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Antiepileptic effect of ethanolic extract of seeds of *Peganum harmala* on maximal electroshock seizure model in albino mice.

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ABSTRACT

Aim of the study

The present study was under taken to evaluate the antiepileptic effect ethanolic extract of seeds of *Peganum harmala* (PHEE) in mice to validate its pharmacological property.

Materials and methods

Ethanolic extract of seeds of *Peganum harmala* was screened for antiepileptic effect by using maximal electroshock seizure threshold test at a dose of 50 mg/kg and 100 mg/kg. Distilled water and diazepam were employed as control and standard groups respectively.

Results

Ethanolic extract of seeds of *Peganum harmala* at the dose of 50 mg/kg and 100 mg/kg in combination with standard drug Diazepam at the dose of 3.0 mg/kg increases the threshold of maximal electroconvulsions in dose dependant manner 11.2±1.52 and 12.3±1.71 respectively.

Conclusion

Ethanolic extract of seeds of *Peganum harmala* was able to potentiate the effect of Diazepam in convulsions.

Keywords: Antiepileptic, Maximal Electroshock Seizure, *Peganum harmala*

INTRODUCTION

Epidemiologic studies on epilepsy show wide variations in prevalence rates (PRs) from 0.9 to 57 per 1000 population .[1–3] The marked differences in the reported PRs may be due to many factors such as accuracy of diagnosis, extent of case ascertainment, sampling criteria, instrument used, and population selection. The introduction of the World Health Organization (WHO) research protocol for neurological disorders in developing countries [4] has been a major breakthrough in the standardization of epidemiological research on epilepsy. [5] Epilepsy is one of the most common neurological diseases and is very prevalent worldwide, affecting more than 50 million people. [6] In the kingdom of Saudi Arabia, the prevalence of epilepsy is 6.54 per 1000. [7] The incidence of epilepsy is substantially greater in developing countries compared with developed countries.[8] Several international studies have demonstrated a lack of awareness regarding epilepsy among the general population and even among health care

professionals.[9,10] Individuals with epilepsy experience problems with employment, education and social relationships. [11, 12] Misguided and false beliefs about epilepsy affect the social acceptance of individuals with epilepsy. [13, 14]

Harmal (Peganum harmala) is a plant of the family Zygophyllacea, native from the eastern Mediterranean region east to India. It is also known as Wild Rue or Syrian Rue because of its resemblance to plants of the rue family. It blossoms between June and August in the Northern Hemisphere. The flowers are white and are about 2.5–3.8 cm in diameter . The round seed capsules measure about 1–1.5 cm in diameter have three chambers and carry more than 50 seeds (Photograph 1). It is a perennial plant which can grow to about 0.8 m tall. [15] The Arabic Names for this herb are Ashqaqil, Aspand, Harmal.

Photograph 1: Seeds of *Peganum harmala*

MATERIALS AND METHODS

Experimental Animals

Swiss albino mice of male sex weighing 22–28 g were used. Animals were maintained under standard conditions in an animal house approved by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). Institutional Animal Ethics Committee approved the experimental protocol. The animals were given standard diet. The animals had free access of standard diet and water and housed in a spacious cage for one week. Mice were housed in cages of 5 at 22 ± 1◦C in a 12- h light/dark cycle. Tap water and food pellets were available as libitum. Groups of 6–11mice were randomly assigned to different treatment groups and were tested in a counter balancing order. Animals were naive to experiment conditions. All experiments were carried out during night cycle of light and the experiments were carried out according to the National Research Council Guide for the Care and Use of Laboratory Animals [16]. All experiments were conducted in accordance with international standards of animal welfare recommended by the Society for Neuroscience [17]. The experimental protocol was approved by the Bioethical Committee on Animal Research. The minimum number of animals and duration of observations required to obtain consistent data were employed.

Drugs and Chemicals

The positive controls were: Diazepam (Lupin, Mumbai) for antiepileptic activity. Ethanol (Hi Media) propylene glycol (Hi Media) was purchased from the respective sources and was of analytical grade.

Treatment

The ethanolic extract of seeds of *Peganum harmala* was freshly dissolved in distilled water to be acutely administered to the rats. Doses of the extract and the time intervals were determined in preliminary tests. Diazepam (3 mg/kg) was dissolved in 40% propylene glycol. Negative control groups received only distilled water. All administrations were performed intraperitoneally (i.p.) in a dose volume of 1 ml/kg body weight. Thirty minutes after i.p. treatment, the animals were submitted to a battery of behavioral tests.

Source of Peganum harmala Seeds

Dried seeds of *Peganum harmala* were purchased from local market. The identity of the seed was confirmed by the Institutional Botanist. A

voucher specimen (PAR-01) was kept in laboratory for future reference.

Preparation of Aqueous Extract

Dried seeds were homogenized to a fine powder. Hundred grams of powdered drug was Infused in 500 ml cold ethanol for 24 h, brought to the boil, then removed from the heat source and allowed to infuse for 15 min. The extract was filtered, concentrated over the water bath and brought to dryness under vacuum. The yield of the extract was 8.7% (w/w).

Acute toxicity study

Acute toxicity study was performed using the limit test dose of 2000 mg/kg as described by Organization for Economic Cooperation and Development guideline and Interagency Research Animal Committee recommendation [18]. Six female mice were dosed sequentially and followed for any signs of toxicity and/ or death within 24 h and then for 14 days thereafter.

Maximal Electroshock Seizure Threshold Test

Electroconvulsions were produced by means of an alternating currrent (0.2 s stimulus duration, 50 Hz, maximum stimulation voltage of 500 V) delivered via ear-clip electrodes by a Rodent Shocker Generator (Type 221, Hugo Sachs Elektronik, Freiburg, Germany). The criterion for the occurrence of seizure activity was the tonic hind limb extension. To evaluate the threshold for maximal electroconvulsions, at least four groups of mice, consisting of six animals per group, were challenged with electroshocks of various intensities to yield 10–30, 30–50, 50–70, and 70–90% of animals with seizures. Then, a current intensity–response relationship curve was constructed, according to a log- probit method by [19], from which a median current strength (CS_{50} in mA) was calculated. Each CS_{50} value represents the current intensity required to induce tonic hind limb extension in 50% of the mice challenged. After administration of a single dose of each drug to 6 groups of animals, the mice were subjected to Electroconvulsions (each group with constant current intensity) and the threshold for maximal Electroconvulsions was recorded. [20]

Experimental groups

Control group: mice were injected with distilled water then 30 min later CS_{50} was recorded.

Standard group: mice were injected with Diazepam 3.0 mg/kg intraperitoneally then 30 min later CS_{50} was recorded.

Test group I: mice were injected with ethanolic extract of seeds of *Peganum harmala* (PHEE) at dose of 50 mg/kg, 30 min later CS_{50} was recorded.

Test group II: mice were injected with ethanolic extract of seeds of *Peganum harmala* (PHEE) at dose of 100 mg/kg, 30 min later CS_{50} was recorded.

Test group III: mice were injected with 3.0 mg/kg Diazepam + ethanolic extract of seeds of *Peganum harmala* (PHEE) at dose of 50 mg/kg, 30 min later CS_{50} was recorded.

Test group IV: mice were injected with 3.0 mg/kg Diazepam + ethanolic extract of seeds of *Peganum harmala* (PHEE) at dose of 100 mg/kg, 30 min later CS_{50} was recorded.

Statistical Analysis

The statistical significance was assessed using one way analysis of variance (ANOVA) followed by Dunnet comparison test. The values are expressed as mean ± SEM and $p<0.05$ was considered significant.

RESULT

Acute toxicity test

At a single oral dose of 2000 mg/kg, seeds of *Peganum harmala* Ethanol Extract does not showed signs of toxicity or death in mice within the first 24 h and during the 14 days observation period.

Maximal Electroshock Seizure Threshold Test

Ethanolic extracts of seeds of *Peganum harmala* were evaluated using maximal electroshock seizure threshold model on mice, in which PHEE at 50 mg/kg and 100 mg/kg did not affect the threshold of maximal electroconvulsions in MEST test as compared to control and standard group. However ethanolic extract of *Peganum harmala* at the dose of 50 mg/kg and 100 mg/kg in combination with standard drug Diazepam at the dose of 3.0 mg/kg increases the threshold of maximal electroconvulsions in dose dependant manner 11.2±1.52 and 12.3±1.71 respectively. Present study also showed that administration of ethanolic

extract of *Peganum harmala* at the dose of 50 mg/kg and 100 mg/kg alone does not affect the mean latency period as compared to control group. However ethanolic extract of *Peganum harmala* at the dose of 50 mg/kg and 100 mg/kg in combination with standard drug Diazepam at the dose of 3.0 mg/kg increases the mean latency period 17.9±1.5 and 18.3±1.1 respectively in dose dependant manner as compared to control and standard group.

Table1: Effect of ethanolic extract of seeds of *Peganum harmala* on the threshold of maximal electro convulsions in mice in MEST test.

Treatment	Dose	CS_{50} mA ± S.E.M.
Control (distilled water)		7.9±0.61
Standard (Diazepam)	3.0 mg/kg	10.1±0.93
Test 1 PHEE	50 mg/kg	8.2±1.2
Test 2 PHEE	100 mg /kg	8.5±1.01
Test 3 Diazepam + PHEE	3.0 mg/kg + 50 mg/kg	11.2±1.52*
Test 4 Diazepam + PHEE	3.0 mg/kg + 100 mg/kg	12.3±1.71*

(Data are presented as median current strengths (CS_{50} values in mA ± S.E.M.) required to evoke seizure activity (tonic hind limb extension) in 50% of animals tested. The CS_{50} values were calculated according to the log-probit method followed by the method transforming 95% confidence limits into S.E.M.)

Table 2: : Effect of ethanolic extract of seeds of *Peganum harmala* on mean latency period, number of convulsed mice to total number of mice and Percent Protection.

Treatment	Mean Latency period (Min) ± S.E.M.	Number of convulsed mice / total number of mice	Percent Protection
Control (distilled water)	14.1±1.2	6/6	0%
Standard (Diazepam 3mg/kg)	17.1±0.96	4/6	33.3%
Test 1 PHEE (50 mg/kg)	13.8±1.1	6/6	0%
Test 2 PHEE (100 mg/kg)	14.6±1.3	6/6	0%
Test 3 Diazepam + PHEE (3.0 mg/kg + 50 mg/kg)	17.9±1.5*	4/6	33.3%
Test 4 Diazepam + PHEE (3.0 mg/kg + 100 mg/kg)	18.3±1.1*	4/6	33.3%

(Results are presented as mean latency period (min) of convulsion.

Statistical analysis of data was performed with one way ANOVA.

S.E.M. = standard error of mean, $P < 0.05$

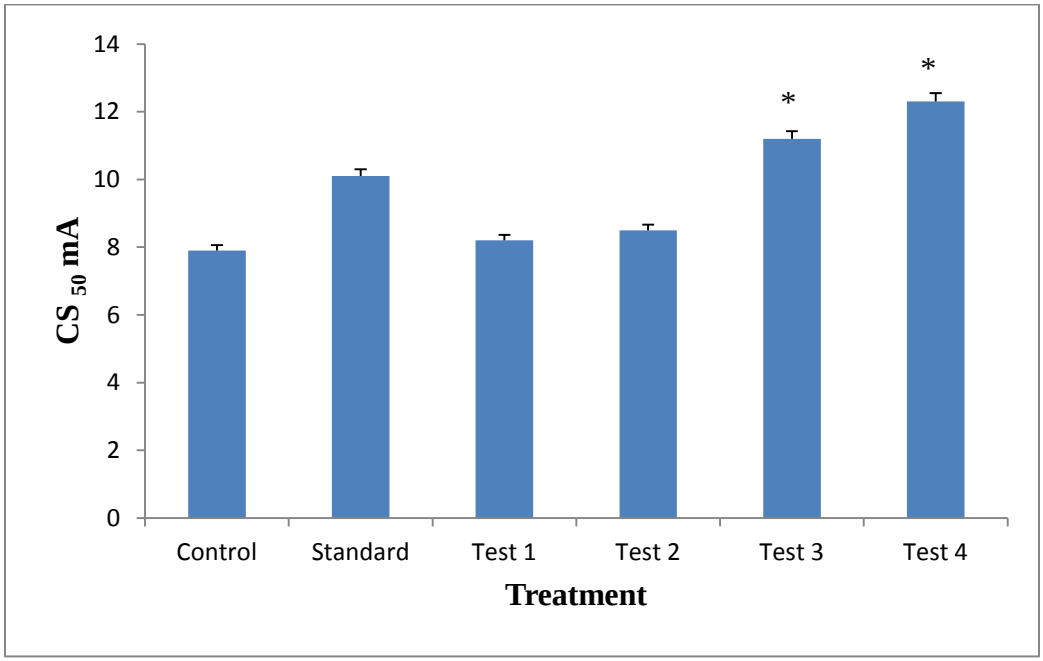

Figure 1: Effect of ethanolic extract of seeds of *Peganum harmala* on the threshold of maximal electro convulsions in mice in MEST test.

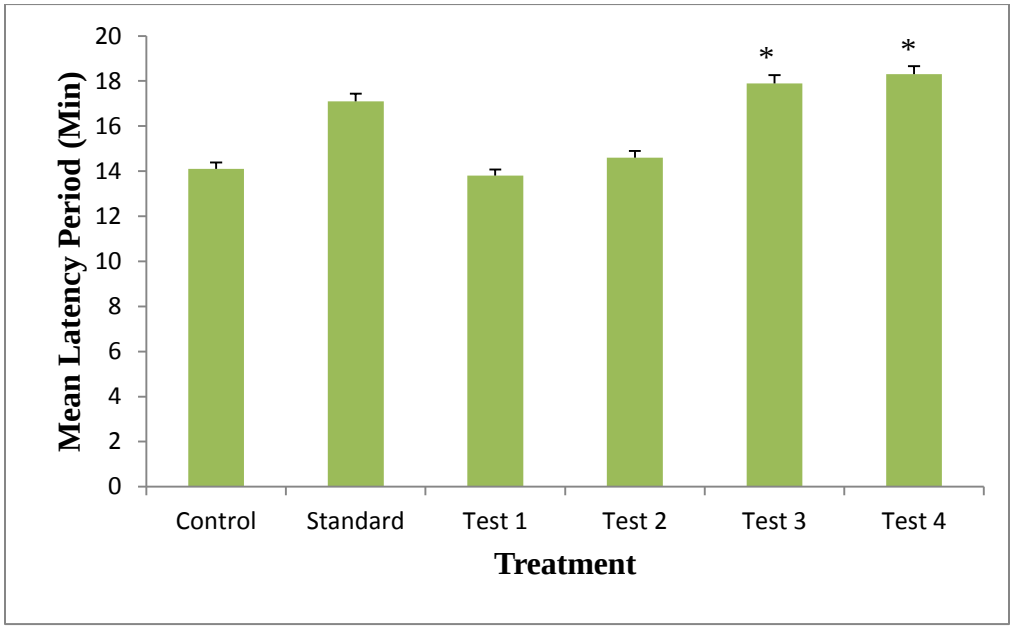

Figure 2: Effect of ethanolic extract of seeds of *Peganum harmala* on mean latency period.

DISCUSSION

Epilepsy disorders comprise a major public health problem are the most prevalent psychiatric disorders worldwide. Because of the fact that the synthetic drugs are endowed with a plethora of problems; these arch for therapeutic alternatives has been conducted largely by means of the study of medicinal plants. In this context, there has been a resurgence of interesting medicine from natural sources with the hope that drugs of plant origin will have significantly lesser side effects than that observed with synthetic drugs while having comparable efficacy. In the present study, the antiepileptic effects of ethanolic extracts of seeds

of *Peganum harmala* were evaluated using maximal electroshock seizure threshold model on mice, in which PHEE at 50 mg/kg and 100 mg/kg did not affect the threshold of maximal electro convulsions in MEST test as compared to control and standard group. However ethanolic extract of *Peganum harmala* at the dose of 50 mg/kg and 100 mg/kg in combination with standard drug Diazepam at the dose of 3.0 mg/kg affect the threshold of maximal electro convulsions in dose dependent manner as shown in Table 1 and Figure 1. Present study also showed that administration of ethanolic extract of *Peganum harmala* at the dose of 50 mg/kg and 100 mg/kg alone does not affect the mean latency period as compared to control group. However ethanolic extract of *Peganum harmala* at the dose of 50 mg/kg and 100 mg/kg in combination with standard drug Diazepam at the dose of 3.0 mg/kg increases the mean latency period (Table 2 & Figure 2) in dose dependant manner as compared to control and standard group indicates that the ethanolic extract of *Peganum harmala* potentiates the effect of Diazepam in convulsions.

CONCLUSION

The present study investigated the putative effects of ethanolic extract of seeds of *Peganum harmala*. The extract was able to potentiate the effect of Diazepam in convulsions.

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Conflict of interest statement

I declare that I have no conflict of interest.

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