Evaluation of the Anticonvulsant Activities of *Rosa damascena* on the PTZ Induced Seizures in Wistar Rats

Masoumeh Kheirabadi, Ali Moghimi, Hassan Rakshandeh and Morteza Behnam Rassoulie

Department of Biology, Faculty of Sciences, Ferdowsi University of Mashhad, Iran

Department of Physiology and Pharmacology, Qaem Hospital, Mashhad Medical University, Iran

**Abstract:** The goal of the present study was to evaluate the anticonvulsant property of *Rosa damascena* essence as a natural origin compound on the PTZ induced seizures in rats. To produce animal models with different graded of seizures (partial to generalized tonic-clonic seizures), PTZ was injected in 40 male Wistar rats. Prior to PTZ injections, the experimental groups of rats were received 500, 750 and 1000 mg kg⁻¹ body weight essential oil. During the experimental period the epileptiform behaviors of all rats of different groups, was evaluated before and after essential injections. The results showed that the Rosa essential can attenuate the latent periods of the beginning of convulsions as well as the severity of seizures in a dose dependent manner. The flavonoids of *Rosa damascena* may act via GABA<sub>A</sub> receptors as nervious studies have proposed for flavonoids of other medicinal plants.

**Key words:** *Rosa damascena*, essential, Seizure, PTZ pentylten tetrazol

**INTRODUCTION**

*Rosa damascena* Mill is one of the most important Rosa species for the flavor and fragrance industries that has been traditionally used for centuries as an odor with relaxant and anxiolytic effects. In Iranian tradition Rosa leaves are using as choleretic and laxative (Zargari, 1992). And several therapeutic effects including hypnotic (Rakhshandeh et al., 2004, 2008), antispasmodic and relaxant (Boskabady et al., 2006), treatment of abdominal and chest pain and strengthening of the heart have been described for the flowers of *Rosa damascena* (Boskabady et al., 2006). Recent studies show that *Rosa damascena* has anti-HIV (Mahmood et al., 1996), antioxidant and antibacterial activities (Ozkan et al., 2004; Basim and Barim, 2004), hepatoprotective (Achuthan et al., 2003) and antitussive effects (Shafei et al., 2003). The toxicological studies of Rose essential have confirmed its safety (Tisserand, 1995).

The objective of the present study was to investigate anticonvulsant effects of essential oil of *Rosa damascena* on different forms of PTZ-induced seizure.

Epilepsy is complicated neurological disorder with various and in some cases undefined etiologies. Although many Attempts have been done to the exact neurochemical basis of human or experimental model epilepsy but the result are disappointing. It has been postulated that an imbalance between the (excitatory Glutamate and inhibitory GABA) neurotransmitters may be the case (Kandel et al., 2000; Matthew and Charles, 2003).

Epileptic patients have impaired physical, psychological and social functions, which may lead them to the loss of job, economic opportunities and therefore lowering of their quality of life. The treatment of this illness is also complicated. During the last few decades many pharmacological investigations have been undertaken to explore new antiepileptic drugs with minimal side effects, but it is still necessary to find out the new effective drugs capable to diminish the various symptoms of seizures.

The objective of the present study was to investigate the anticonvulsant effects of essential oil of *Rosa damascena* as a natural origin compound on different forms of PTZ-induced seizure.

**MATERIALS AND METHODS**

**Plant material:** *Rosa damascena* Shrubs were collected from Kashan (middle part of Iran) in spring and were identified by the botanists of Ferdowsi University of Mashhad, Iran (Rakhshandeh et al., 2008).
The essential oil of *Rosa damascena* from the petals was extracted using a Cleverger apparatus. (Loghamani-Khouzani *et al.*, 2007) and was stored in refrigerator (±4°C). Before every injection, the essence was reconstituted in normal sterile saline and filtered before injections (using bacterial filter).

**Animals:** Male Albino Wistar rats mean body weights (250-300 g) were purchased from Razi research Institute of Mashhad Iran. Animals were kept under controlled environmental conditions ambient (temperature 22-24°C, 12 h light/dark cycle) and were accustomed to laboratory conditions one week before experiments. Rats received food and tap water *ad libitum*. All experiments were performed in midday to avoid the bias of circadian rhythms in endogenous hormonal and transmitter systems. Animals were cared for and handled in accordance with the Iranian society of animal care (member of international animal care society) and also local university animal ethics directions.

**Seizure induction:** Basically, for an *in vivo* evaluation of anticonvulsant or the convulsive effects of a substance, the latent periods of different stages of seizures and the duration of each stage were recorded.

There are different models of induction of experimental epilepsy but in the present investigation the set up of epilepsy induction was based on the application of PTZ. To induce different graded seizures, from the mildest partial to very severe generalized tonic-clonic forms, PTZ administration procedures were as follows:

- Acute model of seizure was induced via an injection (i.p) of 45 mg kg$^{-1}$ body weight of PTZ, dissolved in normal saline. These rats (40 rats) were divided in 4 subgroups and each received 250, 500, 750 and 1000 mg kg$^{-1}$ body weight essential, respectively.

- To produce the chronic model of PTZ-induced seizure, the rats underwent of three PTZ 25 mg kg$^{-1}$ body weight injections, by a 15 min interval. In this procedure, if after the first or the second injection, the tonic-clonic convulsions appeared the next injection/s was ignored. These rats (30 rats) were divided in to 3 subgroups and each received 500, 750 and 1000 mg kg$^{-1}$ body weight essential, respectively.

The time of PTZ injection, in all groups, was 30 min after the injection i.p) of *Rosa damascena* essential oil. After PTZ injection/s, the epileptiform convulsions were recorded for a period of 1 h. The gradation of convulsions was done as indicated in Table 1.

It is necessary to mention that in the acute form of seizure only the latent period of generalized tonic-clonic convulsions was recorded and the evaluation of chronic form of seizure was based on Table 1.

<table>
<thead>
<tr>
<th>Stages</th>
<th>Type of seizures</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>No symptoms</td>
</tr>
<tr>
<td>1</td>
<td>Head myoclonic jerks</td>
</tr>
<tr>
<td>2</td>
<td>More frequent head jerks with forelimb clonus</td>
</tr>
<tr>
<td>3</td>
<td>Total weak body jerks, kangaroo pose, dog shaking</td>
</tr>
<tr>
<td>4</td>
<td>Generalized tonic-clonic convulsions</td>
</tr>
<tr>
<td>5</td>
<td>Lateral recumbency</td>
</tr>
</tbody>
</table>

For the assessment of the beneficial effects of essential administration 3 control groups (*n* = 10) were considered: PTZ, PTZ plus toin and normal saline as sham group.

During the experimental period the epileptiform behavior of all rats of different groups, before and after essential injections, was evaluated.

**Statistical analysis:** The data are presented as mean±SEM. The data of PTZ test were analyzed by one-way analysis of variance ANOVA. The differences were considered significant at 5% level.

**RESULTS AND DISCUSSION**

The results of experiments for all groups show that administration of 750 mg kg$^{-1}$ of essential oil delays the start of epileptic seizures.

Significant differences (*p*<0.05) between control and experimental group (acute model of PTZ injection) were observed in the onset of the seizures (Fig. 1).

Investigation of the duration of convulsive stages indicates that administration of 750 and 1000 mg kg$^{-1}$ of essential decrease the duration of tonic-clonic (seizures stage 4) (*p*<0.05).

The results of the second test (chronic form of PTZ injections) show that administration of 750 mg kg$^{-1}$ of essential has significant antiepileptic effects that evaluated by prolongation of latent periods before tonic-clonic generalized seizures (*p*<0.05) (Fig. 2).

While the injection of 500 mg kg$^{-1}$ of essence significantly doesn't affect the duration of tonic-clonic seizures, the results show that injection of 750 and 1000 mg kg$^{-1}$ essence has decreased this time obviously (Fig. 3).

PTZ a tetrazol derivative initially produces monoclonic jerks which subsequently become sustained and lead to a generalized tonic-clonic seizure. Its systemic administration has consistent convulsant actions in many animal species. At a synaptic level, PTZ appears to interact with the GABA$_A$ receptors complex and acts as a hyperpolarizing agent, so it is a suitable proconvulsive model to evaluate the anti-convulsant effect of different compounds (Jefferys John, 2003; Sarkisian, 2001).
The results of this study show that effects of *Rosa damascena* on PTZ-induced epileptiform seizures is dose dependent. So that, administration of 750 mg kg$^{-1}$ of essence has increased latent period of seizures in all 5 stages and there is a significant difference between the latent period of seizures in control and experimental groups (p<0.05).

The result of injection of 1000 mg kg$^{-1}$ of essence show that the average latent period of seizures in stages 1 to 4 hasn't changed significantly, but the fifth stage is delayed significantly. On the other hand, lateral recumbency was observed rarely in this group.

While the injection of 500 mg kg$^{-1}$ dose of essence doesn't affect on the duration of tonic-clonic seizures, the results show that injection of 750 and 1000 mg kg$^{-1}$ essence has decreased this duration obviously.

According to these results, it can be suggested that *Rosa damascena* essence is effective in convulsions such as status epilepticus.

The statistical results show that administration of 750 mg kg$^{-1}$ of essence causes significant difference between control and experimental groups and latent period of tonic-clonic seizures has increased. Injection of 250, 500 and 1000 mg kg$^{-1}$ of essence don't show any significant difference in the latent period of tonic-clonic seizures.

According to the results it is generally predicted that *Rosa damascena* has anticonvulsant effects in PTZ induced seizures.

The effective component of essence will be count as a suitable drug if it can increase the duration of latent period or decrease the intensity of seizures.

Samples which have shown the stages 1 and 2 of experimental seizures are considered resistant, but they have somehow the capacity to show partial seizures.

Human seizures respond to different treatments in different ways for example, partial seizures cure with specific type of drugs and generalized seizures respond to another type of treatments. Similarly this essence can't affect on all kinds of seizures. On the basis of results although 1000 mg kg$^{-1}$ of essence doesn't have any significant effect on latent period of stages but significantly it has decreased the intensity or duration of stages. Also less number of rats showed lateral recumbency (p<0.05).

Because there are some cases of epileptics resistant to all treatments, if a compound with natural origin and less side effects can decrease the intensity or the duration of seizures it would be very hopeful for patient's future.

Aromatic components in *Rosa damascena* and orange blossoms may be responsible for the effect of these components on anxiolytic behaviors (Erica et al.,...
Nowadays this effect is used in traditionally plant medicine (Mahmoodi et al., 2003). As a result, it is needed to distinguish the exact aromatic components of Rosa damascena essence.

Amygdaloid complex, hippocampus and hypothalamus are involved in sniffing so it is suggested that the essential has had effects on hippocampal systems. On the other hand, researches show that this system has a main role in epilepsy in human and animals so it can be suggested that expanded GABAergic systems in the hippocampus affected by essence. As noted in the past sections flavonoids are an important component of Rosa and as suggested by Dekemendjian (1999), Paladin et al. (1999) and Marder and Paladini (2002), we can propose that such components of Rosa damascena act on the GABA_α receptors in the brain of rats. In addition researchers have shown that flavonoids can enhancing the effects of benzodiazepines on GABA receptors that may confirm the anticonvulsive effects of Rosa damascena (Erica et al., 2004).

The effect of essence on the excitatory neurotransmitter system of the brain (glutamatergic) should be considered too. This system is wide spread in all parts of the brain, from olfactory structures to cortical areas and epilepsy can be originated from these different areas (Sugiyama et al., 1996). So it is proposed that this system may be affected by essence.

Mental challenges can induce and start different kinds of seizures. This fact shows the roll of cortical systems on seizures. Therefore we can't relate the effect of essence just to limbic system.

Rosa damascena contains large amount of flavonoid glycosides. Recent studies have shown the anti-convulsive effect of flavanoids. The effects of sour orange flower extract, which contains flavonoids, on the PTZ induced seizures have been proved (Mahmoodi et al., 2003).

Other component of Rosa damascena essence is geraniol that behavioral studies have shown its hypnotic and anti-epileptic effects (Sugiyama et al., 1996; Rahshandeh et al., 2004).

The other component of Rosa damascena is eugenol, which many studies have shown its anti-epileptic effects (Wie et al., 1996).

In conclusion, according to results obtained from the present investigation, it can be concluded that Rosa damascena essence is effective in convulsions such as status epilepticus. More detailed studies are recommended to define the effective components and if so, the essence of Rosa damascena will be counted as an effective drug in increasing the duration of latent period or decreasing the intensity of seizures.

ACKNOWLEDGMENTS

This research was supported by the grant of the research office of Mashhad, Ferdowsi University and special helps from the research center for medicinal plants of Mashhad Medical University, Iran.

REFERENCES


