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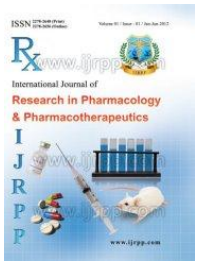

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Studying the effects of *Ruta graveolens* on spontaneous motor activity, skeletal muscle tone and strychnine induced convulsions in albino mice and rats.

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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 epilepsy and some other illnesses. The acute and sub-acute effects of alcoholic extract residue were tested for possible antiepileptic and skeletal muscle relaxation activity. The effect of extract on rat spontaneous motor activity (SMA) was also investigated using open filed. We previously proved the anticonvulsant activity of the plant against pentylenetetrazol and electrically induced convulsions. Therefore in this study strychnine was used to induce convulsions in order to explore the mechanism of anticonvulsant activity of the plant. The skeletal muscle relaxation activity of *Ruta graveolens* was studied using pull-up and rod hanging tests in rats. At concentration of 5%w/v the extract protected mice against strychnine induced myoclonic jerks and death. The pull-up and rod hanging tests pointed to a skeletal muscle relaxant activity at higher concentrations. *Ruta graveolens* extract also significantly decreased the number of squares visited by rats in open field apparatus at all tested concentrations (7.5-20%w/v). However, the significant decrease in number of rearings was noticed at concentrations of above 10%w/v. The results indicate that *Ruta graveolens* contains compound(s) capable to inhibit convulsions, decrease SMA and/or diminish skeletal muscle tone in animal models. This data and the previously generated data together point to a general depression trend of CNS produced by *Ruta graveolens*.

Keywords; *Ruta graveolens*, Strychnine, Spontaneous motor activity, Skeletal muscle relaxation.

INTRODUCTION

The pilot studies on *Ruta graveolens* in our lab showed induction of sedation-like action and depression in motor activity in animal models. We also showed that the plant was able to protect animals against PTZ induced convulsions [1]. 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 [2]. Scopoletin is also present in *Ruta graveolens* [3]. Furthermore, many of anticonvulsant drugs such as barbiturates and benzodiazepines posses skeletal muscle relaxation activity beside the anticonvulsant activity. Therefore we were prompted to investigate the anticonvulsant action of the plant and the action on muscle tone as well.

MATERIAL AND METHODS

Animals

Male Albino mice and rats were used for different experiments. Animals were bred in the animal house of Tripoli University, 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 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 methods

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.

Drug administration

The test drug (*Ruta graveolens* alcoholic extract residue suspended in 5% gum accacia) or vehicle control (5% w/v gum accacia) was administrated by oral gavaging. The volume of administration was 2.5 ml/100g [4]. All the experiments were undertaken 2 hours after drug administration.

Spontaneous motor activity measurements

The spontaneous motor activity (SMA) measurement was done using open field. SMA was studied in a closed room under a low level of illumination [5-7].

Measurement of skeletal muscle activity

The pull-up test was carried out according to [8]. A cut-off time was fixed at 90 seconds.

Rod-hanging test was performed by hanging, the animal clinging to the tight horizontal plastic cord by its fore-limbs. The time taken to lose the grip and falling down was recorded, the cut-off time was fixed of 90 seconds [9].

Strychnine induced convulsion

Strychnine was injected to the mice (2 hours after *Ruta graveolens* treatment) intraperitoneally in a dose of 1.4 mg/kg . The animals were observed for 15 minutes after strychnine administration. Myoclonic jerks, straub tail, clonic convulsion, tonic convulsion, swimming movement, or death were recorded [10].

Statistical analysis

One-way ANOVA, followed by Post-Hoc tests (LSD and Duncan tests) was applied using SPSS version 19 . If the parameters were not normally distributed, treatments were compared by applying the Mann-Whitney two samples (non-matched) test. difference were to be significant at $p<0.05$.

RESULTS

Spontaneous motor activity

The effect of *Ruta graveolens* alcoholic extract on rats is detailed in table.1. It shows a decrease in number of squares visited and rearings by increasing the concentration of *Ruta graveolens*. The decrease in number of squares visited was significant at all tested concentrations. However, the decrease in number of rearing was only significant at concentrations of 10 %w/v and above.

Table 1 Effect of *Ruta graveolens* on spontaneous motor activity in rats.

%w/v *Ruta graveolens* extract	Square visit	Rearing
Control	41.1 ± 2.9 (6)	13.33 ± 1.89 (6)
7.5	24 ± 6.44* (6)	9.83 ± 1.24 (6)
10	22.83 ± 2.12* (6)	6.66 ± 1.94* (6)
15	6.8 ± 3.05* (5)	2 ± 0.7* (5)
20	8.8 ± 3.33* (4)	1.8 ± 0.96* (4)

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

Skeletal muscle relaxation

As can bee seen in table.2, *Ruta graveolens* alcoholic extract resulted in a decrease in skeletal muscle tone in both the pull-up and rodhanging tests. However, rodhanging test was more sensitive to *Ruta graveolens* extract than pull-up test. The times of rodhanging test were significantly reduced at concenrations of 10 and above, while pull-up test times were reduced significantly only at the concentrations of 15 and 20 %w/v.

Table 2 Effect of *Ruta graveolens* on skeletal muscles tone in rats.

%w/v *Ruta graveolens* extract	Pull-up test time	Rod-hanging test time
Control	8.33 ± 1.11 (6)	75 ± 6.31 (6)
7.5	6.66 ± 4.6 (6)	55.5 ± 10.96 (6)
10	10.16 ± 0.74 (6)	43.33 ± 5.32* (6)
15	11.5 ± 0.88* (5)	34.6 ± 3.09* (5)
20	18.75 ± 2.05* (4)	13.6 ± 1.96* (4)

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

Strychnine induced convulsions

The effect of *Ruta graveolens* on strychnine induced convusion was tested in mice, the results are detailed in table. 3. *Ruta graveolens* alcoholic extract significantly reduced the number of deaths in mice from 100% to only 33%, It was also completly protected mice from myclonic jerks produced by strychnine.

Table 3 Effect of *Ruta graveolens* on strychnine induced convulsions in mice.

	Control	(5% acacia)	5% w/v *Ruta graveolens* extract
Swimming movement	3.16 ± 0.04 (6)		2.33 ± 0.49(6)
Myoclonic jerks	1 ± 0.36 (6)		0* (6)
Clonic convulsion	1.83 ± 0.47 (6)		2.33 ± 0.49 (6)
Tonic convulsion	1 ± 0 (6)		1.5 ± 0.34 (6)
Death within 15 min	100% (6)		33%* (6)

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

DISCUSSION

Open field is the most commonly used observational method for the study of spontaneous motor activity. The main advantage of the open field for measuring motor activity is that the pattern and the quantitative profile can be observed. The open field has been used as a test for exploration of locomotion and rearing. This is a classical forced-exploratory situation, however choice measurement is not available [11]. Locomotion in open field may reflect not only exploration , but "emotional reactivity".

All used concentrations (7.5, 10, 15, and 20%) produced significant decrease in number of squares visited **(**Table 1**)** denoting a decrease in SMA. This general activity be differentiated from the exploratory behavior though the most of the spontaneously emitted activities in rats can be associated with exploration [12]. The SMA is decreased by many pharmacologically active substances. Tricyclic antidepressants [13], anti-psychotics), kappa opioid receptors [14] and benzodiazepines [15] also inhibiting exploration. C.N.S stimulants in general increase SMA. The SMA and individual behavioral variability may be determined by the action of "investigation", "fear" and "shifted activity" [16]. In the present investigation there is a dose-dependent decrease in SMA. The extract also decreases the exploratory behavior as evidenced by the change in rearing.

The effect of *Ruta graveolens* on muscle tone was nearly liner effect (Table 2). Concentrations (10 to 20%) decreased rod-hanging time while pull-up time increased with concentrations (15 and 20%). Both testes are pointing to a decrease in skeletal muscle tone.

The general trend of C.N.S activity with *Ruta graveolens* alcoholic extract observed in this investigation was depression. It is possible that the active ingredient(s) in the extract might have central muscle relaxant activity. Most of the C.N.S depressants produce muscle relaxation through their action by inhibition of polysynaptic reflexes at the level of spinal cord [17] .

The chemically induced convulsions are commonly used in the investigation of anti-epileptic effects of drugs [18, 19]. The effect of the extract was studied against strychnine-induced convulsions in mice one of the methods to study anticonvulsant action of drugs in mice, strychnine systemic administration effects networks mainly in spinal cord, brain stem and cerebellum [10]. In the present investigation 5% ethanolic extract residue as gum acacia suspension was used; as this concentration produced optimum protective effect in PTZ model [1]. The extract produced significant protection against strychnine induced myoclonic jerks and death **(**Table 3**)**. However it did not offer any significant protection against strychnine induced tonic/clonic convulsions or swimming movements. Strychnine is known to

block glycine receptors which are predominantly present in spinal cord [20]. It is possible that the active ingredient(s) in *Ruta graveolens* extract have no effect on glycine receptors and the non-specific CNS depressant or the action on Na^+-channels of the extract in the concentration used.

The results of these tests and the previously generated results [1] are in favor of the opinion suggests; *Ruta graveolens* has a general depression trend on the central nervous system.

CONCLUSION

Ruta graveolens alcoholic extract might contain substance(s) able to produce skeletal muscle relation and to prevent convulsions.

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