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Skeletal muscle relaxant activity of methanolic extract of *Parthenium hysterophorus* L. leaves in swiss albino mice

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Abstract

The objective of the present study was to evaluate the skeletal muscle relaxant activity of methanolic extract of the leaves of *Parthenium hysterophorus* L (MEPH) using Rota-Rod method and traction test. MEPH was administered orally at dose of 3mg/kg and 6 mg/kg p.o to Swiss Albino mice. The methanolic extract significantly reduces the fall off time (motor coordination), and highly significant (**P<0.01) at 30 min of duration. It also shows significant muscle relaxant effect in traction test. Thus, the result suggested that the MEPH possess skeletal muscle relaxant activity may be due to presence of different chemical compounds present in the extract.

Key-Words: *Parthenium hysterophorus*, methanolic extract, Skeletal muscle relaxant activity

Introduction

Parthenium hysterophorus L., belongs to the dicot family of flowering plants (Asteraceae) to which otherwise ornamentals like dahlia and chrysanthemum and oilseed crop plants like sunflower and safflower belong¹. It was accidentally introduced in India in the year 1956 during congress raj; When India imports a variety of wheat, therefore it is locally known as Congress Grass². Ethnomedicinally, Plant is used as a tonic, febrifuge emmenagogue and as an analgesic in neuralgia; a decoction of root is used in treatment of dysentery. It causes contact dermatitis and respiratory allergies in human beings. The present study was carried out see the effect of methanolic extract of *Parthenium hysterophorus* on Skeletal muscle.

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Material and methods

Plant material

The mature green leaves of *Parthenium hysterophorus* L. (Asteraceae) were collected from agriculture fields of pune (Maharashtra) India, in the month of September 2010. The leaves were identified and authenticated by Indian botanical survey of India, Pune and voucher specimen number Pritesh -1 was deposited in the Herbarium of Charak College of Pharmacy & Research, Wagholi, Pune, India. After authentication, the fresh leaves were collected in bulk, dried under shade and pulverized in a grinder.

Preparation of methanolic extract

For the preparation of methanolic extract of leaves of *Parthenium hysterophorus* L (MEPH), the dried coarse powdered of leaves were extracted with methanol (95%) in a Soxhlet apparatus. The extract was concentrated and dried using Rotary flash evaporator and stored in a refrigerator at 5°C for experimentation. (Yield 9.1% w/w with respective to dry starting material).

Animals used

Swiss albino mice of either sex with weighing 18-26 g were used. The animals were maintained on the suitable nutritional and environmental condition throughout the experiment. The animals were housed in polypropylene cages with paddy house bedding under standard laboratory condition for an acclimatization periods of 7 days prior to performing the experiment. The animals were fed with commercially available rat pelleted diet. Water was allowed *ad libitum* under strict hygienic conditions.

Acute toxicity study³

Acute toxicity was conducted according to OECD fixed dose guideline. Swiss albino mice were fasted overnight. On the next following morning the animal was administered with 300 mg/kg using distilled water as vehicle. The animal was died on 8th hour. The next lower 50mg/kg was administered to another animal the animal shown no mortality sign after 24 hours of treatment. The 50 mg/kg was selected as sighting study. The main study was conducted with three another animals and there was no sign of mortality were seen after laps of 24 hour the animal was observed for 14 days. After 14 days the study was terminated and the LD₅₀ dose was found between 50-300 mg/kg. For screening its muscle relaxant affect the 1/10 of the LD₅₀ Dose was selected.

Selection of dose for pharmacological screening

The methanolic extract *Parthenium hysterophorus* (MEPH), was found to be non-toxic up to the dose of mg/kg and did not cause any death, therefore it is considered as safe. Hence 1/10th of this dose i.e. 5 mg/kg body weight was used for the activity.

Rotarod

The rotarod apparatus consists of a metal rod (3 cm diameter) coated with rubber attached to a motor with the speed adjusted to 2 rotations per minute. The rod is 75 cm in length and is divided into 6 sections by metallic discs, allowing the simultaneous testing of 6 mice. The rod is in a height of about 50 cm above the tabletop in order to discourage the animals from jumping off the roller. Cages below the section serve to restrict the movements of the animals when they fall from the roller. Swiss albino mice underwent a pretest on the apparatus. Only those animals, which had demonstrated their ability to remain on the revolving rod (20 rpm) for 5 min, were used for the test^{4,5,6}. Swiss albino mice were divided into four groups consisting of six animals each. Group I served as control which received saline solution, animals of group II received standard drug Diazepam at a dose of (10mg/kg, i.p.) while Group III & IV received the MEPH at a dose of 6 and 3 mg/kg, p.o. The animals were placed on the rotating rod and fall off time i.e, when the animal falls from the rotating rod, was recorded, which was taken as grip strength.

Traction test⁷

Placing the forepaws of the mice in a small twisted wire rigidly supported above the bench top did the screening of animal. Normally the mice grasp the wire with the forepaws, and place at least one hind foot on the wire without 5 second when allowed to hang free. The test was conducted on four groups of animals (n=8) that were previously screened, 30 min after the

injection of methanol extract of *Parthenium hysterophorus* (MEPH), diazepam (5 mg/kg) and saline solution as a vehicle control. Inability to put up at least one hind foot on the wire is counted as negative value.

Statistical analysis

The data obtained in present investigation was subjected to statistical analysis. All results are expressed as Mean \pm SEM (standard error of mean); Six animals in each group. All statistical comparisons were made by Bonferroni's test after conducting one-way ANOVA.

Results and Conclusion**Rotorod test**

In this test, MPHE (5 and 3 mg/kg) both significantly reduced the time spent by the animals on revolving rod when compared to Control ($P < 0.05$). The standard drug (diazepam) also showed significant effect when compared to control ($P < 0.01$). (Table I).

Traction test

In traction test, MEPH (5 and 3 mg/kg) both significantly decreases the muscle co-ordination activity of mice compared with Control ($p < 0.05$). (Table II).

The methanolic extract of *Parthenium hysterophorus* was pharmacologically screened for its muscle relaxant study. The result indicates that methanol extract possess a significant skeletal muscle relaxant activity in experimental animals. At dose of 5 and 3 mg/kg it showed highly significant skeletal muscle relaxant activity at 30min of duration. Preliminary phytochemical screening reveals the presence of anthraquinone, steroids, saponins, reducing sugars and tannins in the plant extract⁶. Therefore, the observed skeletal muscle relaxant activity may be attributed to these compounds. Further studies are in progress to isolate the active constituents responsible for this activity. The studies indicate that parthenium one of the major components of methanolic extract shows depolarizing neuromuscular junction blocking effect⁸. Thus, the muscle relaxation may be produced due to depolarizing blockage of neuromuscular junction.

Based on the results of the present study, we conclude that the methanolic extract of *Parthenium hysterophorus* (MEPH) possess significant skeletal muscle relaxant activity. However, further studies are necessary to find the exact mechanism of skeletal muscle relaxant effect and to isolate the active compound(s) responsible for this pharmacological activity.

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Table I: Effect different treatment on duration of time spent on rotarod

Group	Dose	0 minutes	30 minutes
Control	Saline soln.	328.17 ± 1.62	322.7±24.85
Diazepam	10 mg/kg <i>i.p.</i>	340.50 ±18.93	104.8±2.85**
MEPH	5 mg/kg <i>p.o.</i>	342.23 ±18.60	189.5±41.57*
MEPH	3mg/kg <i>p.o.</i>	342.50 ±12.23	201.00±32.45*

Values are expressed in mean ± SEM; n=6; *p<0.05, **p<0.01 considered highly significant.

Table II: Effect of different treatment on motor co-ordination in mice

Group	Dose	% response
Control	Saline soln.	0
Diazepam	5 mg/kg <i>i.p.</i>	100
MEPH	5 mg/kg <i>p.o.</i>	70*
MEPH	3 mg/kg <i>p.o.</i>	60*

Values are the percentage animals showing negative results; n = 8; *p< 0.05 compared with control (Chi-square test).