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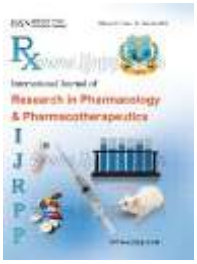

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Effect of *Ruta graveolens* on pentylenetetrazol and electrically induced convulsions in albino mice

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ABSTRACT

Ruta graveolens is a plant commonly found in north Africa and south Europe. It is reported that *Ruta graveolens* is used traditionally for children epilepsy. The chemically induced convulsion by pentylenetetrazol (PTZ) is commonly used for the investigation of anti-epileptic effects of drugs. *Ruta graveolens* alcoholic extract residue (whole plant) was tested against PTZ and electrically induced convulsions in albino mice. The supramaximal electroshock (MES) method was applied in this study as electrically induced convulsion model; it is a simple model of generalized or partial seizures. Two concentrations of alcoholic extract were used (5 and 10%) orally. The treated animals showed significant inhibition of straub tail reaction, myoclonic jerks, clonic convulsions, final tonic extensor spasm and a significant protection against death (100% protection at 10% w/v concentration). The results indicated that *Ruta graveolens* contains compound(s) capable to inhibit convulsions.

Key words: *Ruta graveolens*; pentylenetetrazol; albino mice; anticonvulsant.

INTRODUCTION

The pilot studies on *Ruta graveolens* in our laboratory showed induction of sedation-like action in animal models. In addition, there are some reports pointing to the use of *Ruta graveolens* in traditional medicine for children epilepsy. Furthermore, it has been reported that the anticonvulsant effect of fruit of terapleura tetraptere is due to a furanocoumarine scopoletin (Karamat et al., 2012). Scopoletin is also present in *Ruta graveolens* (Vialart et al., 2012). Therefore we were prompted to investigate the anticonvulsant action of the plant.

MATERIALS AND METHODS

Drugs and chemicals

Ethanol (95% v/v) and formaldehyde were obtained from BDH Limited, England. Gum acacia was obtained from Riedel-De Haen AG, Germany. Normal saline was from Egypt Otsuka Pharmaceuticals Co., Egypt. Pentylenetetrazol from Knoll Co., Germany.

Animals

Male Albino mice (20-40g) were used for different experiments. Mice were bred in the animal house of Tripoli University where each group was housed separately in a cage. Standard food pallet diet (Beeky company, Austria) and water were available ad lib. The animals were kept at constant room temperature (20-25ºC), with 12 hours dark/light cycle. The institutional animal ethical committee (IAEC) has approved the protocol to conduct experiments on these animals.

Collection and preparation of the plant

Ruta graveolens was collected as whole plant from Tripoli area. The taxonomic identification of the plant was done in the department of Botany Tripoli University, Libya. The whole plant was shade-dried for 10 days, then was cut into small pieces and ground to rough powder using grinding machine. The powder was kept in stoppered amber color glass bottles till the time of extraction.

Extraction method

Maceration was applied using ethanol (95%v/v). *Ruta graveolens* powder (500g) was soaked in 3 liters of ethanol for 5 days in a closed glass chromatography jar. The crude total ethanolic extract was filtered and the powder was squeezed to obtain the entrapped solvent. The filtrate was evaporated at 77ºC temperature using rotary evaporator till dryness. After evaporation, 17g of dark brown residue was obtained which was used for animal experiments.

Alcoholic extract dosage form in 5%w/v gum acacia

The alcoholic extract residue was suspended in 5% w/v gum acacia to improve the homogeneity of residue dose. Different concentrations of the suspension were used in the experiments (Carter, 1977).

Drug administration

The test drug (*Ruta graveolens* alcoholic extract residue) was administrated by oral gavaging. The volume of administration was 2.5 ml/100g (Waynforth and Flecknell, 1992).

Pentylenetetrazol induced convulsions

Pentylenetetrazol (PTZ) 100 mg/kg was injected in mice subcutaneously 2 h after the administration of *Ruta graveolens* or vehicle control. The animals were observed for 15 minutes after PTZ administration. Locomotor activity (increase or decrease), myoclonic jerks, straub tail, unconsciousness, clonic convulsion, tonic convulsion, swimming movement, or death were recorded (Everett and Richards, 1945; Koella, 1985).

Electrically induced convulsions

Seizures were induced in mice with an electric current (50 Hz, 50 mA, duration of impulse 0.2 s, earclips electrodes) 2 hours after administration of vehicle control or *Ruta graveolens*, according to the method of Swinyard et al. End point is characterized by tonic flexion, followed by tonic extension including hind legs followed by terminal clonus of all four legs and trunk (Swinyard et al., 1952).

Statistical analysis

Descriptive statistical analysis was applied on the parameters of different samples using SPSS (software package, version 14). Normality test was undertaken for samples, using Kolmogorov-Smirnov maximum deviation test for goodness of fit. If the parameters were normally distributed, treatments were compared by applying One-way ANOVA, followed by Post-Hoc tests (LSD and Duncan tests). If the parameters were not normally distributed, treatments were compared by applying the Mann-Whitney two samples (non-matched) test.

The difference were considered to be significant at $p<0.05$.

RESULTS AND FINDINGS

Pentylenetetrazol induced convulsions

The effect of *Ruta graveolens* extract was tested on pentylenetetrazol induced convulsions in mice. As can be seen in table 1, straub tail was present in all control animals. It was significantly decreased in the group treated with 10%w/v *Ruta graveolens.* However, 5%w/v *Ruta graveolens* treated group did not show significant difference from the control group. The means of myoclonic jerks were 54.33, 21.50 and 17.00 in control, 5% and 10% extract treated groups respectively. The statistical analysis showed that *Ruta graveolens* in the doses used protected the animals significantly from myoclonic jerks compared to the control group. The clonic convulsions number decreased from 4.66 (control) to 3.33 (5%w/v) and to 2.33 (10%w/v) groups. There was a significant protective effect of 10%w/v against clonic convulsions at $p<0.05$, while 5%w/v *Ruta graveolens* had no significant effect compared to the control group. The mean tonic convulsion number was 2.16 for control which decreased substantialy to 0.33 with 5%w/v group and 0.16 in 10% residue group. Death occured in all control animals within 15 minutes after PTZ administration, whereas with 5% residue administration, the death rate decreased to 16% and with 10%w/v there was complete protection of the animals from death (Table. 1).

Table 1: **The effect of *Ruta graveolens* residue suspended in acacia on the convulsion induced by PTZ in albino mice.**

	Control, 5% acacia (n=6)	**5% w/v residue in acacia (n=6)**	**10%w/v residue in acacia (n=4)**
Straub tail	100.00%	83.00%	50.00%*
Myoclonic jerks	54.33 ± 3.62	21.50 ± 4.06*	17.00 ± 7.75*
Clonic convulsion	4.66 ± 2.16	3.33 ± 1.52	2.33 ± 2.33*
Tonic convulsion	2.16 ± 0.30	0.33 ± 0.33*	0.16 ± 0.16*
Death within 15 min	100.00%	16.00%*	0.00*

The values are the means of (times or percentage) ± S.E for the number of animals shown in the parentheses.
*Significantly different from the control group.

Electrically induced convulsion in albino mice

The results indicated that the end point time (tonic flexion followed by tonic extension including the hind legs) for control group was (8.33) while for 5% residue treated group it was 27.66. The death incidence in control group was (66%), while in *Ruta graveolens* groups it was much lower (16.6) (Table 2).

Table 2: The effect of residue on electrically induced convulsion in albino mice.

Treatment	**End point, sec (n=6)**	**Death (n=6)**
Control	8.33 ± 0.71	66%
5%w/v residue	27.66 ± 2.33*	16%*

The values are the means of time (sec) or percentage ± S.E for the number of animals shown in the parentheses.
*Significantly different from the control group

DISCUSSION

The chemically induced convulsions are commonly used in the investigation of anti-epileptic effects of drugs (Prince, 1972, Stone, 1972). PTZ is probably the most commonly employed chemical means of inducing convulsions for the evaluation of anticonvulsive drug activity. It has been found that compounds which are clinically effective in the treatment of petit mal epilepsy are effective in counteracting the convulsant effect of PTZ in mice (Everett and Richards, 1945). Therefore, this has proven to be a useful screening method for the compounds effective in the treatment of petit mal epilepsy.

The alcoholic extract as gum acacia suspension produced significant inhibition of straub tail reaction, myoclonic jerks, clonic convulsion and final tonic extensor spasm a prelude to the final lethality induced by PTZ (Table 1). Since *Ruta graveolens* contains scopoletin (Karamat et al., 2012) which possess anti-convulsant effect (Nwaiwu and Akah, 1986), the present study suggests the presence of anticonvulsant material(s). Whether this protective effect of *Ruta graveolens* is related to non-specific CNS depression like benzodiazepines and barbiturates (Hobbs et al., 1996) or inhibition of T-current in thalamic neurons (like ethosuximide)(Coulter et al., 1989) etc. The mechanism of action has to be further studied. This is important as the extract blocking the tonic spasm in PTZ induced convulsions-reminiscent of phenytoin effect. It may be recalled here that both phenytoin (Macdonald et al., 1989) and *Ruta graveolens* (Bethge et al., 1991) have blocking effect in neuronal Na+-channels. However the protection against clonic convulsions due to PTZ is at variance with the effect of phenytoin-like drugs which protect against tonic spasm in PTZ convulsion but potentate clonic convulsions with PTZ (Keiji Nakamura et al., 1966).

The maximal electroshock seizure is the most efficient method for inducing convulsion against which clinically effective anticonvulsants in the treatment of grand mal are active. Compounds which prevent the maximal electroshock seizure in mice are usually found to have anticonvulsant activity clinically (Domer, 1971). The electrically-induced convulsions are commonly used to study anticonvulsant drugs specially those useful against tonic-clonic generalized seizures. The 5% ethanolic extract residue of *Ruta graveolens* as gum acacia suspension produced significant prolongation against the onset of tonic extensor spasm and protection against death (Table 2). Keeping the results of the two experimental models viz. PTZ induced and electrically induced convulsions, it looks like that the extract produces the protective effect most probably either through the non-specific depression of CNS, acts through prolongation of inactivated state of Na+ channels (Bethge et al., 1991) or utilizing GABA mechanisms (Macdonald et al., 1989, Bonanno and Raiteri, 1993, Franks and Lieb, 1994). This is important as the extract blocking the tonic spasm in PTZ induced convulsions as well as in electrically induced convulsions-reminiscent of phenytoin effect. It may be recalled here that both phenytoin (Macdonald et al., 1989) and *Ruta graveolens* have blocking effect in neuronal Na+-channels. However the protection against clonic convulsions due to PTZ is at variance with the effect of phenytoin-like drugs which protect against tonic spasm both in MES and PTZ convulsion but potentate clonic convulsions with PTZ (Keiji Nakamura et al., 1966). This leads us to believe that protective effect of the extract both in chemically induced and electrically induced convulsions could be due to the non-specific CNS depression like that produced by barbiturates (Hobbs et al., 1996).

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