Pharmacogn. Mag.
A multifaceted peer reviewed journal in the field of Pharmacognosy and Natural Products
www.phcog.com | www.phcog.net

ORIGINAL ARTICLE

Neuropharmacological Effects of Methanolic Extract of *Clerodendrum viscosum* Leaves on Wistar Albino Rats

Meera Rath†, Ayon Bhattacharya[1]†, Soumya Santra, Karmajeet Rath, Goutam Ghosh[2], Bijaya B. Nanda[3]

Department of Pharmacology, Institute of Medical Sciences, Siksha 'O' Anusandhan University, [1]Department of Pharmacology, KPC Medical College, WBUHS, Kolkata, West Bengal, [2]Department of Pharmacognosy, School of Pharmaceutical Sciences, S'O'A University, [3]Regional Institute of Planning Applied Economics and Statistics, Government of Odisha, Bhubaneswar, Odisha, India
†These authors contributed equally to this work

Submitted: 20-05-2018 Revised: 05-09-2018 Published: 17-01-2019

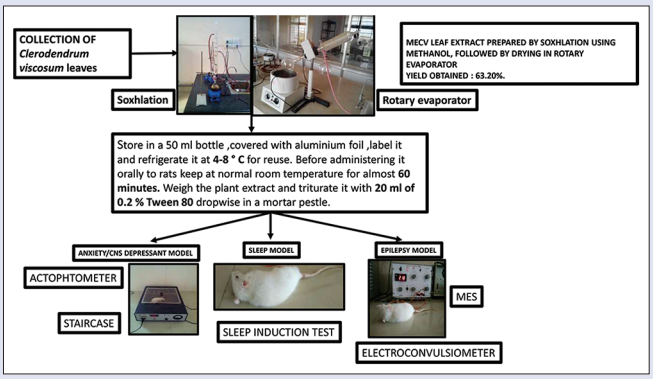
ABSTRACT

Background: The aim is to study the central nervous system (CNS) depressant, anxiolytic, and antiepileptic activities of methanolic extract of *Clerodendrum viscosum* (MECV) leaves. **Materials and Methods:** The study comprised five groups each with 6 Wistar albino rats. The Group 1 or the positive control (phenytoin 30 mg/kg for the epilepsy model, and diazepam 1 mg/kg for the other models), Group 2 or negative control (0.9% NaCl at 10 ml/kg), and Groups 3–5 or the test drug group (MECV at 200, 400, and 800 mg/kg). Actophotometer test, staircase test, maximal electroshock seizure (MES) test, and the thiopentone sodium-induced sleep test, were used to study the various parameters. **Results:** The actophotometer test, MES, and staircase test (steps climbed) were analyzed using parametric tests. The sleep induction and staircase test (number of rears) were evaluated using nonparametric tests. A dose-dependent significant ($P < 0.05$) response was observed in the actophotometer test, sleep induction, and MES tests in comparison to control. In the staircase test at 200 and 400 mg/kg, increase in the number of steps climbed, and at 800 mg/kg decrease in both the number of steps climbed and rears were observed in comparison to control. **Conclusion:** MECV showed a dose-dependent CNS depressant action in the sleep induction and antiepileptic tests. A CNS stimulant action was observed at low dose (200 mg/kg) and depressant action at a higher dose (800 mg/kg) in the staircase test in comparison to control.

Key words: Actophotometer, *Clerodendrum viscosum*, leaf, maximal electroshock seizure, sleep

SUMMARY

- The aim is to study the central nervous system depressant, anxiolytic and antiepileptic activities of methanolic extract of *Clerodendrum viscosum* leaves. In this study, five groups, each comprising 6 Wistar albino rats were used. The Group 1 was the positive control group where phenytoin 30 mg/kg was considered for the MES model and diazepam 1 mg/kg for all other models. Group 2 was the negative control (0.9% NaCl at 10 ml/kg) and Group 3, 4, 5 the test drug group (methanolic extract of *Clerodendrum viscosum* at - 200, 400, 800 mg/kg). Actophotometer test, staircase test, and MES test were used to study the parameters. The variables of the actophotometer test, MES test, and staircase test (steps climbed), were analyzed using parametric tests. In the sleep induction test and the number of rears analysis, nonparametric tests were used. A dose-dependent statistically significant ($P < 0.05$) response was observed in the actophotometer test, sleep induction test and MES test. In the sleep induction test, the extract showed a 188.63%, 237.13% and 514.94% effect at 200, 400, and 800 mg/kg, while the positive control displayed 242.92% effect. In the staircase test, low doses increased the number of steps climbed, and higher doses decreased both the number of steps climbed and rears. Thus, methanolic extract of *Clerodendrum viscosum* showed a dose-dependent central nervous system depressant, antiepileptic action and in the staircase test a central nervous system stimulant action at low doses of 200 mg/kg and depressant action at higher doses of 400 and 800 mg/kg.



Abbreviations used: GLP: Good laboratory practice; CPCSEA: Committee for the Purpose of Control and Supervision of Experiments on Animals; IAEC: Institutional Animal Ethical Committee; CNS: Central nervous system; BZDs: Benzodiazepines; IP: Intraperitoneal ANOVA: Analysis of variance; MECV: *Clerodendrum viscosum*; HLE: Hind limb extension; HLF: Hind limb flexion; NMDA: N-methyl-D-aspartate; MES: Maximal electroshock seizures; MECV: Methanolic extract of *Clerodendrum viscosum*; GABA: Gamma-Aminobutyric acid; CCK: Cholecystokinin; PME: *Pseudospondias microcarpa*; ECG: Electrocardiography; REM: Rapid eye movement; NREM: Nonrapid eye movement.

Correspondence:

Dr. Ayon Bhattacharya,
Department of Pharmacology, KPC Medical College, 1F, Raja Subodh Chandra Mullick Road, Jadavpur, Kolkata - 700 032, West Bengal, India.
E-mail: ayon.bhattacharya23@gmail.com
DOI: 10.4103/pm.pm_267_18

Access this article online
Website: www.phcog.com
Quick Response Code:

INTRODUCTION

Statistical data states 20%–40% of the general population, comprising more than 7% of the adult population, showing a prevalence figure of 14% is suffering from anxiety disorders.[1,2] Insomnia is another serious health issue in both the geriatric and adolescent population. Recent data established the prevalence of insomnia to be comparable to primary psychiatric disease such as depression. Moreover, 88% of

Cite this article as: Rath M, Bhattacharya A, Santra S, Rath K, Ghosh G, Nanda BB. Neuropharmacological effects of methanolic extract of *Clerodendrum viscosum* leaves on wistar albino rats. Phcog Mag 2018;14:S507-14.

an adolescent presenting with insomnia are known to have chronic insomnia.[3]

About 70 million people worldwide have epilepsy, and 80% of this data is from the contributions of developing countries.[4] Moreover, recently, it was found that one-third of the patients with epilepsy do not respond to modern allopathic medicine, thus increasing the incidence to 1% worldwide.[5] These alarming figures on anxiety, insomnia, and epilepsy along with the prevalence in developing countries have prompted us into exploring for safer and cost-effective remedies, and thus lies the motivation for this research.

Clerodendrum viscosum line belongs to the family of Verbenaceae.[6] About 580 species have been identified so far and grown worldwide.[7] It is a perennial shrub, about 4 feet height and widely distributed in the Asian subcontinent (Sri-Lanka, Bangladesh, India, Myanmar, Indonesia, and Thailand).[8,9] The morphology of the leaf is unique, being large, oval in shape, arranged in whorls, with denticulate margins and an acuminate apex.[8] The plant also displays allelopathic effect. This allelopathic effect has been reported a few decades back in the year 1982, where the decaying parts of the plant had effects on five weed species. This allelopathic property displayed a profound impact on the agroecosystem, and thus *C. viscosum* could be a possible option for promoting agroforestry in future.[10]

Folklore medicine has enumerated a multitude of uses of *Clerodendrum* plant, in different medical disciplines such as Ayurveda, Yunani, and homeopathy. The plant leaves were used in various ailments in folklore medicine, however, apart from the antinociceptive and antioxidant property of the leaf, no other activities have been recorded in favor of central nervous system (CNS) activity of this plant.[7] The phytochemistry of the plant is well established. The leaf harbors a factory of phytochemicals such as flavonoids, terpenes such as monoterpenes (myrcene, pinene, limonene, and cymene), sesquiterpenes, triterpenoids, fixed oil, saponins, carbohydrates, tannins, phenolic compounds, and phytosterols.[6,11,12] The reported pharmacological activities of the leaves are as follows: antihelminthic (aqueous leaf and root),[13] antinociceptive and antioxidant (methanolic, leaves),[9] antihyperglycemic (methanolic, leaf).[14]

The standard drugs benzodiazepines (BZDs) and newer non-BZDs used for anxiety, insomnia, and epilepsy are notorious for their daytime fatigue, cognitive impairment, gastrointestinal disturbances, physical dependence, and abuse liability. Moreover, patients operating machinery, elderly, children and with heart disease should be cautioned on the use of these medicines.[15]

Recent findings from our laboratory confirmed the presence of pharmacologically active compounds possessing CNS activities in the methanolic extract of *C. viscosum* (MECV).[8] In this study, we have tried to establish a link with the deciphered compounds from Gas chromatography-mass spectrometry (GCMS) in our laboratory to the pharmacological effects in animal models. However, due to lack of resource and funding, we could not execute the studies using these bioactive compounds, and we plan to do it in the future. Moreover, considering the advantages in cultivation, affordable cost, safety profile, and the novelty, we decided to conduct a neuropharmacological evaluation of the *C. viscosum* leaf on anxiety, insomnia, and epilepsy using animal models.

MATERIALS AND METHODS

Preparation of extract

The leaves (50 g) were kept under open air and dried. After that, the leaves were coarsely powdered and extracted with methanol (250 ml) in Soxhlet apparatus for 24 h. The ratio of the leaf powder and methanol used was 1:5. The extract was filtered and concentrated under reduced pressure at 40°C using a rotary evaporator to obtain a viscous semi-solid mass. The yield generated was 63.20% (63.20 g of extract per 100 g of leaves).

Chemicals

Thiopentone sodium (Thiosol, Neon Laboratories Limited, Andheri, Mumbai, Maharashtra, India), diazepam (Calmpose inj, Ranbaxy Laboratories Ltd, Nehru Place, New Delhi, India), phenytoin (Eptoin, Akums Drugs and Pharmaceuticals Ltd., Ranipur, Haridwar, Uttarakhand, India), and other solvent chemicals of analytical grade were used.

Animals

Wistar albino rats weighing between 120 and 150 g irrespective of sex were taken in this study. They were singly housed and acclimatized to the laboratory conditions for 1 week before the tests. Food and water were delivered *ad libitum*. The animals were kept in a 12 h light/dark cycle. The temperature (26°C ± 1°C) was maintained in the animal house. Behavioral experiments were conducted in the morning hours, and the animals were monitored for 1-h after the experiments.

Grouping

This study is a randomized control experimental study. The study was divided into five groups. The route of administration was intraperitoneal (IP). The Group 1 was the positive control group or the reference/standard drug group where phenytoin 30 mg/kg was considered for the epileptic model, and for all the other models diazepam 1 mg/kg was taken. Group 2 was the negative control group considering 0.9% NaCl at 10 ml/kg, and groups 3–5 was the test drug group (MECV at 200, 400, and 800 mg/kg).

Animal experiments

Actophotometer test

An actophotometer is a device used to test locomotor activity on rats and mice. The apparatus consists of six inbuilt light sources, a photosensor for detecting the movement of animals and a digital counter for recording the locomotor activity**.** The photosensor works on the principle that when the animal blocks a beam of light falling on the photocells, a count is recorded and displayed on the digital counter.[16] In this study, rats were placed in the actophotometer and their basal activity recorded over the period of 300 and 600 s. The recording of data for activity score was done before and after 30 min of administration of the various treatment groups, for 300 s and 600 s. The results were interpreted by decreased or increased activity score. The percentage decrease in motor activity was calculated using the formula:

% reduction in motor activity = $(W_a - W_b/W_a) \times 100\%$

Where; W_a: Basal score, W_b: Score after treatment.[17]

Staircase method

The staircase test is a method used for the evaluation of anxiety in rats and mice. The principle of the test lies in the fact that when rats are placed in a new environment, they experience anxiety and exhibits increased locomotor activity. The apparatus consists of 5 identical steps, 2.5 cm high, 10 cm wide, and 7.5 cm deep. The height of each step is equal throughout the apparatus. The interpretation of this test lies in the number of steps climbed by the rat and the number of years after the test drug administration. Rearing is typical behavior in rats when it stands on its hind leg on a step.[18] Each animal was used only once. The test or the standard drug was given 30 min IP before the experiment. The animals were placed on the floor, with their back facing the staircase device and

observed for 5 min. The number of steps climbed, and the number of rears was recorded. The steps climbed by the animal were considered only when all the four paws of the rat were on a step. The number of steps descended by the animal was not counted. The staircase device was cleaned after each animal to remove feces and other olfactory cues which could manipulate the behavior of the animals.[19]

Prolongation of sleep induction test

In this experiment, the test drug MECV (200, 400, and 800 mg/kg) was administered IP, 30 min before administration of 40 mg/kg IP of thiopentone sodium. The purpose of this experiment was to observe the loss of righting reflex in the animals. Loss of righting reflex is defined as when on turning the animal, the animal does not roll back, and this is interpreted as the animal to be asleep. The duration of sleep noted in this study was taken from the time of onset of sleep (loss of righting reflex) to the recovery measured in seconds.[20]

The percentage effect was calculated using the formula:[21]

$$\text{Effect}(\%) = \frac{\text{Average duration of loss of righting reflex in the test group}}{\text{Average duration of loss of righting reflex in the control group}} \times 100$$

Anti-epileptic activity

Maximal electroshock seizure model

This method was followed as per the description of Giardina and Gasior, 2009.[22] The test drug MECV (200, 400, and 800 mg/kg) was administered IP 30 min before the induction of seizures by an electroconvulsiometer. Seizures were induced with ear electrodes at 60 Hz of alternating current for 0.2 s. The parameters recorded were the onset of seizures, hind limb flexion (HLF) and hindlimb extension (HLE), stupor and recovery. A drug is considered a potent antiepileptic on abolishing the hind limb tonic extension phase of the convulsions. This fact has been given due importance in this experiment and considered as a positive criterion. Moreover, we have also recorded the death and survival data of the rats. The degree of protection afforded by the test drug was calculated using the following formula:[23]

% inhibition of seizure = 100 (1-[D_T/D_C]).

D_T = Duration of seizure after test drug treatment.

D_C = Duration of seizure without any drug treatment.

Ethical committee approval

Institutional Animal Ethical Committee (IAEC) (No. 003/IAEC/IMS and SH/SOAU) permission was obtained before conducting the experiments. Animal experiments and care were supervised and ensured with strict adherence to the committee for the purpose of control and supervision of experiments on animals (CPCSEA) and good laboratory practice (GLP) guidelines.

Sample size estimation

Sample size estimation was done using G*Power software version 3.1.9.2. For one-way analysis of variance (ANOVA), effect size $f = 0.70$, α error $P = 0.05$, power (1-β error probability) =0.80, and the number of groups five were taken into consideration.[24] The total sample size was calculated to be 30 which was equally distributed among the five groups comprising of 6 in each group.

Statistical tests

The variables of the actophotometer test, maximal electroshock seizure (MES), and staircase test (steps climbed) satisfied the normality assumption after the Shapiro–Wilk normality test. Hence, parametric test such as one-way ANOVA followed by a *post hoc* Bonferroni test for pair-wise comparison of various group means was undertaken. The variables in the sleep induction test and the staircase test (number of rears) did not satisfy normality assumptions. Hence, nonparametric tests such as Kruskal–Wallis test followed by Mann–Whitney's U-test were done.

RESULTS

Actophotometer test

In the actophotometer test, the mean activity scores for 300 s and 600 s before drug administration is displayed in Table 1. The results reveal the six groups to be comparable for 300 and 600 s ($P = 0.73$ and $P = 0.92$, respectively), implying that the treatment groups were not significantly different before the administration of test drug. The mean activity scores for 300 s and 600 s after drug administration is displayed in Table 2. The difference among the treatment groups was significant ($P = 0.00$). Moreover, with the increase in the dose of MECV from 200 to 800 mg/kg, a decrease in the mean activity score is observed on comparison to control. This pattern of decrease in the response with increase in dose or dose-dependent decrease can be appreciated in Figure 1. Bonferroni's test for the mean activity scores in the actophotometer test for 300 s and 600 s were done. The mean activity score for positive control was significantly lower than all other treatment groups in a dose-dependent manner, except that the negative control was significantly lower than MECV 200. There were significant differences among the groups ($P = 0.00$). The percentage reduction of motor activity offered by MECV 800 mg/kg at 300 s and 600 s was 79.45% and 72.63% respectively, while diazepam showed 83.22% and 81.29%. MECV 400 mg/kg displaying 21.05% and 7.01% at 300 s and 600 s, respectively.

Staircase test

The mean number of steps climbed among the treatment groups varied significantly ($P = 0.00$) and displayed in Table 3. Here, a unique pattern was observed. MECV 200 mg/kg has the highest mean number of steps climbed (24.5 ± 1.37) followed by MECV 400 mg/kg (13.17 ± 1.47), positive control (12.67 ± 1.21), negative control (6.17 ± 1.47) and MECV 800 mg/kg (5.33 ± 1.63). Pairwise multiple comparisons were done. Significant ($P = 0.00$) differences among all the group comparisons were noted except, between groups MECV 400 mg/kg and positive control ($P = 1.00$) and MECV 800 mg/kg and negative control ($P = 1.00$). A visual representation of the same is displayed in Figure 2. The comparison of mean number of rears revealed significant difference among the treatments ($P = 0.00$) [Table 4]. Mann–Whitney U-test for pairwise comparison of no. of rears revealed that MECV

Table 1: Descriptive data of the activity score before methanolic extract of *Clerodendrum viscosum* administration in the actophotometer test

Treatments (*n*=6)	Activity score for 300 s		Activity score for 600 s	
	Mean±SD	SE	Mean±SD	SE
Positive control	221.17±3.25	1.32	311.50±3.78	1.54
Negative control	220.17±5.84	2.38	309.83±2.48	1.01
MECV 200	218.50±4.59	1.87	310.67±4.59	1.87
MECV 400	219.83±3.18	1.30	310.00±4.29	1.75
MECV 800	221.67±3.88	1.58	311.00±2.53	1.03
P	0.73		0.92	

Positive control; diazepam 1 mg/kg. Negative control; 0.9% NaCl at 10 ml/kg. Statistical test used; ANOVA. Level of significance; $P<0.05$. No significant difference seen among the group mean activity scores before MECV administration. MECV doses expressed; mg/kg. Route of administration; intraperitoneal. SD: Standard deviation; SE: Standard error; MECV: Methanolic extract of *Clerodendrum viscosum;* ANOVA: Analysis of variance

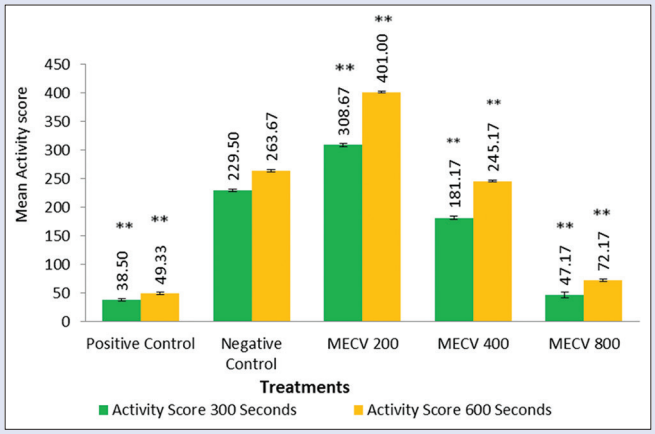


Figure 1: Bar diagram with error bars showing comparison of activity score after methanolic extract of *Clerodendrum viscosum* (MECV) administration in the actophotometer test. Positive control; Diazepam 1 mg/kg. Negative control; 0.9% NaCl at 10 ml/kg; *$P < 0.05$, **$P < 0.01$, group means differing significantly for both 300 seconds and 600 seconds on comparison to negative control. Data expressed as error bar ± standard deviation ($n = 6$). Methanolic extract of *Clerodendrum viscosum* doses expressed; mg/kg. Route of administration: intraperitoneal

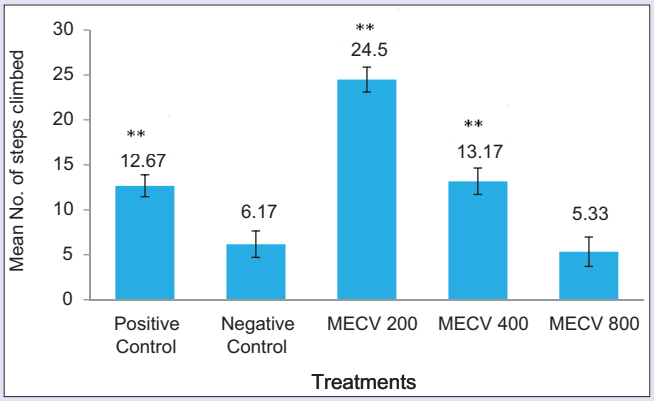


Figure 2: Bar diagram with error bars showing the comparison of the mean no. of steps climbed among treatments in the Staircase test. Positive control; Diazepam 1mg/kg. Negative control; 0.9 % NaCl at 10ml/kg; *$P < 0.05$, **$P < 0.01$, group means differing significantly in comparison to negative control except methanolic extract of *Clerodendrum viscosum* (MECV) 800. (MECV) 800. Data expressed as Error bar ± SD ($n = 6$). MECV doses expressed; mg/kg. Route of administration; intraperitoneal

Table 2: Descriptive data of the activity scores after methanolic extract of *Clerodendrum viscosum* administration in the actophotometer test

Treatments (*n*=6)	Activity score for 300 s		Activity score 600 s	
	Mean±SD	SE	Mean±SD	SE
Positive control	38.50±2.17	0.89	49.33±4.32	1.76
Negative control	229.50±2.51	1.03	263.67±3.93	1.61
MECV 200	308.67±2.73	1.12	401.00±3.03	1.24
MECV 400	181.17±2.99	1.22	245.17±2.99	1.22
MECV 800	47.17±5.12	2.09	72.17±5.12	2.09
Total	161.00±106.59	19.46	206.27±132.92	24.26
P	0.00		0.00	

Positive control; diazepam 1 mg/kg. Negative control; 0.9% NaCl at 10 ml/kg. Statistical test used; ANOVA. Level of significance; $P<0.05$. Significant difference among the treatments seen ($P=0.00$) for activity scores at 300 and 600 s. MECV doses expressed; mg/kg. Route of administration; intraperitoneal. SD: Standard deviation; SE: Standard error; MECV: Methanolic extract of *Clerodendrum viscosum;* ANOVA: Analysis of variance

Table 3: Descriptive data of mean number of steps climbed among treatments in the staircase test

Treatments (*n*=6)	No steps climbed
	Mean±SD
Positive control	12.67±1.21
Negative control	6.17±1.47
MECV 200	24.50±1.37
MECV 400	13.17±1.47
MECV 800	5.33±1.63
P	0.00

Positive control; diazepam 1 mg/kg. Negative control; 0.9% NaCl at 10 ml/kg. Statistical test used; ANOVA. Level of significance; $P<0.05$. Significant difference among the groups seen ($P=0.00$). MECV doses expressed; mg/kg. Route of administration; intraperitoneal. MECV: Methanolic extract of *Clerodendrum viscosum*, SD: Standard deviation; ANOVA: Analysis of variance

200 mg/kg, MECV 400 mg/kg and MECV 800 mg/kg did not have a significantly different number of rears ($P > 0.05$). The positive control has a significantly lower number of rears than the negative control. Positive control and MECV 200 mg/kg did not have significant difference ($P = 0.07$). Positive control has significantly higher mean for the number of rears than MECV 400 mg/kg and MECV 800 mg/kg. Negative control has significantly higher number of rears than MECV 200 mg/kg, MECV 400 mg/kg and MECV 800 mg/kg ($P < 0.01$). The graphical representation of the same is illustrated in Figure 3.

Sleep induction test

Comparison of the mean time of onset of sleep and recovery among the treatment groups are furnished in Table 5. The mean onset of sleep and recovery differ significantly among the treatment groups as revealed by Kruskal Wallis test ($P = 0.00$). The dose-dependent decrease in the onset of sleep with increase in dose of MECV and the dose-dependent increase in the mean recovery time by MECV can be appreciated in Figures 4 and 5. The percent effect in the sleep induction test was also calculated. MECV showed a 188.63%, 237.13%, and 514.94% effect at 200, 400, and 800 mg/kg, respectively, while the positive control displayed 242.92% effect. In the Mann–Whitney's test, significant differences ($P < 0.05$) among all the group comparisons in the onset of sleep were seen except MECV 200 mg/kg and positive control ($P = 0.05$). On analyzing the recovery time, all the groups showed a significant difference ($P < 0.05$) except MECV 400 mg/kg and positive control ($P = 0.80$).

Maximal electroshock seizure test

The comparison of epileptic parameters such as the onset of epilepsy, HLE, HLF, stupor and recovery, among treatments is presented in Table 6. Significant ($P = 0.00$) difference in all the four parameters among all the groups was noted. As shown in Table 6, we can appreciate that the mean onset of epilepsy gradually increased with the increase in the doses of MECV or showing a dose-dependent increase, such that MECV at 800 mg/kg is almost comparable to Phenytoin. The other parameters such as the HLE, HLF, stupor, and recovery showed a gradual decrease in the duration with the increase in the doses of MECV from 200 to 800 mg/kg or displayed a dose-dependent decrease. In Figure 6, the percentage inhibition of seizures has been portrayed. Bonferroni's pairwise multiple comparison tests were done, and some important findings were noted. On comparing the onset of seizures, all the groups showed statistically significant ($P < 0.05$) difference except MECV

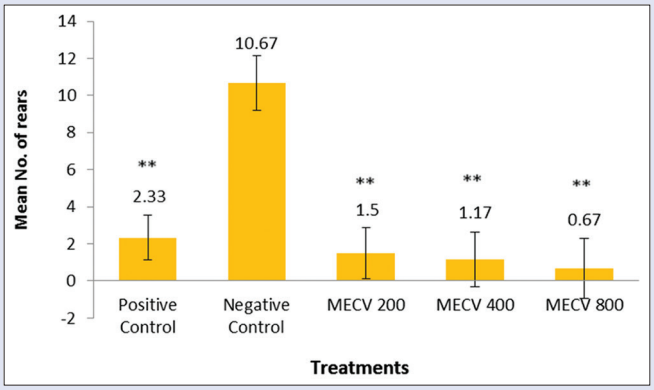


Figure 3: Bar diagram with error bars displaying the comparison of mean no. of rears among treatments in the Staircase Test. Positive control; Diazepam 1mg/kg. Negative control; 0.9% NaCl at 10 ml/kg; *$P < 0.05$, **$P < 0.01$, group means differing significantly in comparison to negative control. Data expressed as error bar ± standard deviation ($n = 6$). Methanolic extract of *Clerodendrum viscosum* (MECV) doses expressed; mg/kg. Route of administration; intraperitoneal

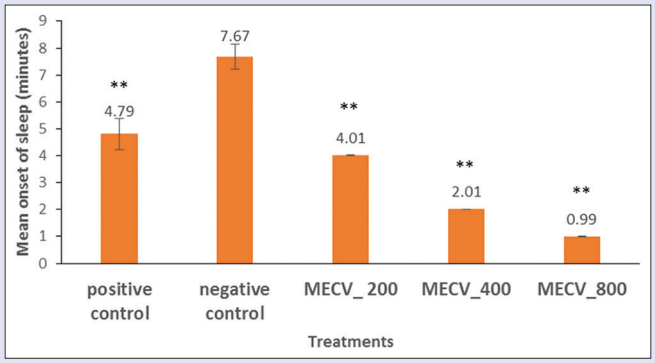


Figure 4: Bar diagram with error bars denoting the comparison of the mean onset of sleep (minutes) among the different groups. Positive control; Diazepam 1 mg/kg. Negative control; 0.9% NaCl at 10 ml/kg; *$P < 0.05$, **$P < 0.01$, group means differing significantly on comparison to negative control. Data expressed as error bar ± standard deviation ($n = 6$). Methanolic extract of *Clerodendrum viscosum* (MECV) doses expressed; mg/kg. Route of administration; intraperitoneal

Table 4: Descriptive statistics and mean ranks of number of rears among treatments in the staircase test

Treatments (n=6)	Number of rears	
	Mean±SD	Mean rank
Positive control	2.33±0.81	18.67
Negative control	10.67±2.42	27.50
MECV 200	1.50±0.54	13.00
MECV 400	1.17±0.75	10.67
MECV 800	0.67±1.03	7.67
Kruskal–Wallis (P)	0.00	

Positive control; diazepam 1 mg/kg. Negative control; 0.9% NaCl at 10 ml/kg. Level of significance; P<0.05. Significant difference among the groups (P=0.00) observed. MECV doses expressed; mg/kg. Route of administration; intraperitoneal. MECV: Methanolic extract of *Clerodendrum viscosum*, SD: Standard deviation

Table 5: Descriptive statistics and mean ranks of the time of onset of sleep and recovery (min) among the different groups in the sleep induction test

Treatments (n=6)	Onset of sleep (min)		Time of recovery (min)	
	Mean±SD	Mean rank	Mean±SD	Mean rank
Positive control	4.79±0.58	20.50	217±22.63	18.75
Negative control	7.67±0.47	27.50	89.33±3.33	3.50
MECV 200	4.01±0.02	16.50	168.50±5.32	9.50
MECV 400	2.01±0.01	9.50	211.83±7.49	18.25
MECV 800	0.99±0.01	3.50	460±33.47	27.50
Kruskal–Wallis P	0.00		0.00	

Positive control; diazepam 1 mg/kg. Negative control; 0.9% NaCl at 10 ml/kg. Level of significance; P<0.05. Significant difference among the groups seen (P=0.00) for both study parameters. MECV doses expressed; mg/kg. Route of administration; intraperitoneal. MECV: Methanolic extract of *Clerodendrum viscosum*, SD: Standard deviation

800 mg/kg and phenytoin 30 mg/kg ($P = 1.00$). In the HLE parameter no significant differences were noted comparing phenytoin with MECV 400 mg/kg ($P = 1.00$) and MECV 800 mg/kg ($P = 1.00$). Similarly, in the HLF, all groups showed significant ($P < 0.05$) difference except phenytoin and MECV 800 mg/kg ($P = 1.00$). The pattern was repeated in the following parameters such as stupor, recovery, and total duration where phenytoin on comparing with MECV 200, 400, and 800 mg/kg showed no significant difference ($P = 1.00$).

DISCUSSION

Actophotometer is a reputed method for screening locomotor and antianxiety activity on rodents. The principle of this test being locomotor activity is an index of alertness, and thus, a decrease in the same after drug administration indicates a sedative action or CNS depressant action.[25] The staircase test is a specific and reliable model for measuring anxiety. Rats display anxiety behavior by increased awareness of surroundings, behavioral alterations, and thigmotaxis.[26] The number of steps climbed portrays exploratory behavior, or the number of rears indicates anxiety in rats.[27] The dissociation of these parameters is the goal of anti-anxiety drugs. Anxiolytic agents will decrease the rears and increase the number of steps climbed. However, in this study at lower doses of MECV such as 200 and 400 mg/kg, the number of steps climbed was more than the control group and phenytoin suggesting a CNS stimulant action, but at the highest dose which is 800 mg/kg the steps climbed showed a phenomenal decrease compared to the positive and negative control groups. The number of rears gradually decreased with the increase in the doses of MECV compared to the positive and negative control. This decrease in both the number of steps climbed and rears at high dose (MECV 800 mg/kg) suggests a CNS-depressant action. A similar pattern has also emerged in the Actophotometer test. One of the possible explanations for such statistical insignificance in the number of steps climbed at 800 mg/kg MECV could be due to the differences in the expression of bioactive ingredients in MECV. Increasing the concentration of MECV could have led to increase in the concentration of certain bioactive components which could have masked the expression of active ingredients in the MECV extract responsible for displaying anti-depressant action.[28] The biphasic profile of anxiolytics such as diazepam belonging to the BZD group, which increases exploratory behavior in low doses and inhibits the same at higher doses have long been reported. Our extract, MECV could be mimicking the pattern of action as diazepam, but further studies are needed to prove the same.[29,30]

Thus, in this study, the mechanism mediated by MECV could be due to CNS depression or muscle relaxation or anxiolytic or due to multiple actions.[31] However, certain questions do arise such as does anxiety and depression override each other? Is anxiety an earlier manifestation of depression? There are studies which have confronted the same questions and similarly needs further exploration to confirm such postulations.[32]

Table 6: Descriptive data of the various epileptic parameters (onset, hind limb extension, flexion, stupor and recovery, and total duration) among different groups

Treatments*	Mean±SD				
	Onset epileptic activity	HLE (s)	HLF (s)	Stupor and recovery(s)	Total duration(s)
Positive control	7.00±0.89	3.83±1.94	7.50±1.87	41.00±4.94	52.33±2.25
Negative control	1.28±0.02	32.17±7.41	46.00±3.90	373.00±279.63	443.33±288.18
MECV 200	2.75±0.44	9.33±1.63	17.33±1.86	98.50±2.59	125.17±2.86
MECV 400	4.60±0.44	6.00±1.26	11.83±0.75	51.67±4.50	69.50±3.21
MECV 800	7.50±0.48	2.08±0.80	8.00±1.27	33.25±3.66	43.33±3.33
P	0.00	0.00	0.00	0.00	0.00

Positive control: Phenytoin 30 mg/kg. Negative control; 0.9% NaCl at 10 ml/kg. *Sample size; 6 for each treatment groups except for parameters (stupor and total duration); 3 animals died. Level of significance; $P<0.05$. Significant difference among the groups seen as $P=0.00$ for all the parameters among the groups. MECV doses expressed; mg/kg. Route of administration; intraperitoneal. MECV: Methanolic extract of *Clerodendrum viscosum*; HLF: Hind limb flexion, HLE: Hind limb extension, SD: Standard deviation

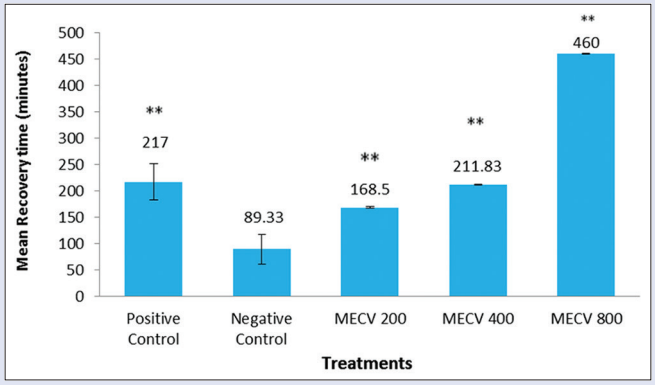


Figure 5: Bar diagram with error bars comparing the mean recovery time (minutes) in the sleep induction test among the groups. Positive control; Diazepam 1 mg/kg. Negative control; 0.9% NaCl at 10 ml/kg; *$P < 0.05$, **$P < 0.01$, group means differ significantly in comparison to negative control. Data expressed as error bar ± standard deviation ($n = 6$). Methanolic extract of *Clerodendrum viscosum* (MECV) doses expressed; mg/kg. Route of administration; intraperitoneal

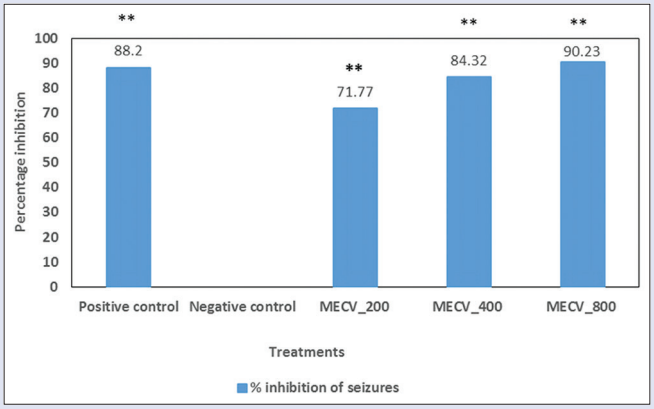


Figure 6: Bar diagram showing the percentage inhibition of seizures among the groups in the MES model. Positive control; Phenytoin 30 mg/kg, Negative control; 0.9% NaCl at 10 ml/kg. *$P < 0.05$, **$P < 0.01$. Percentage inhibition of seizures in each group ($n = 6$) on comparison to negative control. Methanolic extract of *Clerodendrum viscosum* (MECV) doses expressed; mg/kg. Route of administration; intraperitoneal

There are many mechanisms for anxiety, and the pathogenesis remains confounding. The alterations of neurotransmitters and transporters (such as noradrenergic, serotonergic, glutaminergic, and gamma-aminobutyric acid [GABA]-ergic neurons), the impact of hormonal undulations (such as neuropeptide Y and cholecystokinin), and free radicals are some of the mechanisms for anxiety generation in our body. Among the neurotransmitters, low levels of GABA play a significant role in the biogenesis of anxiety. The GABA-A receptor type-ionophore complex is a voltage-gated receptor involved in sedation, as a muscle relaxant and anxiety in the CNS.[33] GABA is an inhibitory neurotransmitter affecting both GABA-A and GABA-B receptor.[34] In fact, various neuropsychiatric and neurodegenerative disorders such as Parkinson syndrome, epilepsy, depression, Alzheimer's disease are mediated by GABA receptor. GABA binds to the alpha subunit, leading to an increase in chloride ion conductance and inhibition of the action potential.[35] Diazepam a BZD used in the actophotometer test and staircase test act via this receptor. Diazepam causes structural change and potentiates the GABA-A receptors, causing membrane hyperpolarization, and eventually resulting in a decrease in the firing rate of neurons in the brain. Another way of elucidating this mechanism is that the increase in GABA neurotransmission has a damping effect on the stimulatory pathways giving a calming psychological impact on the subject.[25]

Nevertheless, other mechanisms involving free radicals cannot be ignored. Free radical damages the noradrenergic and serotonergic nervous systems. Understanding the symptoms of anxiety such as restlessness, feeling of impending doom, tiredness, inability to concentrate, irritability, increased muscle tension, and sleep disturbance is essential to appreciate the results on animals. In this study, the dilemma lies in what could be the mechanism of action of our extract. The effect of the present research directs us to speculate that there must be some ingredients in MECV which could facilitate this inhibitory system or alter the hormonal levels, with or without parallel antioxidant action or could be by using several mechanisms.[31]

CNS depressant drugs such as thiopental sodium are a barbiturate and activate the inhibitory GABAergic system. It binds to the barbiturate binding site on the GABA-A chloride-channel receptor complex and potentiates the GABA-mediated hyperpolarization of postsynaptic neurons. Our findings corroborate to the sedative action inducing capacity of diazepam, and the possible mechanism could be by enhancing the effect of thiopentone sodium.[19] Moreover, thiopental also blocks excitatory glutamate receptors.[36] Hence, exploring whether MECV has an impact on the glutamate receptors further study is required.

The fundamentals of the MES-induced convulsion model are that convulsions generated here are due to the influx of sodium through the opening of sodium channels and subsequent increase in the glutamate levels, an excitatory neurotransmitter. After that, glutamate binds to N-methyl-D-aspartate receptors, exhibiting epilepsy in humans. Based on the underlying mechanism of MES convulsions, MECV could act either by blocking the sodium channel or by reducing the excitatory neurotransmitter.

Compounds isolated from GCMS of *Clerodendrum* leaves revealed compounds such as 5-Hydroxymethylfurfural,[8] xylitol, n hexadecanoic acid, and 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl, possessing antioxidant property.[37,38] Benzofuran, 2,3-dihydro-has been reported as an analgesic and anti-inflammatory agent.[39] Glycerol has been published with an anti-inflammatory effect.[40] Orcinol,[41] 5-Hydroxymethylfurfural,[42] N, N-dimethylglycine,[43] and 4-Pyranone, 2,3-dihydro[44] have been found with CNS activities. These compounds could be responsible for the neuropharmacological activities of MECV.

There are shortcomings to this study. For some test parameters, nonparametric tests were used, which could be replaced with a parametric analysis with a larger sample size. In arriving at a conclusion that BZD receptor is participating in the hypnotic effects of MECV, flumazenil, a specific antagonist could be used and incorporating such as step would lend further credence to the study. Thiopental sodium is a potent enzyme inducer. Hence, we should have studied the enzyme-inducing property of thiopental sodium. The cytochrome P450 enzyme system metabolizes majority of the drugs/xenobiotics. Therefore, there are chances of interactions and toxicities between herbal products and drugs. A study was done where pretreatment with phenobarbitone for two consecutive days shortened the duration of sleep in *Pseudospondias microcarpa* (PME) extract treated mice. This reduction in sleeping time suggests that PME induced cytochrome P450 enzyme activity. Thus, the possibility of interactions between thiopental and MECV must be considered before establishing a concluding statement.[45] Electrocardiography stracing and the effect of the extract on specific stages of sleep such as rapid eye movement (REM) and non-REM sleep needs to be done. Furthermore, LD50/ED50 studies are also essential studies that need to be done. The challenge also lies in not having much information on the bioactive compounds directly responsible for antiepileptic, anxiolytic, and CNS depressant actions. *C. viscosum* has shown very potent CNS depressant action henceforth, the search for the bioactive ingredients accountable for this action should begin vigorously. These are some of our drawbacks and our future quest to undertake it later.

CONCLUSION

Thus, we would conclude on the note that MECV displayed a statistically significant ($P < 0.05$) dose-dependent CNS depressant response and thereby exhibiting phenomenal hypnotic, anti-anxiety, and antiepileptic action in respect to control. Nevertheless, MECV also displayed a unique property of a CNS stimulant nature at low dose (200 mg/kg) and CNS-depressant action at a higher dose (800 mg/kg) in comparison to control.

Acknowledgments

Our sincere gratitude to the animal caretakers, laboratory technicians, official clerks, research scholars, veterinary officers, faculty members, and our deep heartfelt reverence for the animals used in this study at Dept. of Pharmacology, IMS and SUM Hospital and School of Pharmaceutical Sciences, SOA University.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Viggiano A, Cacciola G, Widmer DA, Viggiano D. Anxiety as a neurodevelopmental disorder in a neuronal subpopulation: Evidence from gene expression data. Psychiatry Res 2015;228:729-40.
2. Pignon B, Amad A, Pelissolo A, Fovet T, Thomas P, Vaiva G, *et al*. Increased prevalence of anxiety disorders in third-generation migrants in comparison to natives and to first-generation migrants. J Psychiatr Res 2018;102:38-43.
3. de Zambotti M, Goldstone A, Colrain IM, Baker FC. Insomnia disorder in adolescence: Diagnosis, impact, and treatment. Sleep Med Rev 2018;39:12-24.
4. Auditeau E, Moyano LM, Bourdy G, Nizard M, Jost J, Ratsimbazafy V, *et al*. Herbal medicine uses to treat people with epilepsy: A survey in rural communities of Northern Peru. J Ethnopharmacol 2018;215:184-90.
5. Cano A, Ettcheto M, Espina M, Auladell C, Calpena AC, Folch J, *et al*. Epigallocatechin-3-gallate loaded PEGylated-PLGA nanoparticles: A new anti-seizure strategy for temporal lobe epilepsy. Nanomedicine 2018;14:1073-85.
6. Wang JH, Luan F, He XD, Wang Y, Li MX. Traditional uses and pharmacological properties of *Clerodendrum* phytochemicals. J Tradit Complement Med 2018;8:24-38.
7. Nandi S, Lyndem LM. *Clerodendrum viscosum*: Traditional uses, pharmacological activities and phytochemical constituents. Nat Prod Res 2016;30:497-506.
8. Ghosh G, Panda P, Rath M, Pal A, Sharma T, Das D, *et al*. GC-MS analysis of bioactive compounds in the methanol extract of *Clerodendrum viscosum* leaves. Pharmacognosy Res 2015;7:110-3.
9. Rahman MM, Rumzhum NN, Zinna K, Koh ET, McDonald F, Pitt Ford TR, *et al*. Cellular response to mineral trioxide aggregate. J Endod 1998;24:543-7.
10. Datta SC, Chakrabarti SD. Allelopathic potential of *Clerodendrum viscosum* vent. in relation to germination and seedling growth of weeds. Flora 1982;172:89-95.
11. Lobo R, Chandrshakar KS, Jaykumar. B, Mamatha B. *In vitro* antimicrobial activity of *Clerodendrum viscosum* (Vent). Der Pharm Lett 2010;2:257-60.
12. Dey P, Dutta S, Chaudhuri TK. Phytochemical analysis of the leaves of *Clerodendrum viscossum* vent. Int J Pharm Pharm Sci 2014;6:254-8.
13. Nandi S, Ukil B, Roy S, Kundu S, Lyndem LM. Anthelmintic efficacy of *Clerodendrum viscosum* on fowl tapeworm *Raillietina tetragona*. Pharm Biol 2017;55:1401-6.
14. Rifat US, Rony H, Nabid A, Shahnaz R, Mohammed R. Antihyperglycemic and analgesic activity studies with *Clerodendrum viscosum* vent (*Verbenaceae*) leaves. World J Pharm Pharm Sci 2015;4:216-24.
15. Brunton L, Chabner B, Knollman B. Goodman and Gilman's Pharmacological Basis of Therapeutics. 12th ed. New York: McGraw Hill; 2011. p. 457-80.
16. Dews PB. The measurement of the influence of drugs on voluntary activity in mice. Br J Pharmacol Chemother 1953;8:46-8.
17. Protapaditya D, Sangita C, Sanjib B. Neuropharmacological activities of *Mikania scandens* root. Glob J Pharmacol 2012;6:193-8.
18. Ago Y, Takahashi K, Nakamura S, Hashimoto H, Baba A, Matsuda T, *et al*. Anxiety-like and exploratory behaviors of isolation-reared mice in the staircase test. J Pharmacol Sci 2007;104:153-8.
19. Vogel HG, Vogel WH, editors. Drug Discovery and Evaluation Pharmacological Assays. Vol. 2. Germany: Springer; 2000. p. E235.
20. Moniruzzaman M, Atikur Rahman M, Ferdous A. Evaluation of sedative and hypnotic activity of ethanolic extract of *Scoparia dulcis* linn. Evid Based Complement Alternat Med 2015;2015:873954.
21. Ali MS, Dash PR, Nasrin M. Study of sedative activity of different extracts of *Kaempferia galanga* in swiss albino mice. BMC Complement Altern Med 2015;15:158.
22. Giardina WJ, Gasior M. Acute seizure tests in epilepsy research: Electroshock- and chemical-induced convulsions in the mouse. Curr Protoc Pharmacol 2009;45:5.22.1-22.37.
23. Florek-Luszczki M, Wlaz A, Kondrat-Wrobel MW, Tutka P, Luszczki JJ. Effects of WIN 55,212-2 (a non-selective cannabinoid CB1 and CB 2 receptor agonist) on the protective action of various classical antiepileptic drugs in the mouse 6 Hz psychomotor seizure model. J Neural Transm (Vienna) 2014;121:707-15.
24. Faul F, Erdfelder E, Lang AG, Buchner A. G*Power 3: A flexible statistical power analysis program for the social, behavioral, and biomedical sciences. Behav Res Methods 2007;39:175-91.
25. Bhattacharya A, Naik MR, Agrawal D, Sahu PK, Kumar S, Mishra SS. CNS depressant and muscle relaxant effect of ethanolic leaf extract of *Moringa oleifera* on albino rats. Int J PharmTech Res 2014;6:1441-9.
26. Treit D, Fundytus M. Thigmotaxis as a test for anxiolytic activity in rats. Pharmacol Biochem Behav 1988;31:959-62.
27. Gnanasekar N, Reddy CU, Narayanan N, Chamundeeswari C, Gopal TK. Anxiolytic activity of *Flacourtia indica* using staircase and light dark exploration methods in mice. J Chem Pharm

Sci 2014;7:29-33.

28. Onasanwo SA, Chatterjee M, Palit G. Antidepressant and anxiolytic potentials of dichloromethane fraction from *Hedranthera barteri*. Afr J Biomed Res 2010;13:76-81.
29. Treit D. Animal models for the study of anti-anxiety agents: A review. Neurosci Biobehav Rev 1985;9:203-22.
30. Santos FJ, Lima SG, Cerqueira GS, Citó AM, Cavalcante AA, Marques TH, *et al*. Chemical composition and anxiolytic-like effects of the *Bauhinia platypetala*. Rev Bras Farmacogn 2012;22:507-16.
31. Bhattacharya A, Santra S, Mahapatra S, Sahu PK, Agrawal D, Kumar S. Study of anxiolytic effect of ethanolic extract of drumstick tree leaves on albino mice in a basic neuropharmacology laboratory of a postgraduate teaching institute. J Health Res Rev 2016;3:41-7.
32. He D, Wang X, Zhang P, Luo X, Li X, Wang L, *et al*. Evaluation of the anxiolytic and antidepressant activities of the aqueous extract from *Camellia euphlebia* merr. ex sealy in mice. Evid Based Complement Alternat Med 2015;2015:618409.
33. Tirumalasetti J, Patel M, Shaikh U, Harini K, Shankar J. Evaluation of skeletal muscle relaxant activity of aqueous extract of nerium oleander flowers in albino rats. Indian J Pharmacol 2015;47:409-13.
34. Sieghart W, Sperk G. Subunit composition, distribution and function of GABA (A) receptor subtypes. Curr Top Med Chem 2002;2:795-816.
35. Muhammad N, Saeed M, Khan H, Adhikari A, Khan KM. Muscle relaxant and sedative-hypnotic activities of extract of *Viola betonicifolia* in animal models supported by its isolated compound, 4-hydroxy coumarin. J Chem 2013;28:997-1001.
36. Khan IN, Sarker MM, Ajrin M. Sedative and anxiolytic effects of ethanolic extract of *Calotropis gigantea* (*Asclepiadaceae*) leaves. Asian Pac J Trop Biomed 2014;4:S400-4.
37. Yu X, Zhao M, Liu F, Zeng S, Hu J. Identification of 2, 3-dihydro-3, 5-dihydroxy-6-methyl-4H-pyran-4-one as a strong antioxidant in glucose – Histidine maillard reaction products. Food Res Int 2013;51:397-403.
38. Bhattacharya A, Ghosh G, Agrawal D, Sahu PK, Kumar S, Mishra SS. GCMS profiling of ethanolic extract of *Moringa oleifera* leaf. Int J Pharm Bio Sci 2014;5:263-75.
39. Idan SA, Al-Marzoqi AH, Hameed IH. Spectral analysis and anti-bacterial activity of methanolic fruit extract of *Citrullus colocynthis* using gas chromatography-mass spectrometry. Afr J Biotechnol 2015;14:3131-58.
40. Jananie RK, Priya V, Vijayalaksmi K. Determination of bioactive components of cynodon dactylon by GC-MS analysis. N Y Sci J 2011;4:16-20.
41. Wang X, Li G, Li P, Huang L, Huang J, Zhai H, *et al*. Anxiolytic effects of orcinol glucoside and orcinol monohydrate in mice. Pharm Biol 2015;53:876-81.
42. Ya BL, Li HF, Wang HY, Wu F, Xin Q, Cheng HJ, *et al*. 5-HMF attenuates striatum oxidative damage via Nrf2/ARE signaling pathway following transient global cerebral ischemia. Cell Stress Chaperones 2017;22:55-65.
43. Lee MY, Lin YR, Tu YS, Tseng YJ, Chan MH, Chen HH, *et al*. Effects of sarcosine and N, N-dimethylglycine on NMDA receptor-mediated excitatory field potentials. J Biomed Sci 2017;24:18.
44. Eiden F, Denk F. Synthesis of CNS-activity of pyran derivatives: 6,8-dioxabicyclo(3,2,1) octane. Arch Pharm (Weinheim) 1991;324:353-4.
45. Adongo DW, Mante PK, Woode E, Ameyaw EO, Kukuia KK. Effects of hydroethanolic leaf extract of *Pseudospondias microcarpa* (A. Rich.) Engl. (*Anacardiaceae*) on the central nervous system in mice. J Phytopharmacol 2014;3:410-7.