVA), followed by a Tukey's multiple comparison test and $p \leq 0.05$ was considered to be statistically significant. The statistical analysis was carried out with Graph Pad Prism 5.0 software.

RESULTS AND DISCUSSION

Acute toxicity studies: The results reveal no toxic symptoms/ mortality in animals treated with GgLME (2000 mg/kg, b.w.) until the 14th day of the study period, according to the OECD 423-2d guidelines. Based on the experimental results, we have preferred 100, 200 and 400 mg/kg, b.w as low, moderate and high doses to investigate the central nervous system depressant and muscle relaxant activity.

Preliminary phytochemical screening: Qualitative preliminary phytochemical screening of GgLME discloses the presence of primary and secondary metabolites like carbohydrates, proteins and amino acids, phenolic compounds, tannins and flavonoids, terpenoids and steroidal compounds.

Central nervous system depressant activity

Open field test: The central nervous system depressant effect of GgLME was proved by the behavioural response of mice in the open field test. The GgLME significantly ($P \leq 0.001$) decreased the rearing and spontaneous ambulatory activity of the mice. The results are shown in Fig 1.

Hole-board test: The sedative action of GgLME was proved in the hole-board test. Fig. 2 shows the actions of both standard drug diazepam given 1.0 mg/kg dose and GgLME of varying doses on the performance of mice in this test. *Galphimia gracilis* leaf methanol extract (400 mg/kg) significantly decreased the head-dipping number (13.1 ± 0.4), showing $p \leq 0.001$ and the number of rears (9.3 ± 0.3), showing $p \leq 0.001$, in a dose dependent manner when compared to the negative control group.

Test for sodium pentobarbital induced sleeping time: This test is accepted as a standard method for the screening of sedative-hypnotic drugs. The sedative and hypnotic effects of GgLME given orally to mice in combination with sodium pentobarbital are shown in Fig 3. *Galphimia gracilis* leaf methanol extract in combination with sodium pentobarbital exhibited significant ($P \leq 0.05$) synergistic sedative and hypnotic effects. The time span of sleeping produced by sodium pentobarbital was significantly ($P \leq 0.05$) prolonged with GgLME (400 mg/kg, bw.)

Pentylenetetrazole (PTZ) induced convulsions in mice: In this test, the latency period of myoclonus was delayed by diazepam (1.0 mg/kg); tonic seizures were prevented and the percentage mortality was reduced in comparison to the negative control group, whereas GgLME did not inhibit the appearance of myoclonic seizure but prevented the tonic seizures ($P \leq 0.001$). Nevertheless, GgLME significantly delayed the beginning of pentylenetetrazole induced convulsions in a dose dependent manner. The methanolic extract of *Galphimia gracilis* leaf reduced the percentage of mortality to 0 % at 400 mg/kg, 10 % at 200 mg/kg and 20 % at 100 mg/kg (Table-1).

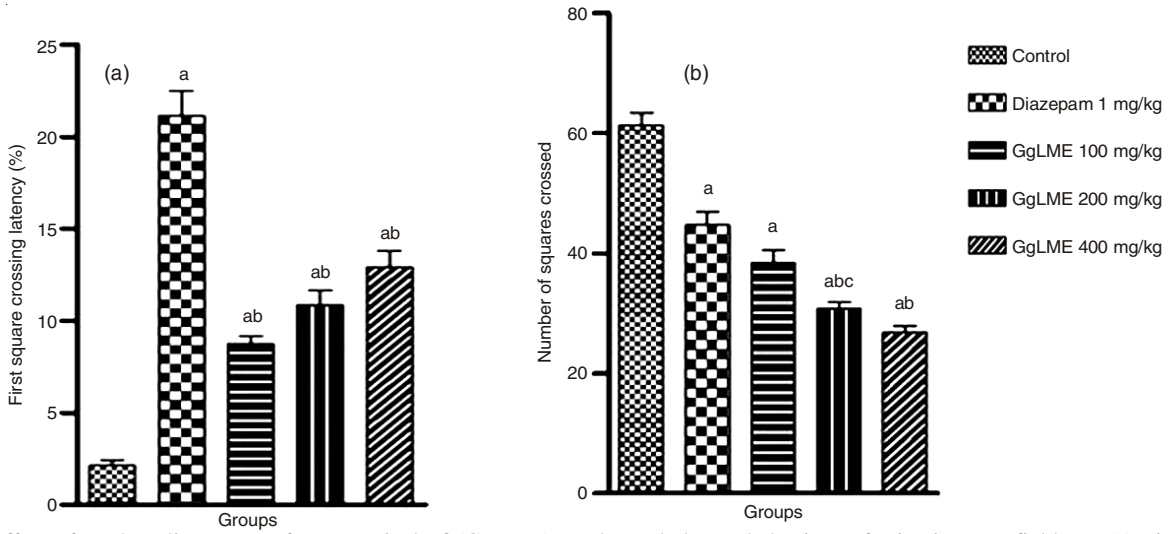

Fig. 1. Effect of methanolic extract of *G. gracilis* leaf (GgLME) on the ambulatory behaviour of mice in open field test (a) First square crossing latency (s); (b) Number of squares crossed; [a]P ≤ 0.001 indicates comparison with group I; [b]P ≤ 0.001 indicates comparison with group II; [c]P ≤ 0.001 indicates the dose dependent activity on comparison of the high dose with respective low dose of the extracts

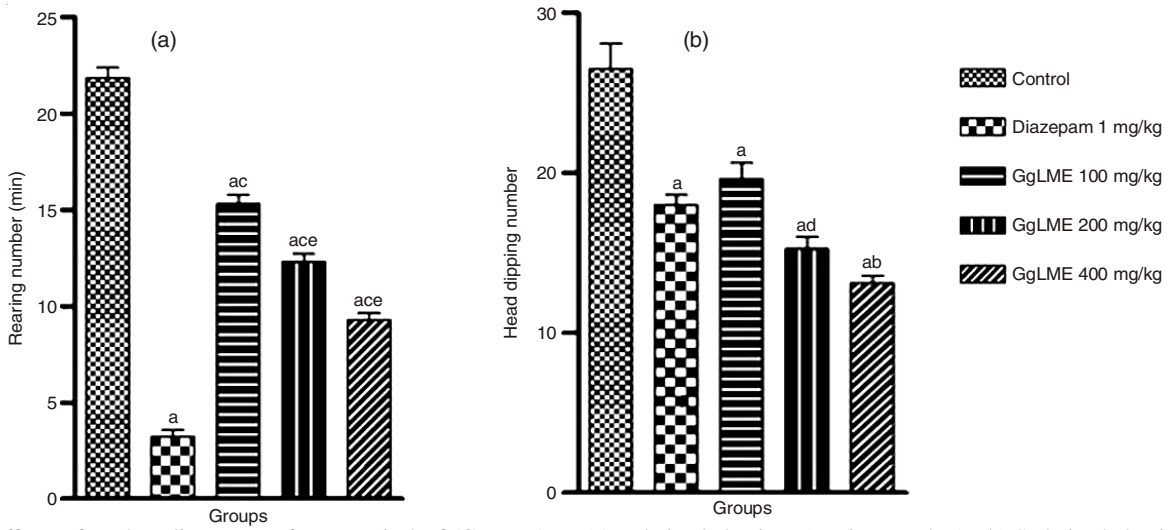

Fig. 2. Effects of methanolic extract of *G. gracilis* leaf (GgLME) on (a) sedative behaviour (rearing number); (b) Sedative behaviour (head dipping number); [a]P ≤ 0.001 indicates comparison with group I; [b]P ≤ 0.05 indicates comparison with group II; [c]P ≤ 0.001 indicates comparison with group II; [d]P ≤ 0.05 indicates the dose dependent activity on comparison of the high dose with respective low dose of the extract; [e]P ≤ 0.001 indicates the dose dependent activity on comparison of the high dose with respective low dose of the extract

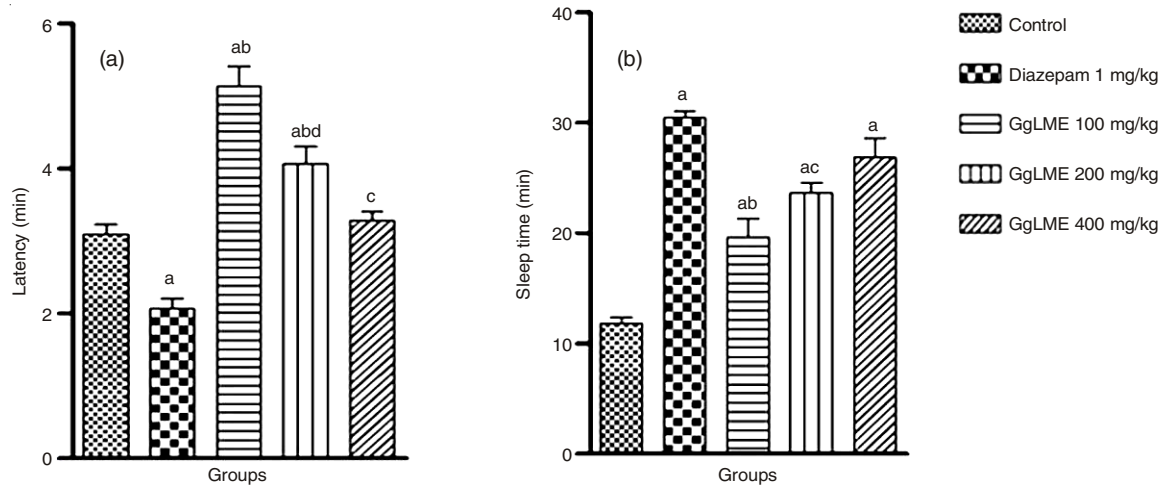

Fig. 3. Effects of methanolic extract of *G. gracilis* leaf on sodium pentobarbital induced sleeping time (a) effect of GgLME on the latency of sodium pentobarbital induced sleep; (b) effect of GgLME on the time of sleep induced by sodium pentobarbital; [a]P ≤ 0.05 indicates comparison with group I; [b]P ≤ 0.001 indicates comparison with group II; [c]P ≤ 0.01 indicates comparison with group II; [d]P ≤ 0.01 indicates the dose dependent activity on comparison of the high dose with respective low dose of the extract

Effect on picrotoxin induced convulsions in mice: *Galphimia gracilis* leaf methanol extract administered, significantly slowed the appearance of both clonic and tonic seizures and the GgLME reduced the mortality induced by picrotoxin to 10, 0 and 0 % at low, medium and high doses respectively (Table-2).

Muscle relaxant activity

Rota rod test: The results are shown in Fig. 4. The percentage of mice falling down from the revolving rod in diazepam (1 mg/kg) and GgLME treated groups exhibited significant ($P < 0.001$) decline in time spent by the mice when compared to the negative control group.

Grip strength test: The GgLME exhibited muscle relaxant activity with grip strength test in mice; the activity was shown by the poor act of the animals when remained hanging to a metal rod. When compared to the negative control group, the percentage of animals loosing their catching reflex are found to be significant ($P \leq 0.001$) and dose dependent in mice treated with GgLME (Fig. 5).

The shrub *Galphimia gracilis* is chosen for the current study to investigate its possible pharmacological importance related to central nervous system depressant and muscle relaxant properties in the leaf methanol extract. This investigation in planned based on the traditional uses of the genus Galphimia in treating conditions like fear, phobia, anxiety, stress and it is as well used for its calming effects [7-10].

Central nervous system depressant drugs are used in various disorder states of human health like sleeplessness, stress states and panic attacks effecting an individual's physical and mental health. These drugs are used to treat symptoms of the above disorder states like nervousness, tension, worried thoughts and increased blood pressure that is observed in conditions of anxiety by their relaxation effects that slows usual brain functions [9,10,24].

TABLE-1
EFFECT OF *G. gracilis* LEAF METHANOL EXTRACT (GgLME) ON PENTYLENETETRAZOLE (PTZ) 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	5.5 ± 0.3	100
II.	Diazepam	1	0/10	–[b]	0
III.	GgLME	100	2/10	16.3 ± 1.1[ab]	20
IV.	GgLME	200	1/10	29.1 ± 1.1[abc]	10
V.	GgLME	400	0/10	38.8 ± 1.8[abc]	0

Values are expressed as Mean ± SEM.; n = 6; the statistical significance done by ANOVA, followed by Tukey's multiple comparison tests and is represented by a symbol. [a]$P \leq 0.001$ indicates comparison with group I; [b]$P \leq 0.001$ indicates comparison with group II; [c]$P \leq 0.001$ indicates the dose dependent activity on comparison of the high dose with respective low dose of the extracts.

TABLE-2
EFFECT OF *G. gracilis* LEAF METHANOL EXTRACT (GgLME) 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	–[a]	0
III.	GgLME	100	1/10	15.6 ± 1.0[ab]	10
IV.	GgLME	200	1/10	23.8 ± 0.9[abc]	0
V.	GgLME	400	0/10	32.8.1 ± 1.4[abc]	0

Values are expressed as Mean ± SEM.; n = 6; the statistical significance done by ANOVA, followed by Tukey's multiple comparison tests and is represented by a symbol. [a]$P \leq 0.001$ indicates comparison with group I; [b]$P \leq 0.001$ indicates comparison with group II; [c]$P \leq 0.001$ indicates the dose dependent activity on comparison of the high dose with respective low dose of the extracts.

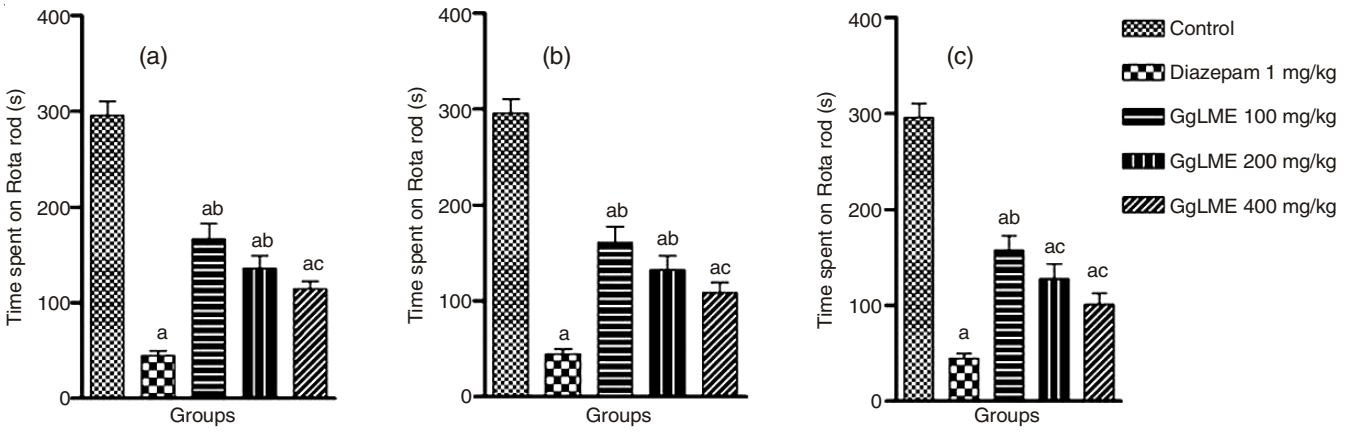

Fig. 4. Effects of methanolic extract of *G. gracilis* leaf (GgLME) on muscle relaxant activity in rota rod (a) time spent (s) by the mice on rota rod after 30 min; (b) time spent (s) by the mice on rota rod after 60 min; (c) time spent (s) by the mice on rota rod after 90 min; [a]P ≤ 0.001 indicates comparison with group I; [b]P ≤ 0.001 indicates comparison with group II; [c]P ≤ 0.05 indicates comparison with group II

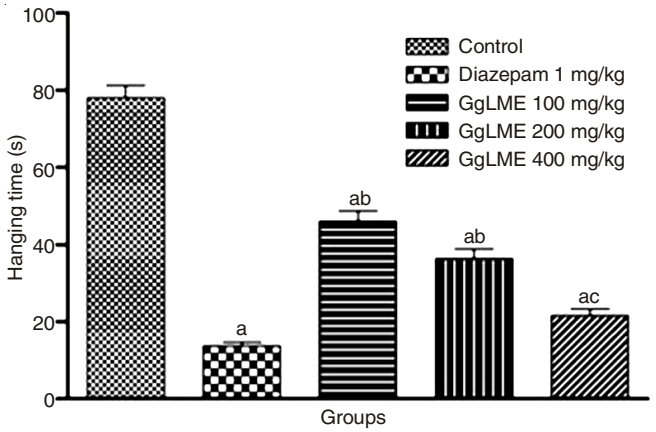

Fig. 5. Effects of methanolic extract of *G. gracilis* leaf (GgLME) on grip test in mice; [a]P ≤ 0.001 indicates comparison with group I; [b]P ≤ 0.001 indicates comparison with group II; [c]P ≤ 0.05 indicates the dose dependent activity on comparison of the high dose with respective low dose of the extract

The central nervous system depressant effect of GgLME in mice is proved by the declined locomotor activity and exploratory behaviour. Open field test helped in the concurrent measure of exploration, locomotion and anxiety [25]. Rearing and locomotion is a reaction to the different levels of excitement of central nervous system. Present results proved the central nervous system depressant effects of GgLME through decreased locomotion (the number of times the mice had crossed the squares) and the exploration reaction of the mice (object sniffing and rearing) respectively.

The hole-board test is usually carried out in mice to investigate the emotional status, stress and anxiety of the mice by recording its behaviour reaction in unusual locations. The central nervous system depressant actions are proved by the decreased number of head dips observed in the experimental animals. Mice administered with GgLME exhibited decreased number of head dips suggesting the lowered levels of anxiety and improved exploratory behaviour in animals.

In this investigation, GgLME exhibited significant central nervous system depressant actions. The mice administered with the GgLME were found to be relaxed, calm and awoke. Nevertheless, treatment with GgLME at single doses of 100, 200 and 400 mg/kg, 60 min earlier the treatment of sodium pentobarbital resulted in decreased sleep latency and increased sleep time. These effects are compared to the effect of diazepam. The GgLME reinforced sodium pentobarbital-induced hypnosis. This action, perhaps due to its effects on central nervous system that are involved in the control of sleep or it perhaps due to inhibition of pentobarbital metabolism [26].

Commonly, the sedative actions of the medication are assessed by checking the sleep time, which is induced by the drug sodium pentobarbital in testing animals [27,28]. During this investigation, it was noticed that the GgLME lengthened sodium pentobarbital induced hypnosis, which perhaps a proof of central nervous system depressant activity was recited. It is usually well-known that these central nervous system depressant actions are mediated through the benzodiazepine receptor complex/GABA or it perhaps due to the participation of the GABAergic system in GgLME induced augmented the effects of sodium pentobarbital [29].

In picrotoxin and pentylenetetrazole (PTZ) induced convulsion, the beginning of a seizure is the time span from the dose of picrotoxin/pentylenetetrazole to the early myoclonic jerks of the forelimbs observed in mice which is treated as the early indication of the inception of a seizure action [30]. The generalized seizures include both clonic and tonic seizures. Stiff extension of the forelimbs/hind limbs with or without loss of posture was noticed in clonic seizures while the tonic seizures are identified by rhythmic contractions of forelimbs/hind limbs. Different seizure phases like an orofacial seizure, head twitches, tremor/confusion, jumps/jerks, clonic seizure, tonic seizure, straub tail and death were noticed in mice administered with pentylenetetrazole/picrotoxin. Drugs that decrease $GABA_A$ synaptic functions/reduction in GABA mediated opening of the chloride ion channel/excitatory amino acids like glutamate and aspartate are treated as hypothesis for seizures.

Pentylenetetrazole exhibits its action by inhibiting GABA mediated activity. The central nervous system stimulant picrotoxin is a non-competitive antagonist at $GABA_A$ receptors and thus a convulsant. It acts as an antagonist at $GABA_A$ receptors, changing the permeability and decreasing the transmission of the chloride channel. The standard, diazepam acts by inhibiting the picrotoxin/pentylenetetrazole induced seizure and thereby improving the action of $GABA_A$ receptor, thus promoting the $GABA_A$ receptor mediated opening of chloride ion channels [31].

The methanolic extract of *Galphimia gracilis* leaf at 100, 200 and 400 mg/kg doses limited the occurrence of mortality to 10-20 % in both picrotoxin and pentylenetetrazole induced convulsions in mice. The GgLME exhibited a significant protection against convulsions induced by pentylenetetrazole with an increased threshold of clonic seizures and delayed the progression to tonic convulsion. The above results confess that the activity exhibited by the GgLME may be due to the above discussed mechanisms.

Relaxation of the skeletal muscles is caused by the drugs acting centrally, peripherally and through direct actions on the muscle fibers. Diazepam, a centrally acting drug usually employed to relieve muscle spasms. It exhibits its action by depressing the motor system of both the spinal cord and brain stem reticular formation.

The muscle relaxant activity was studied using Grip-strength test and Rota rod test in mice. The Rota rod test is performed to evaluate the drugs interfering with motor coordination while the grip strength test is done to assess the muscular strength and neuromuscular functions in mice [16]. The mice were placed on the revolving rod are allowed to stay on it. The mice, which stay less time signify the muscle relaxant actions of the test compound [22]. Most of the central nervous system depressant drugs are effective in this test [31]. Our results are similar to that of standard drug diazepam, thus confirming GgLME perhaps act similar to that of standard drug diazepam.

Conclusion

The research work concludes that the methanolic extract of *Galphimia gracilis* leaf (GgLME) has significant central nervous system depressant actions and muscle relaxant effects

against all the tested animal models. The results obtained supports the traditional claims of the plant in the treatment of fear, phobia, anxiety, stress and it is as well used for its calming effects on the nerves. This work suggests that the phyto-constituents present in the leaf methanol extract are worthy of further investigation and structure elucidation.

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