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ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
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308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 8 (12): 1807-1811, 2005
ISSN 1028-8880

Effect of *Rosmarinus officinalis* L. Extract on the Seizure Induced by Picrotoxin in Mice

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Abstract: In this investigation, the effects of percolated and suxhelet Methanolic extract of aerial parts of *Rosmarinus officinalis* L. on generalized seizure induced by picrotoxin was studied. The study was performed on groups of 10 animals pretreated with different doses of percolated (50, 200, 500 and 1000 mg kg^{-1}) and suxhelet (50 and 100 mg kg^{-1}) extracts of this plant by intraperitoneally (i.p.) injection. After 20 min each animal received picrotoxin 12 mg kg^{-1} for induction of seizure. Latency of onset time of seizure, duration of seizure, death time and mortality rate were determined. The results showed that latency of seizure was increased in groups that pretreated with different doses of extract ($p<0.01$). The doses of 200 and 500 mg kg^{-1} of extract increased the duration of seizure compared with control group ($p<0.01$). The severity of seizure was milder in treated group with extract. The death time also increased with doses of 50, 200 and 500 mg kg^{-1}. The percolated extract of *R. officinalis* was more effective but not significant than suxhelet extract. The extracts have no effect on mortality rate. It seems that the *R. officinalis* extract has anticonvulsant activity on generalized seizure induced by picrotoxin in mice. Overall the more effective dose of extract was 50 mg kg^{-1} and more experiments are needed in this field.

Key words: *Rosmarinus officinalis* L., seizure, picrotoxin, mice, rosemary

INTRODUCTION

The use of plants is as old as the mankind. Natural products are cheap and claimed to be safe. They are also suitable raw material for production of new synthetic agents. Rosemary (*Rosmarinus officinalis* L.) is native to the Mediterranean area. It is now cultivated widely in other parts of the world which frequently used with meat and fish in Western and Asian countries. This plant is an erect evergreen shrub which grows in a warm and relatively dry climate. In Iran, this plant grows in the northwest especially Azerbaijan and Kurdistan provinces[1-3]. It is used for flavoring food, a beverage drink, as well as a fragrant additive in soaps and other cosmetics. Extracts of *R. officinalis* L. have been widely used as a preservative in food industry due to the antioxidant activity and slowing the growth of a number of bacteria such as *E. coli* and *S. aureus* that are involved in food spoilage[3,4]. These effects are attributed to the some of its constituents such as carnosol and carnosic acid[5,6].

In folk medicine it is believed that the extract of this plant affect the menstrual cycle, relieves menstrual cramps, increase urine flow, reduce kidney pain (for example, from kidney stones), in relieving respiratory disorders, stimulate growth of hair and to support the circulatory and nervous systems[7]. Multiple pharmacological activities such as antimicrobial effects[5,6], antiviral activities[8], antiulcerogenicity[9], anti-tumerogenic and antimutagenesis activities[10,11], hyperglycaemic action[12], diuretic effect[13], antioxidant effects[8,14-16], hepatoprotective activities[11,17] and acting as an abortifacient[4] (inducing miscarriage) have been reported for this plant. The plant has relaxing effect on the tracheal and vascular smooth muscle[18,19]. In Iranian traditional medicine the calming effects on nervous system and anticonvulsant activity[3,20-22] have been attributed to this plant. It is used for nervous headache and has beneficial effect on brain and nervous tension[3,23].

Gas chromatography-mass spectroscopy analysis of the essential oil showed the presence of alpha-pinene, 1, 8-cineole, camphor, verbenone and borneol, constituting

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ca. 80% of the total oil[5]. The other phytochemical studies have been shown the presence of saponins, tannins and flavonoids in this plant[6,24-26].

This is an attempt to establish a scientific basis for the use of this plant as antiepileptic in Iranian traditional medicine. On the basis of the above facts and our interest for antiepileptic drugs, this study was initiated to investigate the effect of *R. officinalis* on generalized convulsive seizure induced by picrotoxin, a widely used as a model for chemically-induced convulsion[27-29] in mice.

MATERIALS AND METHODS

Animal: Male albino mice weighing 20-25 g were employed. The animals were obtained from Neuroscience Research Center of Kerman University of Medical Sciences. They were housed in a room temperature 22±2 at 12/12h light/dark cycle. They had free access to food and water except during the time of experiments. Animals were acclimatized to the laboratory for at least 1 h before testing and were used for once experiment only. The experiments were carried out between 9.00 and 14.00. The animals were distributed into groups of 10 as controls and test groups[27,29]. According to international rules considering animal experiments, all efforts were made to minimize animal suffering and to reduce the number of animal used[29,30].

Plant material: *R. officinalis* were purchased from an herbal drug shop in June 2002. Voucher specimens (No. 1002) were authenticated by botanist and then deposited in Herbarium of the Department of Pharmacognosy, Faculty of Pharmacy, Kerman University of Medical Sciences, Kerman, Iran.

Preparation of extract: The dried leaves and aerial flowered parts of *R. officinalis* were powdered and 50 g of powder were extracted with 80% aqueous methanol by percolation (72 h) and Sauxhelet method[2]. The extract, filtered and evaporated under vacuum. The weights of dried extract were 10.5 and 12.5 g for percolated and Sauxheleted extracts, respectively. The residue was dissolved in normal saline for final suitable concentrations to give the desired concentration (50, 200, 500 and 1000 mg 10 mL).

Convulsion test and data recording: Picrotoxin with dose of 12 mg kg^{-1} was injected intraperitoneally (i.p.) for induction of generalized seizure[27]. The animals received experimental doses of 50, 200, 500 and 1000 mg kg^{-1} of *R. officinalis* extract 20 min before picrotxin 12 mg kg^{-1}. The onset time of seizure, duration of seizure, time of death and mortality was measured in test and control groups[27,29]. Mice were observed for 90 min after picrotoxin injection. Normal saline 10 mL kg^{-1}, i.p., or phenobarbital 40 mg kg^{-1} i.p. were injected into groups of animals 20 min before picrotoxin, as control and positive control groups, respectively[27,29].

Statistic analysis: Results are presented as Mean±SEM and statistical significance between groups was analyzed by ANOVA followed by Newman-Keuls test. $p<0.05$ were considered significant[27,29].

RESULTS AND DISCUSSION

Pretreatment of animals with different doses of extract delayed the onset of seizure induced by picrotoxin. The most effective dose was 50 mg kg^{-1} ($p<0.01$) (Fig. 1A).

The duration of seizure was increased significantly ($p<0.01$) with doses of 200 and 500 mg kg^{-1}, in compare to the control group (Fig. 1B). The severity of seizure was milder than control group.

Pretreatment of animals with different doses of *R. officinalis* extract delayed the death time significantly ($p<0.01$) (Fig. 1C).

The extract has no effect on percentage of mortality from seizure induced by picrotoxin in this experiment.

Percolated extract of *R. officinalis* was more effective but not significant than Sauxhelet extract in this experiment (Data not shown).

Picrotoxin as a GABA-A antagonist[31] has widely used as a model of chemically-induced convulsion and produces a general tonic-clonic convulsions that leads to death in most cases[27,29].

The present results indicate that the methanol extracts of *R. officinalis* L. aerial parts was effective on generalized seizure induced by picrotoxin. The more effective dose of the both extracts was 50 mg kg^{-1}. This dose of extract delayed the onset time of seizure significantly in comparison to control group ($p<0.01$). It seems that this dose can produce enough concentration for induction of anticonvulsant effect[32]. However doses of higher than this dose decreased the time instead of exceptionally increase it. Higher doses of this extract may produce concentration higher than therapeutic level and produced nonpharmacologic or toxic effect[32]. Pretreatment of animal with different doses of *R. officinalis* extract delayed the death time also significantly ($p<0.01$). In the present study, any doses of methanolic extract of *R. officinalis* did not change mortality rate.

The phytochemical studies on *R. officinalis* extract showed that the extract had alkaloids, saponins, tannins

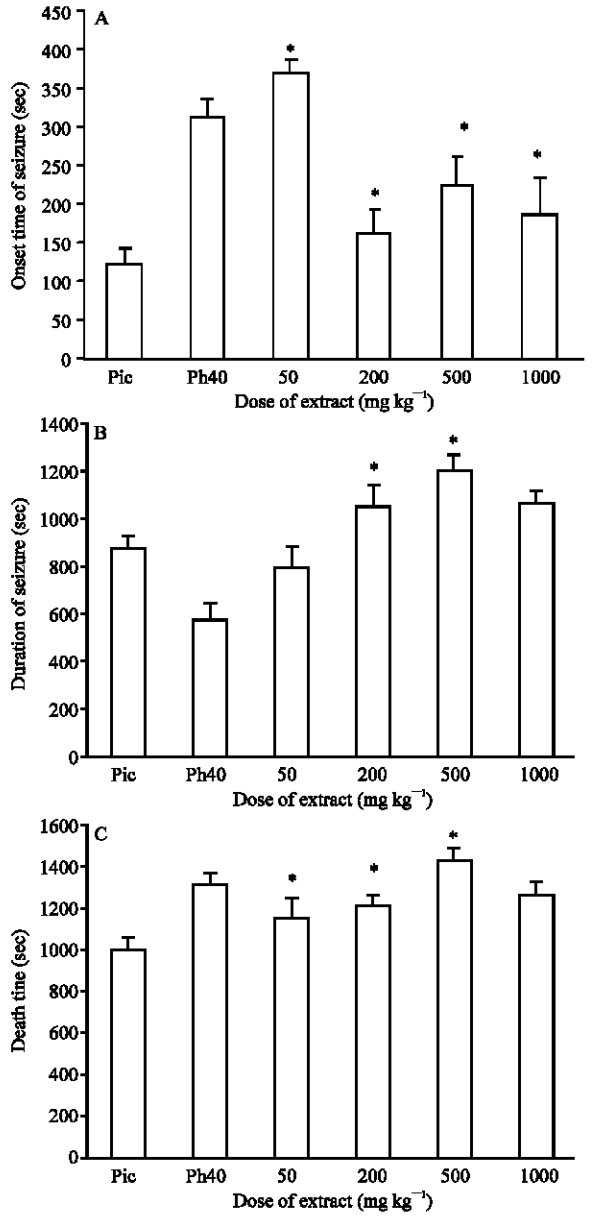

Fig. 1: The effect of *R. officinalis* extract on the A: the onset time of seizure, B: the duration time of seizure and C: the death time
Normal saline (NS) 10 mL kg^{-1}, Phenobarbital (Ph) 40 mg kg^{-1} or different doses of extract (E) were injected intraperitneally 20 min before picrotoxin 12 mg kg^{-1}. Each column indicates the Mean±SEM. n=10
*, $p<0.01$; significant difference from control group

and flavonoids constituents[3,24-26]. There are some evidences about anticonvulsant effect of some flavonoid compounds[33-35]. Salgueiro *et al.*[36] showed anxiolytic effects of some natural and synthetic flavonoids in rats and found that these compounds exerted their effects through the central benzodiazepine receptors. In several other studies selectively binding of some flavonoids to the central benzodiazepine receptors[33,34,36-40] have been shown.

Goutman et al.[37] showed that flavonoid modulated the ionic currents mediated by GABA (A) and GABA (C) receptors. Their results indicated that mechanism/s underlying the modulation of ionotropic GABA receptors by some flavonoids differs from that described for classic benzodiazepine modulation. It is also reported that the extract of rosemary can increase the GABA content in the brain[41] thus, the extract may modulate generalized seizure by potentiation of the GABA system. Gas chromatography-mass spectroscopy analysis of the essential oil showed the presence of α-pinene, 1, 8-cineole, camphor, verbenone and borneol, constituting in the oil[5]. There are some reports regarding the anticonvulsant effects of terpenoids compounds including pinene and 1, 8-cineol[42,43]. Meanwhile, the volatile oil of *R. officinalis* leaves inhibited calcium influx[18,19]. Blocking of calcium channels is one of the mechanisms involved in the anticonvulsant effects of some antiepileptic drugs[44,45], therefore this mechanism can be also one of the other mechanisms involved in the effect of extract. Gathering together these evidents, it seems that the antiseizure effect of *R. officinalis* may be related in part to terpenoids and/or flavonoid compounds present in the extract. Determination of the role of each compound in the anticonvulsant effect of *R. officinalis* extract is a wide field for more investigations.

In conclusion, present results shows that antiseizure activity of *R. officinalis* percolated and Sauxhelet extracts on seizure induced by picrotoxin. The results showed that percolated extract of *R. officinalis* was more effective than Sauxhelet extract in this experiment, but was not significant. The findings of this study confirmed the traditional use of this plant as anticonvulsive. Rosemary is generally considered safe when taken in recommended doses. However, there have been occasional reports of allergic reactions. Large quantities of rosemary leaves, because of their volatile oil content, can cause serious side effects, including vomiting, spasms, coma and, in some cases, pulmonary edema (fluid in the lungs). An overdose of rosemary may induce a miscarriage or cause damage to the fetus. Those who are pregnant or breast feeding should not use rosemary in quantities larger than those normally used in cooking[4]. However more pharmacological and toxicological experiments are needed for use of this plant as an official herbal drug in clinical use.

ACKNOWLEDGMENT

This research was supported partly by interuniversity research, Bahonar University and Kerman University of Medical Sciences, Kerman, Iran (No. 468/41-1381).

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