Effects of different extracts of *Rosa damascena* on pentylenetetrazol-induced seizures in mice

Mahmoud Hosseini¹, Mahboobe Ghasemzadeh Rahbarad¹, Hamid Reza Sadeghnia², Hassan Rakhsandeh²

1. Neuroscience Research Center and Department of Physiology, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran
2. Neuroscience Research Center and Department of Pharmacology, Department of New Sciences and Technologies, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran
3. Pharmacological Research Center of Medicinal Plants and Department of Pharmacology, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran

Objective: In the present study, the effects of aqueous, ethanolic and chloroformic extracts of *Rosa damascena* on pentylenetetrazol (PTZ)-induced seizures were investigated in mice.

Methods: The animals were divided into the following groups: normal saline control group, diazepam group (3 mg/kg), three aqueous extract groups (100, 500 and 1 000 mg/kg), three ethanolic extract groups (100, 500 and 1 000 mg/kg) and three chloroformic extract groups (100, 500 and 1 000 mg/kg). The extracts, normal saline or diazepam were injected intraperitoneally 30 min before PTZ injection. Latency to the first minimal clonic seizure (MCS) and generalized tonic-clonic seizure (GTCS) and the percent of mortality of rats in each group were recorded.

Results: Significant increases in both MCS and GTCS latencies were observed in all the three aqueous extract groups in comparison with the normal saline control group (*P* < 0.05 or *P* < 0.01). The MCS latency in the ethanolic extract (1 000 mg/kg) group and the GTCS latencies in the two ethanolic extract (500 and 1 000 mg/kg) groups higher than those in the normal saline control group (*P* < 0.05, *P* < 0.01). There were no significant differences in MCS and GTCS latencies between the three chloroformic extract groups and the normal saline control group. No significant differences were seen in mortality rate following PTZ administration between the different extracts-treated mice and the control mice.

Conclusion: The results of the present study showed that *R. damascena* has an anticonvulsant effect in a mouse model of PTZ-induced seizures but the exact mechanism of this effect should be clarified in future studies.

Keywords: *Rosa damascena*; Rosaceae; anticonvulsants; pentylenetetrazol; seizures; mice
**Rosa damascena**, an erect shrub with 1 to 2 m in height, belongs to the Rosaceae family. It is usually used for decorative and perfumery purposes\[^{1, 4}\]. The flowers of this plant are large and colorful which are used for production of essential oil and rose water\[^{1, 3, 4}\]. This plant contains carboxylic acid, terpenes, myrcene, vitamin C, flavonoids like kaempferol and quercetin glycosides and terpenoids like geraniol, linalool, eugenol, citral and farnesol\[^{2, 5, 14}\]. It was reported that essential oil of *R. damascena* collected from Kashan region of Iran has more than 95 macro- and micro-components, from which 18 compounds represented more than 95% of the total oil. The most important compounds were β-citronellol (14.5% to 47.5%), nonadecane (10.5% to 40.5%), geraniol (5.5% to 18%), nerol and kaempferol\[^{6}\]. Analyses of rose absolute extract showed that phenyl ethyl alcohol (78.38%), citronellol (9.91%), nonadecane (4.35%), geraniol (3.71%), ethanol (0.00 to 13.43%) and heneicosane were the major compounds\[^{7}\]. In another study, the major constituents of rose were phenyl ethyl alcohol (72.73% to 73.80%), citronellol (10.62% to 11.26%), nerol (2.42% to 2.47%), and geraniol (5.58% to 5.65%). Hydroosol of the flowers was also found to contain four constituents: geraniol was the major compound (30.74%) followed by citronellol (29.44%), phenyl ethyl alcohol (23.74%), and nerol (16.12%)\[^{7, 9}\].

In traditional medicine, various therapeutic effects including treatment of fever and sore throat, constipation and other gastrointestinal complaints, ophthalmic diseases, memory loss and breast tenderness have been suggested for *R. damascena*\[^{11, 4}\]. This plant has also been advised to be as a gentle laxative and to ease coughs, suppress the activity of the hypothalamus-pituitary system and other parts of the central nervous system (CNS)![^14]. Treatment for a long time with high doses of rose oil can lead to stress adjustment and the ability of the brain to compensate by going into a steady state of exhaustion\[^{2}\].

Recent experimental studies showed different pharmacological effects for this plant including anti-HIV effect, anti-microbial and anti-infection in various ophthalmic disorders, bronchodilatory, antitussive, cardiotonic and cardiodeterminating and hypoglycemic effects\[^{15, 5, 13-18}\]. Antioxidant, anti-aging and hepatoprotective effects of *R. damascena* have also been reported which were comparable to α-tocopherol\[^{10-21}\].

Algesic effects of *R. damascena* extract have also been reported\[^{22, 24}\]. The results of previous studies showed that different extracts and fractions of *R. damascena* have hypnotic effects\[^{22, 24, 25}\]. It has also been reported that ethanolic extract of the flowers suppresses the motor activity in rats\[^{24}\]. Anticonvulsant effect of the essence of *R. damascena* has also been reported\[^{26}\]. However, essential oil of *R. damascena* did not show any antinociceptive activity in light tail flick test which is a central model with selectivity for opioid-derived analgesics\[^{27}\]. The essential oil mainly contains lipophilic compounds and therefore, it seems that for hydrophilic substances, the ethanolic and aqueous extracts should be considered\[^{28}\]. Therefore, in this study we used these different extracts for better understanding the nature of active compounds responsible for the anticonvulsant effect of this plant in pentylentetrazol (PTZ)-induced seizures in mice.

### 1 Materials and methods

#### 1.1 Chemicals and plant extract

PTZ was purchased from Sigma (St. Louis, MO, USA) and dissolved in normal saline. *R. damascena* shrubs were collected from Kashan region in the middle part of Iran and identified by botanists. A voucher specimen was preserved in the herbarium of the School of Pharmacy, Mashhad University of Medical Sciences, Iran (herbarium No: 254-1804-01). The chopped, dried flowers (50 g) were extracted using a Soxhlet apparatus with 300 mL distilled water, ethanol and chloroform to prepare aqueous, ethanolic and chloroform extracts.

---

**Related Articles**


respectively. The extracts were reduced to dryness
with a rotary vacuum evaporator, yielded 15%,
17%, and 3.2% for aqueous, ethanolic and chloro-
formic extracts, respectively.

1.2 Experimental animals Eight-week male BALB/
c mice (The Pasteur Institute, Iran), weighing 25
to 30 g each, were used throughout the study. All
of them were housed in the same room in groups
of 8 per cage ((26.5 x 42 x 15) cm³) under a
constant temperature (22 ± 2) °C, a humidity of
55% to 60% and a 12 h light/dark cycle (lights on
at 7:00 AM). Food and water were available ad libitum
properly. Animal handling and all related
procedures were carried out in accordance with
the Ethical Committee Acts, Mashhad University
of Medical Science, Iran.

1.3 PTZ-induced seizures In order to observe ictal behavior, PTZ (90 mg/kg, intraperitoneal
injection (i.p.)) was injected and the animals
were placed in a plexiglas arena ((30 x 30 x
30) cm³) on the day of the experiment. The animals
were observed during 60 min after PTZ admin-
istration. Behavioral responses of the animals to
PTZ administration were evaluated using these
criteria: latency to first minimal clonic seizure
(MCS), latency to the first generalized tonic-
clonic seizures (GTCS) and protection percentage
against mortality.

1.4 Experimental procedure The animals were
divided into 11 groups (n = 10 in each) and were
injected as follows: normal saline (plus 1% Tween
80) in group 1, diazepam (3 mg/kg) in group 2,
aqueous extract (100, 500 and 1000 mg/kg) in
groups 3 to 5, ethanolic extract (100, 500
and 1000 mg/kg) in groups 6 to 8 and chloroformic
extract (100, 500 and 1000 mg/kg) in groups 9
to 11. The extracts, normal saline and diazepam
were injected 30 min before PTZ administration.
All injections were carried out in a volume of 10 mL/kg
(i.p.) between 10:00 AM and 2:00 PM.

1.5 Statistical analysis Data are expressed as
mean ± standard error of mean. Fisher’s exact
probability test, as well as analysis of variance,
followed by Tukey test, was used for statistical
evaluation. The P values less than 0.05 were
considered to be statistically significant.

2 Results

All animals in different treatment groups, except
the diazepam group, showed MCS and GTCS
following PTZ administration. In diazepam

group, the MCS and GTCS in all animals started
after 10 min or the animals did not show any
seizure. Therefore, a 600-second latency
was recorded for all animals of this group.

2.1 Effects of aqueous extract A significant
increase in the MCS latency was seen following
treatment with the aqueous extract (100, 500
and 1000 mg/kg), in comparison with the normal
saline control group (P<0.05 or P<0.01). The

GTCS latencies in all groups treated with the
aqueous extract (100, 500 and 1000 mg/kg) were
higher than the control group (P<0.01) (Table 1).

Table 1 Comparison of latencies to MCS and GTCS onsets
between the control group and the aqueous extract groups
(Mean±standard error of mean, s)

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>MCS</th>
<th>GTCS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal saline control</td>
<td>10</td>
<td>40.46±1.77</td>
<td>49.85±2.27</td>
</tr>
<tr>
<td>Aqueous extract (100 mg/kg, i.p.)</td>
<td>10</td>
<td>56.89±5.63*</td>
<td>77.62±5.79**</td>
</tr>
<tr>
<td>Aqueous extract (500 mg/kg, i.p.)</td>
<td>10</td>
<td>56.80±1.54*</td>
<td>68.80±2.73**</td>
</tr>
<tr>
<td>Aqueous extract (1000 mg/kg, i.p.)</td>
<td>10</td>
<td>64.80±6.71**</td>
<td>70.11±5.39**</td>
</tr>
</tbody>
</table>

* P<0.05, ** P<0.01, vs normal saline control group.
MCS: minimal clonic seizures; GTCS: generalized tonic-clonic seizures; i.p.: intraperitoneal injection.

2.2 Effects of ethanolic extract The MCS latency
in the ethanolic extract (1000 mg/kg) group was
higher than the normal saline control group (P<
0.01). However, there were no significant
differences in the MCS latency between the ethanolic
extract (100 and 500 mg/kg) groups and the
control group. A significant increase in the GTCS
latency was seen in the ethanolic extract (500 and
1000 mg/kg) groups in comparison with the normal
saline control group (P<0.05, P<0.01) (Table 2).

Table 2 Comparison of latencies to MCS and GTCS onsets
between the control group and the ethanolic extract groups
(Mean±standard error of mean, s)

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>MCS</th>
<th>GTCS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal saline control</td>
<td>10</td>
<td>40.46±1.77</td>
<td>49.85±2.27</td>
</tr>
<tr>
<td>Ethanolic extract (100 mg/kg, i.p.)</td>
<td>10</td>
<td>49.20±3.15</td>
<td>49.50±1.80</td>
</tr>
<tr>
<td>Ethanolic extract (500 mg/kg, i.p.)</td>
<td>10</td>
<td>41.70±1.64</td>
<td>48.66±4.48**</td>
</tr>
<tr>
<td>Ethanolic extract (1000 mg/kg, i.p.)</td>
<td>10</td>
<td>60.60±3.14**</td>
<td>72.00±5.61**</td>
</tr>
</tbody>
</table>

* P<0.05, ** P<0.01, vs normal saline control group.
MCS: minimal clonic seizures; GTCS: generalized tonic-clonic seizures; i.p.: intraperitoneal injection.

2.3 Effects of chloroformic extract There was no
significant difference in the MCS and GTCS latencies
of the three chloroformic extract groups in
comparison with the normal saline control group.

2.4 Mortality rate There were also no significant
differences in the mortality rate following
PTZ administration between different treatment
groups and the normal saline control group and no
mortality was seen in the diazepam group.

3 Discussion

The results of the present study show that
aqueous and ethanolic extracts of R. damascena
have potentially anticonvulsant effect in PTZ-
induced seizure model mice. These data confirm
the results of previous studies showing the beneficial
effects of the essential oil of this flower on kindling seizure model\textsuperscript{[25]}, as well as PTZ-induced seizures\textsuperscript{[26]}. In our previous study, the potentiation of pentobarbital-induced hypnosis by the aqueous, ethanolic and chloroformic extracts was shown in mice with the same doses we used in the present study\textsuperscript{[12]}. Both aqueous and ethanolic extracts (500 and 1000 mg/kg, i.p.) significantly potentiated hypnotic effects of pentobarbital. The study of Nyeem et al\textsuperscript{[24]} also showed that 250 and 500 mg/kg of ethanolic extract of \textit{R. damascena} significantly fastened the beginning of sleep and also prolonged the duration of sleeping time in pentobarbital-induced hypnosis test in mice. The results of the present study confirm that all doses of aqueous extract and only the highest dose of ethanolic extract (1000 mg/kg) significantly increased MCS latency while, the chloroformic extract failed to protect against MCS following PTZ administration. On the same way, the aqueous and ethanolic extracts significantly increased GTCS latency while chloroformic extract had no significant protective effect. These results are in good agreement with our previous study\textsuperscript{[12]}.

It has also been shown that ethanolic extract of \textit{R. damascena} decreased the locomotor activity using the open field and hole cross tests\textsuperscript{[28]}. Hypnotic, analgesic and locomotor activities as well as anticonvulsant effect of \textit{R. damascena} might confirm its depressant effects on the nervous system\textsuperscript{[24]}, which has been suggested in traditional medicine\textsuperscript{[11]}, In a single blind, randomized clinical trial over a period of one year, the useful effects of the essential oil of \textit{R. damascena} was shown in patients with chronic sleep disorder\textsuperscript{[31]}. \textit{R. damascena} contains several components such as geraniol, citral, linalool, farnesol, nerol, limonene, eugenol, citral, terpene, myrcene, vitamin C and bioflavonoids\textsuperscript{[11]}. The responsible compounds for anticonvulsant effect of \textit{R. damascena} is uncertain and cannot be concluded from the results of the present study. Regarding the fact that the aqueous extract solubilizes mainly polar constituents and the ethanolic extract solubilizes compounds of intermediate polarity while the chloroformic extract bears non-polar agents, it seems that the responsible compounds are polar or with intermediate polarity\textsuperscript{[28]}. Other plants contained compounds such as flavonoids, terpenes and saponins have been found to have hypnotic and anticonvulsant effects\textsuperscript{[34]}. Therefore, it might be suggested that these compounds are responsible for anticonvulsant effects of \textit{R. damascena}. Flavonoids with anxiolytic and antidepressant properties have been described in many plant species used in folk medicine to depress the CNS activity. These effects have been attributed to their affinity to central benzodiazepine receptors\textsuperscript{[26]}. Geraniol contains methoxyphenol residue in its structure. Behavioral studies have shown that compounds which contain methoxyphenol and alkylphenols in their structures have hypnotic and anticonvulsant effects\textsuperscript{[36]}. It is conceivable that geraniol may be at least partially responsible for the anticonvulsant effects of \textit{R. damascena} through GABA\textsubscript{A} system. The involvement of GABA neurotransmission in convulsion, sleep, analgesia and locomotor activity confirms that this compound or other ingredients of \textit{R. damascena} may have interaction with GABA system. It has also been reported that saponins regulate the effects of sedatives and anticonvulsants\textsuperscript{[37, 38]}; therefore, saponins may also contribute to the anticonvulsant activity of \textit{R. damascena}. On the other hand, eugenol has been found to have analgesic, anesthetic and anticonvulsant effects\textsuperscript{[39-41]}. Thus, this compound may have a role in the results of the present study. Anticonvulsant effect of linalool has also been reported and therefore, the anticonvulsant effects of \textit{R. damascena} extract which was seen in the present study may in part be due to this compound\textsuperscript{[42, 43]}. Antioxidant properties of \textit{R. damascena} have been mainly attributed to quercetin\textsuperscript{[39, 22, 44, 45]}. This compound has shown to have anticonvulsant effects in several animal models\textsuperscript{[46]}. In addition, the contribution of kaempferol which has anticonvulsant effects and presents in \textit{R. damascena} should not be ignored\textsuperscript{[47-49]}.

4 Acknowledgements
The authors would thank the Vice Chancellor of Research Affairs of Mashhad University of Medical Sciences, Iran, for financial supports.

5 Competing interests
The authors declare that they have no competing interests.

REFERENCES


31 Hosseinzadeh H, Nassiri Asl M. Anticonvulsant, sedative and muscle relaxant effects of carbinoxalone in mice.


突厥蔷薇提取物抗戊四唑所致小鼠痉挛的作用

Mahmoud Hosseini1, Mahboobeh Ghasemzadeh Rahbardar1, Hamid Reza Sadeghnia5, Hassan Rakhshandeh3
1. Neuroscience Research Center and Department of Physiology, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran
2. Neuroscience Research Center and Department of Pharmacology, Department of New Sciences and Technologies, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran
3. Pharmacological Research Center of Medicinal Plants and Department of Pharmacology, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran

目的:研究突厥蔷薇(Rosa damascena)的水提物、乙醇提取物及氨甲酰提取物对戊四唑所致小鼠痉挛的作用。
方法:实验动物按如下方案分组;生理盐水对照组,地西泮组(3 mg/kg),水提物组(100, 500 和 1 000 mg/kg),
乙醇提取物(100，500和1000 mg/kg)，三氯甲烷提取物(100，500和1000 mg/kg)。所有药物均在注射戊四唑30 min前皮下注射。记录并比较各组小鼠阵发性强直性抽搐潜伏期及死亡率。

结果：与生理盐水对照组相比，三组水提物组小鼠的阵发性强直性抽搐潜伏期及死亡率显著延长(P<0.05或P<0.01)。一组乙醇提取物(1000 mg/kg)小鼠的阵发性痉挛潜伏期及死亡率较对照组明显延长(P<0.05, P<0.01);三组三氯甲烷提取物组小鼠的阵发性强直性抽搐潜伏期及死亡率无明显变化。不同提取物各组的死亡率与对照组相比无明显差异。

结论：本研究的结果证实了突厥蔷薇具有抗戊四唑所致小鼠惊厥的作用，其具体作用机制有待进一步研究。

关键词：突厥蔷薇；蔷薇科；抗惊厥药；戊四唑；发作；小鼠

Journal of Chinese Integrative Medicine
publishes papers for Study Protocol

Journal of Chinese Integrative Medicine (JCIM) publishes papers for Study Protocol. JCIM believes that publishing clinical study protocols will help improve the standard of medical research by:

- Enabling researchers to obtain feedback on draft study protocols through peer review;
- Enabling readers to compare what was originally intended with what was actually done, thus preventing both “data dredging” and post-hoc revisions of study aims;
- Enabling funders and researchers to see what studies are underway and hence reducing duplication of research effort;
- Enabling systematic reviewers to find trials, which may in turn reduce distortion of the evidence from publication bias;
- Enabling patients to see what studies are underway that they may wish to volunteer for.

Your study protocol published in JCIM becomes a fully citable open-access article — freely and universally accessible online, permanently archived. It will also be included in PubMed, further increasing its visibility.

The study protocol can be for proposed or ongoing research. Study protocols will usually be published without peer review if the study has received ethics approval and a grant from a major funding body (proof will be required). Study protocols without funding or ethical approval will be peer reviewed. Proof of both ethics and funding will be required and we recommend that authors provide the relevant documentation on submission.

Protocols of randomized controlled trials should follow the CONSORT guidelines and must have a trial registration number included as the last line of the abstract.

Publishing your study protocol in JCIM does not commit you to submitting subsequent reports of the study to us, although we do, of course, welcome such submissions.

Protocols should provide a detailed account of the hypothesis, rationale and methodology of the study. Manuscripts for Study Protocol articles submitted to JCIM should be divided into the following sections: Title Page, Abstract (consists of 4 paragraphs, labeled as Background, Methods and Design, Discussion, and Trial Registration), Keywords, Background and Significance/Preliminary Studies, Study Aims, Study Design/Methods, Discussion, Competing interests, Authors’ contributions, Acknowledgements and Funding, References, Figure legends (if any), Tables and captions (if any), Description of additional data files (if any).

Please submit your manuscript at the website of JCIM (http://www.jcimjournal.com) or at http://mc03.manuscriptcentral.com/jcim-en via ScholarOne Manuscripts submitting system.