



Anticonvulsant and Antioxidant Effects of Methanol Extract of Stems of *G. arborea* Roxb.

N. S. Acharya*, S. R. Acharya, V. Kumar, P. Barai

Institute of Pharmacy, Nirma University, Ahmedabad 382 481, India; niyati20103@gmail.com

Abstract

Gmelina arborea Roxb. (Verbenaceae) is greatly valued plant reported in Indian traditional system of medicine for various ailments. In this study, anticonvulsant and antioxidant activity of methanol extract of stems (MES) of *Gmelina arborea* Roxb. was investigated. Protective effects were evaluated in pentylenetetrazole (PTZ) (60 mg/kg, s.c.) and strychnine (STR) (2 mg/kg, i.p.) induced seizure models in adult albino mice (250 mg/kg and 500 mg/kg) using diazepam as a standard. Total phenolics and flavonoids content in MES were determined. The antioxidant activity was evaluated by 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay, scavenging of hydrogen peroxide radical and through reducing power assay. The onset and duration of seizures (tonic-clonic convulsions), mortality rate and number of mice with and without convulsion within the observation period were noted in PTZ and STR induced seizure models. MES showed good reductive capability and free radical scavenging effects with IC_{50} 47.47 μ g/ml for DPPH radical assay and 97.33 μ g/ml for hydrogen peroxide scavenging in a dose dependent manner. MES at the dose of 500 mg/kg, exhibited maximum delay in onset of convulsions (8.188 min in PTZ and 11.81 min in STR induced seizures) in both the models with increased latency period. The suppression of seizures by MES (500 mg/kg) might be observed due to enhanced gamma amino butyric acid neurotransmission in PTZ induced animals. The constituents of MES might also have glycine inhibitory potential to impart protection in STR induces seizures in our study. Good free radical scavenging activity and considerable amount of flavonoids and phenolics in the extracts might have contributed in protection offered with good anticonvulsant effects in both the models. This study justifies the multilevel therapeutic uses of stem bark and heartwood of *G. arborea* in Indian system of medicine.

Keywords: Anticonvulsant, antioxidant, *Gmelina arborea*, pentylenetetrazole, seizures, strychnine

1. Introduction

Epileptic seizures are traditionally characterized as the ultimate expression of monolithic, hypersynchronous neuronal activity arising from unbalanced runaway excitation [1]. It's the involuntary contraction of striated muscles repeatedly, characterized by many clinical disturbances of consciousness, movement and sensation. Convulsion arises due to sudden excessive and rapid discharge of cerebral neurons in the grey matter of the brain.

Epilepsy is the second most common neurological disorder after stroke, overall accounting for 1% of the world's burden of disease and affecting at least 50 million persons worldwide and 7 million people in India. Large numbers of antiepileptic drugs available for the treatment of different types of seizures are frequently reported with side effects like sedation, cognitive dysfunction, chronic toxicity and safety related issues. Further, many drugs are not able to cure the condition or prevent the relapse and repeatedly observed to cause drug interactions [2].

These findings have stimulated a considerable number of research efforts for development of drugs from

*Author for correspondence

Email: niyati20103@gmail.com

natural sources for treatment of the epileptic seizures in order to find safe and effective lead compounds for the treatment of this debilitating neurological disorder. In this regard, medicinal plants with potential antiepileptic activity would be important source for development of newer drugs with possibly lesser side effects [2]. Natural products from folk remedies have contributed significantly in the discovery of modern drugs with better safety and efficacy profiles compared to the conventional medicines [2, 3].

Many medicinal plants from Ayurveda and Traditional Chinese Medicine (TCM) have been reported for their Central Nervous System (CNS) potential and antiepileptic activity for example *Withania somnifera* Linn. (Solanaceae), *Bacopa monnieri* Linn. (Scrophulariaceae), *Berberis vulgaris* Linn. (Berberidaceae), *Nardostachys jatamansi* DC (Valerianaceae), *Caesalpinia sappan* Linn. (Fabaceae), *Valeriana officinalis* Linn. (Valerianaceae). Several traditionally reported plants when evaluated for pharmacological studies using modern *in vitro* and *in vivo* methods for anti convulsant property, have shown promising results [2, 3].

Gmelina arborea Roxb. (Family: Verbenaceae) commonly known as 'Gambhari', 'Candhar tree' or 'White teak' is a moderate sized, deciduous tree found distributed throughout the greater part of India up to an altitude of 1500 meters [4]. The roots and heartwood are used as bitter tonic, stomachic, laxative and prescribed in fever, indigestion, heart disease, piles and liver disorders. [5-7] Major chemical constituents of heartwood and stem include phenolics like paulownin acetate, epieudesmin and lignans such as arboreol, isoarboreol, gmelanone, ketolignans like arborone, hydroxylignan along with iridoid glycoside. [6-9] Heartwood of *Gmelina* is used as an ingredient in many well-known Ayurvedic and herbal preparations like Dashmula and Chyawanprasha and is therefore much used in a variety of diseases. [6, 10] Pharmacological studies indicating anti-inflammatory [11, 12], antioxidant [13], antimicrobial [14], anti-ulcer [15] and wound healing [16] potential of different plant parts are well reported. The plant extracts are also reported for their hypolipidemic activity [17], antidiabetic activity [18], anti-diarrhoeal activity [19] and immunomodulatory activity [20]. The plant is described to be used to improve memory and as a remedy for hallucinations [8, 9] but scientific study supporting the

role of stems and heartwood of *G. arborea* in treatment of nervous system disorders like epilepsy or convulsions have not been reported. Therefore, present study has been undertaken to assess antioxidant and antiepileptic effect of methanol extract of stems of *G. arborea* in adult albino mice.

2. Material and Methods

2.1 Drugs and Chemicals

All the chemicals and reagents used were of analytical grade. Pentylentetrazole, strychnine and diazepam were acquired from MP Biomedicals Pvt. Ltd. (Mumbai, India), Sigma Aldrich Chemicals Pvt. Ltd. (Steinheim, Germany), and Ranbaxy Laboratories Limited (Mumbai, India) respectively.

2.2 Plant Material

Fresh stems were collected from fully-grown trees from fields near the outskirts of Ankleshwar city (Dist. Bharuch), during October 2010. The authenticity was established by comparing its morphological and microscopical characters with the available literature and by an ethanobotanist, Dr. Bhasker L. Punjani, of Smt. S.M. Panchal Science College, Talod (Dist. Sabarkantha). Voucher specimen [PL11NSAHKga001] was submitted to Institute of Pharmacy, Nirma University, Ahmedabad. Stems were shade dried and pulverized for further use.

2.3 Preparation of Extract

100 g powder of stems was subjected separately to continuous and sequential hot solvent extraction using 500 mL 50% methanol in a soxhlet apparatus for 48 h at 50 °C. Filtrate was dried under vacuum using rotary vacuum evaporator at temperature below 50 °C (percentage yield = 20 %w/w) which was stored in air tight container for further use upon drying.

2.4 Phytochemical Analysis

Preliminary phytochemical tests were performed to detect the presence of alkaloids, flavanoids, saponin glycosides, tannins, sterols, carbohydrates, phenolics, coumarins, fixed oils and fats. Total phenolic and flavonoid content of stems of *G. arborea* were determined in methanol extract by following methods [21, 22].

2.4.1 Estimation of Phenolic Substances

One gram of air-dried powder of stems of *G. arborea* was extracted with 100 mL of methanol by maceration for 24 h to prepare methanol extract and then filtered. The final volume of the filtrate was adjusted to 100 mL. Five mL of the filtrate was diluted with an equal volume of methanol and was used for the estimation of phenolics. To 10 mL of stem extract, 10 mL of distilled water and 1.5 mL of diluted (1:2) Folin Ciocalteu reagent was added and the mixture was kept for 5 min. After adding 4 mL of 20 %w/v Na₂CO₃ solution, the final volume was adjusted to 25 mL using distilled water. The absorbance was measured at 765 nm at an interval of 30 min up to 2 h and distilled water was used as a blank. The data was compared with similarly prepared set of standard substance - gallic acid, in concentration range of 50 µg to 300 µg per 25 mL. Total phenolic content was calculated by the method described by Singleton and Rosi, 1965 [21].

2.4.2 Estimation of Flavonoids

One gram of air-dried powder of stems of *G. arborea* was extracted with 100 mL methanol by maceration for 24 h and filtered. The final volume of the filtrate was adjusted to 100 mL and one ml of the filtrate was diluted up to 10 mL with methanol and was used for the estimation of flavonoids. To 3 mL of this extract, 3 mL of methanolic AlCl₃ was added. After 10 min, the absorbance was read at 430 nm and distilled water was used as a blank. The data was compared with similarly prepared set of standard substance - quercetin, in concentration range of 25 µg to 300 µg per 25 mL. Total flavonoid content was calculated by the method described by Bahorun *et al.*, 1996 [22].

2.5 Antioxidant activity MES of *Gmelina arborea*

2.5.1 DPPH Radical Scavenging Activity

The antioxidant activity of MES was assessed on the basis of the radical scavenging effect of the stable DPPH free radical [23]. MES (10-100 µL) was added to 3 ml of DPPH in methanol (0.33%) in a test tube. After incubation at 37 °C for 30 min, the absorbance of each solution was determined at 517 nm using UV visible spectrophotometer [24, 25]. The corresponding blank reading was also taken and the remaining DPPH was calculated using following formula:

$$\text{DPPH radical scavenging activity (\%)} = \left[\frac{\text{Abs (control)} - \text{Abs (MES)}}{\text{Abs (control)}} \right] \times 100$$

Where, Abs (control): Absorbance of DPPH radical + methanol

Abs (MES): Absorbance of DPPH radical + MES

2.5.2 Hydrogen Peroxide Scavenging Potential

The ability of the MES to scavenge hydrogen peroxide was determined according to the method of Ruch *et al.* [26]. A solution of hydrogen peroxide (2 mM/L) was prepared in phosphate buffer (pH 7.4). MES (10– 100 µg/mL) was added to hydrogen peroxide solution (0.6 mL) and absorbance of hydrogen peroxide at 230 nm was determined after 10 min against a blank solution containing phosphate buffer without hydrogen peroxide. For each concentration, a separate blank sample was used for background subtraction. The percentage scavenging activity of hydrogen peroxide was calculated using the following formula,

$$\% \text{ scavenging activity [H}_2\text{O}_2] = \left[\frac{\text{Abs (control)} - \text{Abs (MES)}}{\text{Abs (control)}} \right] \times 100$$

Where, Abs (control): Absorbance of the control and
Abs (MES): Absorbance of the MES

2.5.3 Reducing Power Assay

The reducing power of MES was determined according to the method of Gupta *et al.*, 2007 [27]. Different concentrations of the MES (10–100 µg/mL) in 1.0 mL of deionised water were mixed with phosphate buffer (2.5 mL, 0.2 M, pH 6.6) and potassium ferrocyanide (2.5 mL, 1%). The mixture was incubated at 50 °C for 20 min. A portion of trichloroacetic acid (2.5 mL, 10%) was added to the mixture, which was then centrifuged at 3000 rpm for 10 min. The upper layer of the solution (2.5 mL) was mixed with distilled water (2.5 mL) and ferric chloride (FeCl₃) (0.5 mL, 0.1%) and the absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicated increased reducing capacity of the sample.

2.6 Evaluation of Anticonvulsant Activity of MES of *G. arborea*

2.6.1 Animals

Healthy adult albino mice weighing between 25-30 g were selected for the study and housed in well ventilated propylene cages in the animal house of

Institute of Pharmacy, Nirma University, Ahmedabad, India. Animals were maintained at 25 ± 2 °C, 50-60 % RH and kept under natural photoperiodic condition with free access to food and water. During the period of experiment the animals were fed with the standard diet (M/S Pranav Agro Tech Ltd. Vaghodiya, Vadodara) and water *ad libitum*. They were kept for one week in laboratories before the experiments for acclimatization to the laboratory conditions. The study was conducted after approval from the Institutional Animal Ethics Committee (IAEC) of Institute of Pharmacy and the protocol number given was IPS/PCOG/MPH1011/2016). Animal ethics guidelines and good laboratory practice guidelines were followed and precautions were taken to minimize pain and discomfort to the animals.

2.6.2 Preparation of Test Drug Material

Suspension of methanol extract of stems (MES) of *G. arborea* was prepared freshly using 0.5% carboxymethyl cellulose (CMC) in normal saline and was used for evaluation of anticonvulsant activity.

2.6.3 Experimental Protocol for Anticonvulsant Activity

The mice were randomly divided into four different groups with 6 mice ($n = 6$) in each group for PTZ and STR induced models (Table 1). Two different doses of MES were administered orally using oral gastric gavage tube one hour prior to the administration of the inducers viz. PTZ (60 mg/kg, s.c.) and STR (2 mg/kg, i.p.) [28]. The animals were observed for 1 h by placing in a separate cage. The onset of seizure and duration of tonic-clonic convulsions were recorded and the number of animals convulsing or not convulsing within the observation period were noted [29]. The latency for development of convulsions in mice and mortality rate were also noted. The ability of the plant extract to prevent or delay the onset of convulsions was taken as an indication of

anticonvulsant activity [30]. The doses were decided on basis of literature review wherein these dose levels have been screened for other pharmacological activities of the extracts of same plant including the evaluation of anti diabetic activity of the roots and stems of *G. arborea* at the dose levels i.e. 250 and 500 mg/kg body weight in Wistar rats [31, 32]. The toxicity studies have also been reported for aqueous and methanol extract of bark of *G. arborea* (ME). Acute toxicity study on Swiss albino mice at a dose range of 300–5000 mg/kg, p.o. and repeated dose toxicity study on Wistar rats at the doses of 300, 1000, and 2000 mg/kg/day, p.o. for 28 days have been reported with no symptoms of toxicity or mortality at the doses tested in both acute and repeated dose toxicity study [31, 33, 34].

2.7 Statistical Analysis

Data were expressed as mean \pm S.E.M using Graph pad prism 5. Statistical significance between normal control and diseased control groups was tested using one way ANOVA followed by Post-hoc Dunnett's *t*-test.

3. Results

3.1 Phytochemical Analysis

Results revealed that methanol extract of stems showed considerable amount of flavonoids and phenolics present along with presence of alkaloids, tannins, carbohydrates, sterols, lignans and saponin glycosides. Further estimation of total phenolics and flavonoids clearly indicated phenolic rich composition of stems with 0.634% of phenolics and 0.9% of total flavonoid content in the methanol extract of stems (Fig. 1).

3.2 Antioxidant Activity

3.2.1 DPPH Radical Scavenging Activity

Decrease in the absorbance was read to calculate % inhibition in the presence of extracts of stems of *Gmelina*

Table 1: Treatment protocol for evaluation of anticonvulsant activity of MES of *G. arborea* on PTZ induced convulsions and STR induced convulsions in mice

Sr. no.	Groups	Treatment
1.	Induced control	Pentylentetrazole (PTZ) (60mg/kg, s.c) + Vehicle Strychnine (STR) (2mg/kg, i.p.) + Vehicle
2.	Standard	Diazepam, 5mg/kg, p.o. + PTZ Diazepam, 5mg/kg, p.o. + STR
3.	S250	MES in dose of 250 mg/kg, p.o. + PTZ MES in dose of 250 mg/kg, p.o. + STR
4.	S500	MES in dose of 500 mg/kg, p.o. + PTZ MES in dose of 500 mg/kg, p.o. + STR

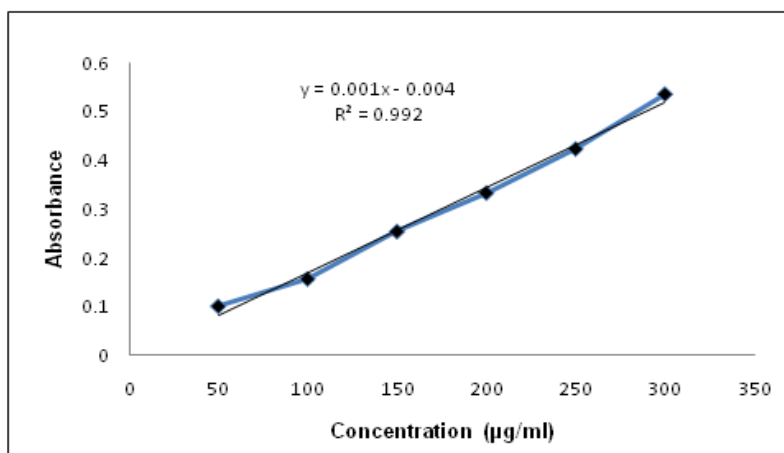


Fig. 1a. Standard curve for total phenolic content estimation (Quercetin).

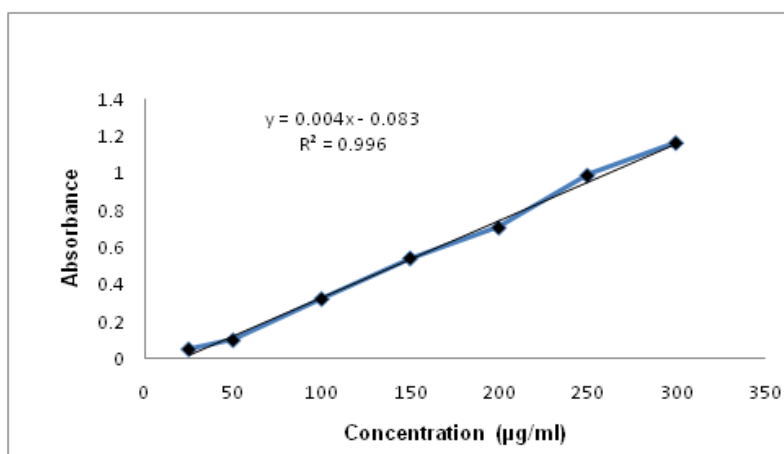


Fig. 1b. Standard curve for total flavonoid content estimation (Gallic acid).

arborea. Results showed concentration dependent response relationship in DPPH radical scavenging activity and the activity was found increased with increased concentration of the extract. IC₅₀ value of MES was found 47.4733 µg/ml (Fig. 2).

3.2.2 Hydrogen Peroxide Scavenging Potential

The Scavenging ability of methanol extract of powdered stem of *G. arborea* is shown in Fig. 3. The extract was capable of scavenging peroxide radical in concentration dependent manner and the IC₅₀ value for scavenging of H₂O₂ for methanol extract was found to be 97.3310 µg/ml (Fig. 3).

3.2.3 Reducing Power Assay

The results of reducing power assay depicted increase in the absorbance observed with corresponding

increase in the concentration of methanol extract as illustrated in the Fig. 4. The conversion of Fe³⁺ to Fe²⁺ in the presence of methanol extract could be considered as the reductive capability of constituents present in the extract. The results are suggestive of Fe³⁺ reducing ability of the constituents of methanol extract of *G. arborea* stems which might be observed due to neutralizing effect on the free radical followed by forming a stable complex.

3.3 Evaluation of Anticonvulsant Activity of MES of *G. arborea*

The anticonvulsant activity of MES (250 and 500 mg/kg) were studied in PTZ and STR induced seizure models. Our data suggests that methanol extract of stems showed significant dose dependant delay in the onset of seizures.

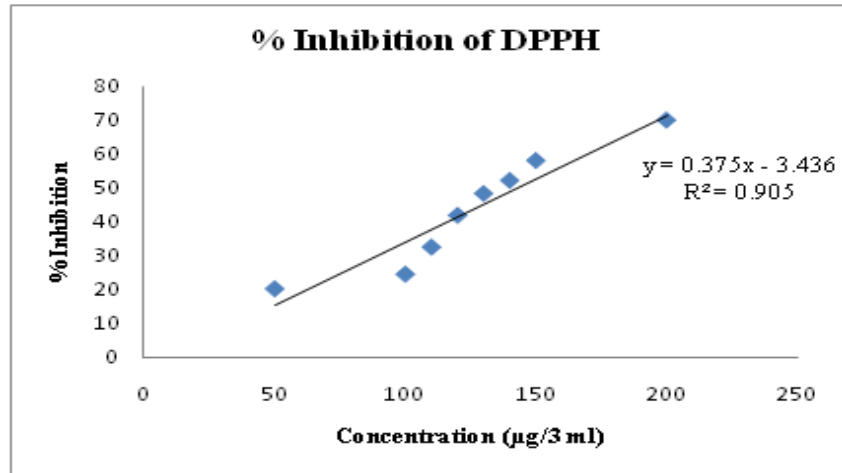


Fig. 2. Effect of MES of *G. arborea* on % inhibition of DPPH radicals.

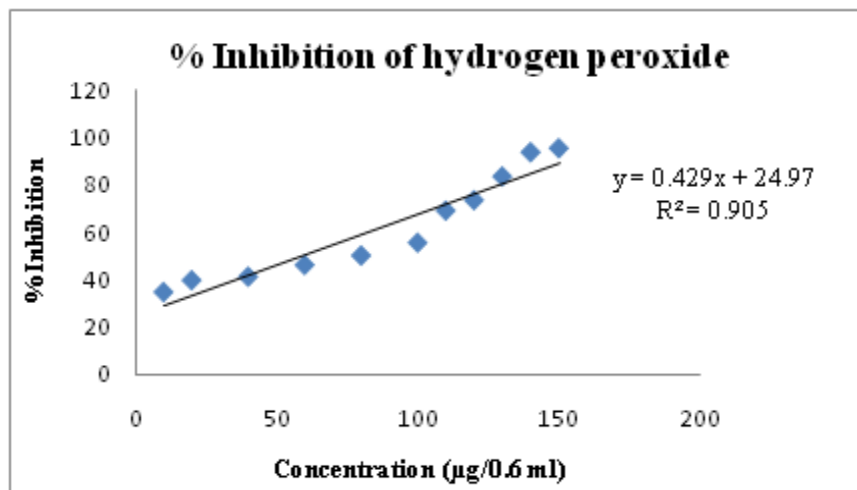


Fig. 3. Effect of MES of *G. arborea* on % inhibition of Hydrogen peroxide.

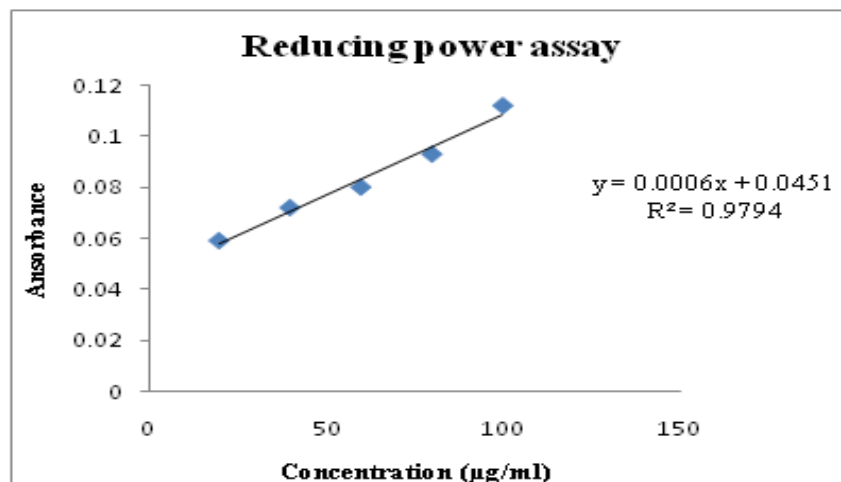


Fig. 4. Reducing power determination of MES of *G. arborea*.

3.3.1 Pentylentetrazole (PTZ) Induced Convulsion Model

Results of our study indicated that administration of MES at dose of 500 mg/kg body weight, one hour prior to injection of PTZ, significantly delayed the onset of convulsions (8.188 min, $P < 0.0001$) which was found even higher than the standard group (3.494 min). However, it was observed that MES at the same dose level were not able to decrease the duration of clonic and tonic convulsions. MES at the dose of 250 mg/kg body weight was also found to be active due to their ability to delay the onset of convulsion by 2.408 min respectively.

Further, pre-treatment with MES was found to prolong the latency of convulsions induced by PTZ dose dependently and protected the animals from

mortality. MES at the dose of 250 mg/kg and 500 mg/kg protected 60% and 80% of the animals while the standard antiepileptic drug, diazepam, offered complete protection to the animals from mortality (Table 2).

3.3.2 Strychnine (STR) Induced Seizure Model

In STR induced seizure model, mortality rate was found to be more than that in PTZ induced seizure model. MES at dose of 500 mg/kg was found to produce significant ($P < 0.0001$) delay in onset of convulsion (11.81 min) while treatment with 250 mg/kg showed less increase in onset of convulsion. MES at dose of 250 mg/kg were found to have minimum protection against convulsion, 40%. MES at dose of 500 mg/kg showed 80% protection (Table 3).

Table 2: Effect of methanol extract of powdered stems of *G. arborea* on PTZ induced convulsions in mice

Groups	Dose (mg/kg)	Onset (min)	Clonic (min)	Tonic (min)	Latency (min)	% Protection
Induced control	1 ml	2.223± 0.0511	4.795± 0.4944	4.685± 0.4628	4.541± 0.5201	0
Standard (Diazepam)	5	3.494± 0.1642	5.516± 0.04155	4.65± 0.1655	3.986± 0.1731	100
S250	250	3.866± 0.2451 [#]	5.322± 0.3021	4.73± 0.2148	3.538± 0.1515	60
S500	500	8.188± 0.7259 [*]	6.4± 0.4259 [#]	5.564± 0.329	4.514± 0.3188	80

N = 5 in each group, all values are mean ± SEM. Induced control: 0.9% NaCl, (1 ml/kg) p.o. and pentylentetrazole (60mg/kg, s.c.), Diazepam (5mg/kg, i.p) as standard, S250: methanol extract of stem (250 mg/kg p.o.), S500: methanol extract of stem (500 mg/kg p.o.). [#]significantly different from solvent control group $P < 0.01$ and ^{*}significantly different from solvent control group $P < 0.0001$

Table 3: Effect of methanol extract of powdered stems of *G. arborea* on STR induced convulsions in mice

Groups	Dose (mg/kg)	Onset (min)	Clonic (min)	Tonic (min)	Latency (min)	% Protection
Induced control	1 ml	3.808± 0.1243	3.846± 0.1277	6.286± 0.2162	2.845± 0.4154	0
Standard (Diazepam)	5	13.35± 0.6638 [*]	12.37± 0.4718 [*]	14.85± 1.211 [*]	1.132± 0.1337 [*]	100
S250	250	7.854± 0.1257 [*]	8.078± 0.0265 [*]	9.578± 2.12	1.594± 0.2669 [#]	40
S500	500	11.81± 0.3921 [*]	11.74± 0.3509 [*]	15.1± 0.2128 [*]	1.684± 0.1841 [#]	80

N = 5 in each group, all values are mean ± SEM. Induced control: 0.9% NaCl, (1 ml/kg) p.o. and STR (2 mg/kg, i.p), Standard: Diazepam (5 mg/kg, i.p), S250: methanol extract of stem (250 mg/kg p.o.), S500: methanol extract of stem (500 mg/kg p.o.). [#]significantly different from solvent control group $P < 0.01$ and ^{*}significantly different from solvent control group $P < 0.001$

4. Discussion

Results of phytochemical analysis revealed that methanol extract of stems showed considerable amount of flavonoids and phenolics which may be responsible for observed antioxidant and anticonvulsant activities of MES. Neuronal hyper excitability and unwarranted production of free radicals have been implicated in the pathogenesis of a substantial array of neurological disorders, including epilepsy [35]. The high rate of oxidative metabolism, coupled with the low antioxidant defences and the richness in polyunsaturated fatty acids, makes the brain highly vulnerable to free radical damage. Further many free radical scavenging assays are utilized to judge antioxidant potential of natural compounds and DPPH radical scavenging activity is considered as a basic and rapid screening method which demonstrated the antioxidant effect by decrease in the absorbance at 517 nm [36]. Free radical quenching has been recognized as one of the pivotal mechanism to prevent lipid peroxidation by various oxidants. Methanol extracts of powdered stem of *G. arborea* was able to reduce free DPPH with IC_{50} value 47.4733 $\mu\text{g/ml}$. The effect of antioxidants on DPPH is thought to be due to their hydrogen donating ability. Through the DPPH radical scavenging activity, the study showed that the extracts have the proton-donating ability due to presence of phenolics and could serve as free radical inhibitors or scavengers, acting possibly as primary antioxidants [36].

Hydrogen peroxide radical is not very much reactive, but can sometimes be noxious to cells due to generation of extremely reactive hydroxyl radicals in the cells which have been implicated as highly damaging reactive species. Scavenging of H_2O_2 by methanol extract of stems might be attributed to their phenolic content, which can donate electrons to H_2O_2 and thus neutralizing it to water [25]. The extracts were capable of scavenging hydrogen peroxide ($IC_{50} = 97.3310 \mu\text{g/ml}$) in a concentration-dependent manner which is considered as an important antioxidant defence parameter in the cells.

Methanol extract of powdered stem of *G. arborea* showed the reductive capabilities in a concentration-dependent manner, as results indicated increase in absorbance in reducing power assay. MES could reduce the most Fe^{3+} ions owing to its phenolic composition. Increased absorbance of the reaction indicated increased reducing capabilities [27]. Phytochemical screening and

estimation of total phenolic content showed considerable amount of phenolics present in stems of this plant. This report suggests likely development of a mechanism behind the observed anticonvulsant activity of stems and the possible role of the polyphenols in anticonvulsant potential with underlying mechanisms like antioxidant activity.

The anticonvulsant activity of MES of *G. arborea* at two dose levels (250 and 500 mg/kg) was studied by PTZ and STR induced seizure model. PTZ is a non-competitive Gamma Amino Butyric Acid (GABA) receptor antagonist and works by inhibiting the activity of GABA at GABA_A receptor. On the other hand STR induces convulsions by antagonising glycine receptor and increases postsynaptic excitability and ongoing activity in dorsal neurons [37, 38]. PTZ is the most popular and widely used chemically induced seizure model and it represents an effective model for human generalized myoclonic and also absence seizure while strychnine model is considered valid for primary generalized seizures [39].

Most of the anti-epileptic drugs act by enhancing GABA neurotransmission. In PTZ induced seizure model, PTZ produced clonic convulsions and lethality in mice, while pre-treatment with MES at two different dose levels has resulted in to delayed onset of convulsions, prolonged latencies of tonic seizures and reduction in lethality.

It has been well reported in literature that flavonoids and phenolics enhance GABA neurotransmission and GABA is the main inhibitory neurotransmitter which is suppressed in epilepsy [40]. Thus, the suppression of seizures by MES dose-dependently might be attributed to phenolics reach composition of the extract through enhancing GABA neurotransmission in brain or by blockade of glutamatergic neurotransmission mediated by the NMDA receptor. However effects of MES of *G. arborea* on glutamatergic neurotransmission was not studied in this study.

Strychnine has been demonstrated to have a well-defined mechanism of convulsant action by directly antagonizing the inhibitory spinal cord and brainstem reflexes of glycine and thus increasing spinal reflexes [41–43]. Glycine is an important inhibitory transmitter to motor neurons and interneurons in the spinal cord, and STR acts as a selective and competitive antagonist

to block the inhibitory effects of glycine at all glycine receptors. STR sensitive synaptic inhibition in higher centres of the CNS is also mediated by glycine [28]. In the STR-induced seizure model, it is known that STR directly antagonises the inhibitory spinal reflexes of glycine [43] and suppression of seizures by MES at dose of 500 mg/kg may be due to, the glycine inhibitory mechanisms of constituents of MES. MES in dose of 500 mg/kg was significantly active against convulsion produced by PTZ and STR.

5. Conclusion

In present study, anti-oxidant activity and protective effects of methanol extract of stems against seizures induced by PTZ and STR were evaluated. The observed antioxidant and anticonvulsant activities are due to the presence of considerable amount of flavonoids and phenolics in the extract of stems. Increased oxidative load is directly implicated as seizures can cause imbalance in oxidant, antioxidant system of brain which leads to oxidation of lipids, DNA and protein ultimately resulting into neurodegeneration. Methanol extract of stems of *G. arborea* showed good anticonvulsant activity in PTZ as well as STR induced convulsions may be through the involvement of GABAergic and glutaminergic transmission and through glycine inhibitory property. However, further studies are needed to develop the exact underlying mechanism of anticonvulsant action of possible constituents of the plant after isolation of bioactives. Thus results of our study showed promising anti-oxidant and anticonvulsant effects of methanol extract of stems against both the toxicants and provided a scientific claim to the usefulness of this traditional plant in neurological disorders like epilepsy.

6. References

1. Jobst BC. What Is a Seizure? Insights from Human Single-Neuron Recordings. *Epilepsy Curr.* 2012; 12:135–7.
2. Kumar S, Madaan R, Bansal G, Jamwal A, Sharma A. Plants and Plant Products with Potential Anticonvulsant Activity – A Review. *Phcog Commn.* 2012; 2(1):3–99.
3. Hasan S, Dwivedi V, Misra M, Singh PK, Hashmi F, Ahmed T. Anti-epileptic activity of some medicinal plants. *Int J Med Arom Plants.* 2012; 2(2):354–60.
4. Anonymous. The Wealth of India, Raw Materials. Volume IV. New Delhi: Council of Scientific and Industrial Research; 1956.
5. Sharma BP, Balakrishnav N. *Flora of India*, 2. India: Botanical survey of Calcutta; 1993.
6. Acharya NS, Acharya SR, Shah MB, Santani DD. Development of Pharmacognostical Parameters, and Estimation of β -sitosterol using HPTLC in Roots of *Gmelina arborea* Roxb *Phcog J.* 2012; 4(30):1–9.
7. Deka L, Majumdar R, Dutta AM. Some Ayurvedic important plants from district Kamrup (Assam). *Ancient Sci Life.* 1983; 3(2):108–15.
8. Rastogi RP, Mehrotra BN. *Compendium of Indian medicinal plants*, Volume 1. New Delhi: Central Drug Research Institute and Publication and Information Directorate; 1990.
9. Chopra RN, Nayar SL, Chopra LC. *Glossary of Indian medicinal plants*. Delhi: Council of Scientific and Industrial Research; 1999.
10. Tewari DN. A monograph on Gamari (*Gmelina arborea* Roxb.). Dehradun, India: International book distributors; 1995.
11. Barik BR, Bhaumik T, Dey AK, Patra A, Chatterjee A. Premnazole, an isolated alkaloid of *Premna integrifolia* L. and *Gmelina arborea* L. with anti-inflammatory activity, *Fitoterapia.* 1976; 63(4):295–9.
12. Singh A, Malhotra S, Subban R. Anti-inflammatory and analgesic agents from Indian medicinal plants, *IJIB.* 2008; 3(1):57–72.
13. Sinha S, Dixit P, Bhargava S, Devasagayam TPA, Ghaskabdi S. Bark and fruit extracts of *Gmelina arborea* protect liver cells from oxidative stress. *Pharm Biology.* 2006; 44(4):237–43.
14. El-Mahmood AM, Doughari JH, Kiman HS. *In vitro* antimicrobial activity of crude leaf and stem bark extracts of *Gmelina arborea* (Roxb) against some pathogenic species of Enterobacteriaceae. *Afr J Pharm Pharmacol.* 2010; 4(6):355–61.
15. Giri M, Divakar K, Goli D, Dighe SB. Anti-ulcer activity of leaves of *Gmelina arborea* plant in experimentally induced ulcer in Wistar rats. *Pharmacologyonline.* 2009; 1:102–10.
16. Shirwaikar A, Ghosh S, Rao PGM. Effect of *Gmelina arborea* Roxb leaves on wound healing in rats. *J Nat Remedies.* 2002; 3(1):45–7.
17. Khanna AK, Chander R, Kapoor NK. Hypolipidemic activity of Abana in rats. *Fitoterapia.* 1991; 62(3):271–5.
18. Kulkarni YA, Veeranjanyulu A. Amelioration of STZ induced Type I diabetic nephropathy in rats by a

- phytomedicine: *Gmelina arborea*. *FASEB J.* 2010; 24:569–75.
19. Agunu A, Yusuf S, Andrew GO, Zezi AU, Abdurahman EM. Evaluation of five medicinal plants used in diarrhoea treatment in Nigeria. *J Ethnopharmacol.* 2005; 101:27–30.
 20. Shukla SH, Saluja AK, Pandya SS. Modulating effect of *Gmelina arborea* Linn. on immunosuppressed albino rats. *Pharmacognosy Res.* 2010; 2(6):359–63.
 21. Singleton VL, Rossi JA. Colorimetry of total phenolics with phosphomolybdic - phosphotungstic acid reagents. *Amer J Enol Viticult.* 1965; 16:144–58.
 22. Bahorun T, Gressier B, Trotin F, Brunete C, Dine T, Gazin J, Pinkas JC, Luycky M, Gazin M. Oxygen species scavenging activity of phenolics extracts from Hawthorn fresh plant organs and pharmaceuticals preparations. *Drug Res.* 1996; 46(11):1086–9.
 23. Blois MS. Antioxidant Determinations by the Use of Stable Free Radical. *Letters to Nature.* 1958; 181:1199–200.
 24. Hwang BY, Kim HS, Lee JH, Hong YS, Ro JS, Lee KS. Antioxidant benzoylated flavan-3-ol glycoside from *Celastrus orbiculatus*. *Journal of Natural Products.* 2001; 64:82–4.
 25. Patil SM, Kadam VJ, Ghosh R. *In vitro* antioxidant activity of methanol extract of stem bark of *Gmelina arborea* ROXB. (Verbenaceae). *International Journal of PharmTech Research.* 2009; 1(4):1408–84.
 26. Ruch RJ, Cheng SJ, Klaunig JE. Prevention of cytotoxicity and inhibition of intracellular communication by antioxidant catechins isolated from Chinese green tea. *Carcinogenesis.* 1989; 10:1003–8.
 27. Gupta M, Mazumdar UK, Gomathi P. *In vitro* antioxidant and free radical scavenging activities of *Galenga purpurea* root. *Phcog Mag.* 2007; 3:219–25.
 28. Vogel HG. *Drug discovery and evaluation.* 2nd ed. New York: Spinger publication; 2002.
 29. Amabeoku GJ, Chikuni O. Cimetidine-induced seizures in mice: Antagonism by some GABAergic agents. *Biochem Pharmacol.* 1993; 46(12):2171–5.
 30. Salahdeen HM, Yemitan OK. Neuropharmacological effects of aqueous leaf extract of *Bryophyllum pinnata* in mice. *Afr J Biomed Res.* 2006; 9:101–7.
 31. Shah K, Kulkarni Y, Chavan D. Toxicity study and analgesic activity of *Gmelina arborea* extract and its fractions. *Journal of Natural Pharmaceuticals.* 2013; 4(1):71–4.
 32. Acharya N, Barai P, Katariya H, Acharya S, Santani D. Evaluation of antidiabetic potential of roots and stems of *G. arborea*. *Int J Pharm Pharm Sci.* 2015; 7(8):355–62.
 33. Kulkarni Y, Veeranjanyulu A. Toxicological studies on aqueous extract of *Gmelina arborea* in rodents. *Pharm Biol.* 2010; 48(12):1413–20.
 34. Kulkarni YA, Veeranjanyulu A. Toxicological evaluation of the methanol extract of *Gmelina arborea* Roxb. bark in mice and rats. *Toxicol Int.* 2012; 19(2):125–31.
 35. Devi PU, Manocha A, Vohora D. Seizures, antiepileptics, antioxidants and oxidative stress: an insight for researchers. *Expert Opin Pharmacother.* 2008; 9(18):3169–77.
 36. Koleva II, Van BTA, Linssen JPH, De GA, Evstatieva LN. Screening of plant extracts for antioxidant activity: A comparative study on three testing methods. *Phytochem Anal.* 2002; 13:8–17.
 37. Park HG, Yoon SY, Choi JY, Lee GS, Choi JH, Shin CY, Son KH, Lee YS, Kim WK, Ryu JH, Ko KH, Cheong JH. Anticonvulsant effect of wogonin isolated from *Scutellaria baicalensis*. *Eur J Pharmacol.* 2007; 574:112–9.
 38. Rogawski MA. Diverse mechanisms of antiepileptic drugs in the development pipeline. *Epilepsy Res.* 2006; 69(3):273–94.
 39. Rasilingam D, Duraisamy S, Subramanian R. Anticonvulsant activity of bioflavonoid gossypin. *Bangladesh J Pharmacol.* 2009; 4(1):51–4.
 40. Salgueiro JB, Ardenghi P, Dias M, Ferreira MB, Izquierdo I, Medina JH. Anxiolytic natural and synthetic flavonoid ligands of the central benzodiazepine receptor have no effect on memory tasks in rats. *Pharmacol Biochem Behav.* 1997; 58(4):887–91.
 41. Biggio G, Cibir M, Diana M, Fadda F, Ferrara SD, Gallimberti L, Gessa GL, Mereu GP, Rossetti ZL, Serra M. Suppression of voluntary alcohol intake in rats and alcoholics by gamma-hydroxybutyric acid: a non-GABAergic mechanism. *Adv Biochem Psychopharmacol.* 1992; 47:281–8.
 42. Rang HP, Dale MM, Ritter JM. *Pharmacology.* 3rd ed. London: Churchill Livingstone, Longman Group; 1998.
 43. Dhayabaran D, Florance J, Krsihnadas N, Indumathi, Muralidhar. Anticonvulsant activity of alcoholic root extract of *Cardiospermum halicacabum*. *Rev Bras Farmacogn.* 2012; 22(3):623–9.