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Short Communication

Protective effect of *Grewia asiatica* leaves extract in animal models of epilepsy and anxiety

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ABSTRACT

Grewia asiatica Linn. is a well-known plant for its nutritional and therapeutic attributes. It has been mentioned in ancient Indian literature as Rasayana due to its stimulant and tonic effects. Thus, present investigation was carried out to evaluate the antiepileptic and anxiolytic action of *G. asiatica* Linn. leaves using animal models. Methanol extract at dose levels of 100 and 200 mg/kg was capable of providing protection against both pentylenetetrazole and maximal electroshock induced seizures in mice. Extract also showed significant anxiolytic activity in elevated plus maze, light/dark box and mirror chamber mice models at same dose levels. Results of this study indicated that the methanol extract of leaves of *G. asiatica* plant possess significant antiepileptic and anxiolytic effect.

1. Introduction

Epilepsy is a major health problem with huge psychosocial impact. The problem of epileptic seizures is very common in neurodegenerative disorders [1]. The psychiatric comorbidity i.e. anxiety is commonly observed in patients with epilepsy and has a profound influence on the quality of life of these patients. Synthetic antiepileptic medicines undoubtedly have good efficacy but their associated side effects, toxicity and narrow safety margin remain the major issue [2–4]. On the other hand, natural products have been emerging as potential alternatives to synthetic antiepileptic and anxiolytic drugs [5]. The use of traditional herbal medications for the treatment of different neurodegenerative disorders is becoming very common among the physicians in Europe and Asian countries [6]. *Grewia asiatica* Linn., commonly known as phalsa in India is a food plant with immense therapeutic potential. The methanol extract of *G. asiatica* Linn. leaves have been reported to possess various pharmacological activities [7]. Earlier studies have indicated the neuroprotective, antidepressant, sedative and hypnotic effect of methanol extract of *G. asiatica* [8,9]. In Indian Materia Medica, it has been mentioned as tonic and stimulant and has been used for the same property by the folk medicinal healers [10], but scientific evidence for these claims has not been reported anywhere. Taking a lead from the above cited findings *G. asiatica* leaves were investigated for its antiepileptic effect and its role in curtailing the anxiety in animal models.

2. Materials and methods

2.1. Plant material

Leaves of *G. asiatica* Linn. were collected in the month of April from the local area of Amritsar located in Punjab and its botanical identity was verified by Dr. Amarjit Soodan having expertise in Plant Systematics, Department of Botanical and Environmental Sciences, Guru Nanak Dev University, Amritsar (India). A voucher specimen No. 1041 has been deposited in herbarium of the department.

Fresh leaves were shade dried, coarsely powdered and subjected to successive Soxhlet extraction with solvents in increasing order of polarity viz. petroleum ether (60–80 °C), chloroform, and methanol. The methanol extract was concentrated using rota-evaporator and dried [11].

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2.2. Animals

Swiss albino mice (25–30 g) were used in the present study. The animals were given standard laboratory feed and water *ad libitum*. The biological studies were performed as per the guidelines of the institutional ethical committee (IAEC/GNDU/2013/19).

2.3. Animal models for epilepsy

For antiepileptic evaluation, pentylenetetrazole (PTZ) induced seizures and maximal electroshock seizures (MES) models were employed. Both models contained four groups, having six animals in each (Table S1). All recordings were done after 30 min of the administration of vehicle/standard/extract. In the MES model, seizures were induced by delivering stimulus of 50 Hz and 50 mA through corneal electrodes. After that, duration of tonic hindlimb flexion, extensor, stupor and convulsions were recorded [12]. In the MES model, phenytoin (25 mg/kg) was used as the standard drug.

In the PTZ model, seizures were induced by administration of PTZ (70 mg/kg, i.p.) to all animals. After that onset and duration of clonic convulsions was recorded [13]. The protection from seizures was calculated by using the following formula-

$$\%\ \text{protection} = \frac{\text{Number of animals in which duration of seizures was less than 20 seconds}}{\text{Total number of animals in that group}} \times 100$$

Evaluation was done by using two dose levels of MEGA (100 and 200 mg/kg per oral). The doses were selected from the previous published reports and studies carried out by various researchers on various animals models [10,14]. In the PTZ model diazepam (5 mg/kg) was used as the standard drug [15].

2.4. Evaluation of anxiolytic activity

Elevated plus maze, Light/dark box and Mirror chamber mice models were employed for the evaluation of anxiolytic activity. All models contained five groups, having six animals in each (Table S2). All recordings were done after 45 min of the administration of vehicle/standard/extract. Diazepam (2 mg/kg) was used as the standard drug in this study. In the Elevated plus maze model, mice were placed individually at the center of the elevated plus maze with their head facing open arm. Number of entries and average time spent in open arms were recorded during test period for 10 min. In the light/dark box model, mice were placed individually at the corner of the lit compartment. Time spent and the average number of entries in the light compartment was recorded during test period (10 min). In the mirror chamber model, mice were placed individually in the chamber of mirrors at a fixed corner. During the five minute test period latency to enter the chamber, the number of entries and the time spent with each entry in the mirrored chamber was recorded [16,17].

2.5. Statistical analysis

The anxiolytic and antiepileptic activities of methanolic extract, fractions, diazepam and control were analyzed by ANOVA. The test groups were compared with control by Tukey's Kramer multiple comparison test. Difference was considered significant at $p < 0.05$.

2.6. Isolation of compounds from bioactive extract

Isolation of phytoconstituents from methanol extract was done using column chromatography. The sample was prepared by first dissolving 0.4 g of ethyl acetate fraction in the small amount of solvent in hot conditions (50–60 °C) in a china dish. 5 g of silica gel of mesh size 60–120 was added slowly with continuous stirring. After proper mixing, the remaining solvent was removed by heating on a water bath and then air dried to get a free flowing sample. The free flowing sample was then loaded to the top of the column. The column was initially eluted with hexane followed by increasing the polarity to 50% and then to 75% with ethyl acetate until desired compounds were eluted. It gave two spots, out of which, one spot appeared in fraction (100–115) while the second spot was in fraction (121–135). These spots were then observed in a UV light (254 nm) and the compounds were designated as SB-7 and SB-8.

3. Results

Anticonvulsant activity of methanol extract of *G. asiatica* Linn. leaves (MEGA) was evaluated by using MES and PTZ mice models.

PTZ induced seizures model was employed for screening anticonvulsant potential of compounds against generalized myoclonic (absence) seizures [18]. This model is acute model of seizures. Various studies using MES and PTZ induced seizures did not report effect of male and female animals on seizures severity [19–22]. Similar results were observed in the present study. Treatment of MEGA significantly ($p < 0.05$) delayed the onset and decreased the duration of clonic convulsions in the PTZ induced seizures model (Table 1). Maximum protection from seizures was shown by the extract at the dose of 200 mg/kg.

MES induced seizures model is used for screening anticonvulsant potential of compounds against tonic-clonic (grand mal) seizures [18]. Treatment of MEGA significantly ($p < 0.05$) decreased the duration of hind limb flexion, extensor and stupor phases as compared to control. Duration of convulsions was also significantly decreased as compared to control and maximum protection from seizures shown by extract was 82% (200 mg/kg). No significant

Table 1
Effect of methanol extract of *Grewia asiatica* Linn. leaves (MEGA) on PTZ induced seizures.

Groups	Onset of clonic convulsions (s)	Duration of clonic convulsions (s)	Protection (%)
Control	100 ± 8.69	55 ± 7.3	2 ± 0.03
Diazepam (5 mg/kg)	510 ± 20.25	11 ± 1.03	98 ± 3.24
MEGA (100 mg/kg)	400 ± 30.25	30 ± 2.34	69 ± 2.45
MEGA (200 mg/kg)	480 ± 35.69	19 ± 1.87	82 ± 4.12

Values were expressed as Mean ± SEM, n = 6. Data was analyzed by one-way ANOVA followed by Tukey's test. a = $p < 0.05$ vs. control group; b = $p < 0.05$ vs. standard.

Table 2
Effect of methanol extract of *Grewia asiatica* Linn. leaves (MEGA) on MES induced seizures.

Groups	Duration of hind limb tonic flexion (s)	Duration of hind limb tonic extensor (s)	Duration of Stupor (s)	Duration of convulsions (s)	Protection (%)
Control	27 ± 1.24	16.5 ± 2.48	100 ± 9.68	31 ± 2.48	1 ± 0.025
Phenytoin (25 mg/kg)	12 ± 1.54	1 ± 0.014	58 ± 4.65	14 ± 1.85	98 ± 4.58
MEGA (100 mg/kg)	16 ± 2.58	10 ± 1.78	63 ± 3.84	19 ± 1.98	64 ± 6.98
MEGA (200 mg/kg)	14 ± 1.14	8.8 ± 1.98	60 ± 4.15	15 ± 1.78	82 ± 7.89

Values were expressed as Mean ± SEM, n = 6. Data was analyzed by one-way ANOVA followed by Tukey's test. a = $p < 0.05$ vs. control group; b = $p < 0.05$ vs. standard.

Table 3
Effect of methanol extract of *Grewia asiatica* Linn. leaves (MEGA) on different anti-anxiety models. Elevated plus maze model (A), Light/dark box model (B), Mirror chamber model (C).

Groups	Elevated plus maze model (A)		Light and dark chamber model (B)		Mirror chamber model (C)	
	No. of entries in open arm	Time spent in open arm (s)	No. of entries in light box	Time spent in light box (s)	Time spent in mirrored chamber (s)	Latency time (s)
Control	4.8 ± 0.189	10 ± 1.34	4 ± 0.64	70 ± 2.48	6 ± 0.98	100 ± 7.41
Diazepam (2 mg/kg)	11.5 ± 1.24	62 ± 4.78	24 ± 2.56	150 ± 8.69	26 ± 1.24	42 ± 3.33
MEGA (100 mg/kg)	7 ± 1.36	28 ± 4.66	12 ± 2.25	99 ± 4.65	14 ± 1.33	79 ± 4.82
MEGA (200 mg/kg)	9 ± 1.86	40 ± 3.31	16 ± 1.36	118 ± 7.69	18 ± 2.22	60 ± 3.45
MEGA (400 mg/kg)	9.8 ± 1.34	42 ± 6.15	18 ± 1.22	119 ± 8.76	16 ± 2.13	61 ± 3.73

Values were expressed as Mean ± SEM, n = 6. Data was analyzed by one-way ANOVA followed by Tukey's test. a = $p < 0.05$ vs. control group; b = $p < 0.05$ vs. standard.

difference was observed between 200 mg/kg dose of MEGA and standard drug in decreasing duration of convulsions as well as flexion and stupor phases (Table 2). Antiepileptic effect was not observed on treatment with 400 mg/kg dose in both MES and PTZ models (Data for 400 mg/kg not shown).

Anxiolytic effect of MEGA was evaluated by using Elevated plus maze, light/dark box and mirror chamber mice models. Evaluation was done by using three dose levels of MEGA (100, 200 and 400 mg/kg). In the elevated plus maze model, treatment of MEGA significantly ($p < 0.05$) increased the number of open arm entries and time spent in the open arm at all dose levels as compared to control (Table 3A). In the light/dark model, treatment of MEGA significantly ($p < 0.05$) increased the number of entries and time spent in light at all dose levels as compared to control (Table 3B). In mirror chamber model, treatment of MEGA significantly ($p < 0.05$) decreased the latency time and increased the time spent in the mirror chamber as compared to control (Table 3C). It was worth to note that there was no significant difference between the activity of 100 and 200 mg/kg dose levels of MEGA in all anxiety models that suggest the dose independent anti-anxiety effect. Overall outcomes from anxiety models suggested that MEGA exhibited anti-anxiety effects by improving emotional and psychomotor aspects in animals. Considering the effectiveness, MEGA was subjected to column chromatography that yielded SB-07 and SB-08 (kaempferol and quercetin), confirmed by NMR spectroscopy (Figs. S1–S4). NMR data was in agreement with those reported in literature [23].

4. Discussion

Results obtained from this study revealed that the methanol extract of leaves of *G. asiatica* possess significant antiepileptic and anxiolytic effect which may be attributed to the presence of flavonoids quercetin and kaempferol isolated from this plant.

Quercetin has been previously reported to reduce generalized seizures duration in PTZ model [24]. In another study, quercetin through its effects on various cell types has been shown to reduce the occurrence of seizures and is preferred for the treatment for electrical discharges and neurotransmitters which are the main causative factors in epilepsy [25]. Quercetin showed inhibition of kainic acid induced epilepsy by microglia cell inactivation and prevented proinflammatory cytokine (TNF-α, NF-κB and IL-1β) release from microglia cells [26]. Also, it was reported that quercetin treatment (at 10 and 20 mg/kg) significantly prolonged the onset and reduced the severity of the seizures in PTZ and picrotoxin models of epilepsy [27].

Kaempferol also possesses a mild effect against PTZ induced seizures and showed protective effects against NMDA induced neurotoxicity in rat neuronal cultures. Antiepileptic activity of flavonoids (polyphenolic class to which quercetin and kaempferol belongs) is considered due to the allosteric modulation of $GABA_A$ receptors and anti-inflammatory action in brain [28,29].

Quercetin has been reported to exhibit anti-anxiety effects via increasing availability of 5-HT in synapse by inhibiting monoamine oxidase (MAO) activity [30]. Kaempferol is a selective inhibitor of MAO-A, increases 5-HT in the brain and exhibits potent anxiolytic activity in mice models. Apart from 5-HT both quercetin and kaempferol are also reported to increase norepinephrine (NE) and dopamine (DA) levels in mice brain [31–33].

All these studies suggested that both kaempferol and quercetin may be responsible for the antiepileptic (via modulation of GABAergic neurotransmission and NMDA activity) and anxiolytic response (via MAO inhibition and elevation of neurotransmitters like 5-HT, NE and DA in CNS) of MEGA in mice models.

Author's contributions

Shabnampreet Kaur: Methodology, Investigation; Atamjit Singh, Hasandeep Singh: Writing original draft; Balbir Singh, Preet Mohinder Singh Bedi: Reviewing and Editing; Kunal Nepali: Analysis; Sarabjit Kaur: Conceptualization and supervision.

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Declaration of competing interest

The authors declared that they have no conflict of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jaim.2022.100616.

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