Effects of different extracts of *Eugenia caryophyllata* on pentylenetetrazole-induced seizures in mice

Mahmoud Hosseini¹, Taha Jafarianheris¹, Navid Seddighi¹, Mohammad Parvaneh², Ahmad Ghorbani², Hamid Reza Sadeghnia², Hassan Rakhshandeh¹

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, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran
3. Pharmacological Research Center of Medicinal Plants, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran

**OBJECTIVE:** To investigate the possible anticonvulsant effect of different extracts of *Eugenia caryophyllata* (clove) on pentylenetetrazole (PTZ)-induced seizures in mice.

**METHODS:** The animals were divided into saline, 50, 100, 250 and 500 mg/kg of aqueous extract, 50, 100, 250 and 500 mg/kg of ethanolic extract, and 50, 100, 250 and 500 mg/kg of chloroformic extract of clove groups. The extracts or saline were injected 60 min before each PTZ injection. Latency to the first minimal clonic seizure (MCS) and generalized tonic-clonic seizure (GTCS) and the percent of mortality were recorded.

**RESULTS:** Aqueous extract of clove at doses of 50, 100, 250 and 500 mg/kg significantly extended the MCS and GTCS latency (*P*<0.05). The MCS latency in mice treated with 50, 100 and 250 mg/kg of the ethanolic extract was significantly increased (*P*<0.05). The GTCS latency in mice treated with 50, 100, 250 and 500 mg/kg of ethanolic extract was significantly higher than that of the saline-treated group (*P*<0.05). There were no significant differences in MCS and GTCS latency between mice treated with different chloroformic extract of clove or saline.

**CONCLUSION:** The aqueous and ethanolic extracts of clove could inhibit the PTZ-induced convulsion, and this plant has the potential to be used as a new therapeutic agent for control of seizures. The exact mechanisms and the active compounds that are responsible for the anticonvulsant effect need to be clarified in further studies.

**KEYWORDS:** *Eugenia caryophyllata,* pentylenetetrazole; seizures; mice
Epilepsy is a common neurological disease that affects about 1% of the population\(^1\). With currently available drugs, in one out of three patients, seizure freedom is not achievable\(^2\). Therefore, the search for more effective anticonvulsant agents with fewer side effects has been continued. For thousands of years, medicinal plants are always good sources to find new remedies for human diseases. \textit{Eugenia caryophyllata} (clove) has acquired a folk reputation due to its medicinal values\(^3,4\).

Clove is a tree from Myrtaecae family with a height ranging from 10 to 20 m that is growing on islands of Malaysia, Indonesia, Madagascar, India, Tanzania and Sri Lanka\(^1\). Traditionally, several parts of this plant such as buds and leaves are used in pharmacy, cooking, food processing, cosmetics and perfumery\(^5\). It has also been used as a remedy for many illnesses such as disorder of digestive systems. It has been shown that some components of clove are beneficial for treating bacterial and fungal infections\(^6,7\). The antimicrobial and antifungal properties of clove oil allow its use for acne, scars, parasites and warts and to treat the oral bacteria which are usually associated with dental cavity and periodontal disease\(^8,9,10\). The cytotoxic and anticancerogenic effects of clove and its components have also been observed\(^6,9,12\). Vaso-relaxant as well as smooth muscle relaxant effects have also been described for clove essential oil\(^11,12\). Useful effects of clove in asthma as well as different allergic diseases have also been demonstrated\(^13\). Results obtained from human research also confirmed the analgesic effect of this plant in patients suffering from anf fissure or toothache\(^6\). The anesthetic effect of essential oil from different parts of this plant has also been observed in fishes\(^14\). Phytochemical analysis of clove essential oil has revealed the eugenol as a main component\(^7,17\). The anesthetic as well as the anti-inflammatory and analgesic effects of eugenol have been well documented in animal models\(^7,18-20\). The anti-stress and anticonvulsant properties of eugenol have also been reported\(^21,22\). In our previous study, the analgesic effect of clove was observed using hot plate test\(^22\). In Iranian traditional medicine, the buds of clove have been used as an antiepileptic drug\(^23\). Thus, the present work was done in order to investigate the possible anticonvulsant effect of different extracts of clove in mice.

1 Materials and methods

1.1 Chemicals and plant extracts Pentylenetetrazole (PTZ) was bought from Sigma (St. Louis, MO) and dissolved in normal saline (0.9%). The clove was kindly provided by Exire Gole Sorkh Co. ( Mashhad, Iran) which was previously identified by herbalists. The chopped and dried flowers (50 g) were extracted using a Soxhlet apparatus with 300 mL distilled water, ethanol and chloroform to prepare aqueous, ethanolic and chloroformic extracts. The extracts were concentrated to dryness with a rotational vacuum evaporator\(^21\). The aqueous extract was dissolved in saline. The ethanolic extract was dissolved in saline supplemented with 1% (volume ratio) of Tween. For better solubility of chloroformic extract, the dried extract was dissolved in saline plus dimethyl sulfoxide (DMSO); final concentration was 0.1%, volume ratio. It has been shown that this concentration was not toxic\(^21\).

1.2 Animals Eight-week male BALB/c mice weighing 25 to 30 g ( Pasteur Institute, Tehran, Iran) were used in this study. All of them were housed in the same room, under stable temperature of (22±2) °C, moisture of 55% to 60% and 12 to 12 h light to dark cycle. Food and water existed \textit{ad libitum} properly. Animal handling and all related procedures were approved by Mashhad University of Medical Science, Ethical Committee Acts and were performed in accordance with the \textit{National Institutes of Health Guidelines for the Care and Use of Laboratory Animals}.

1.3 PTZ-induced seizures In order to observe ictal behavior, PTZ (90 mg/kg, intraperitoneally) was injected and the animals were placed in Plexiglas square (30 cm×30 cm×30 cm) on the day of the experiment\(^15-20\). The animals were observed during 60 min after PTZ accomplishment. Behavioral

### Related Articles


responses of the animals to PTZ were evaluated using criteria including latency to the first minimal clonic seizure (MCS), incidence of MCS, latency to the first generalized tonic-clonic seizure (GTCS), incidence of GTCS, protection percentage against GTCS and protection percentage against mortality.\textsuperscript{26-28}

1.4 Procedure The animals were randomly divided into 15 groups (n = 10) as follows: groups 1 to 3 were given saline, saline plus 1% of Tween 80 and saline plus DMSO as vehicles; groups 4 to 7 were given 50, 100, 250 and 500 mg/kg of aqueous extracts; groups 8 to 11 were given 50, 100, 250 and 500 mg/kg of ethanolic extracts; groups 12 to 15 were given 50, 100, 250 and 500 mg/kg of chloroformic extracts. The extracts and vehicles were injected 60 min before PTZ administration. All injections were done in a volume of 10 mL/kg intraperitoneally between 10 AM and 6 PM.

1.5 Statistical analysis Data were expressed as mean ± standard error of mean and analyzed by using Graphpad InStat software program. Fisher’s exact test as well as one-way analysis of variance, followed by Tukey’s test were used for statistical evaluation. When P < 0.05, it was considered as statistical significance.

2 Results

2.1 Effects of aqueous extract of clove on MSC and GTCS latency All animals in different groups showed MSC and GTCS following PTZ administration. When compared with the saline group, MSC latency was significantly increased in 50, 100, 250 and 500 mg/kg of aqueous extract of clove groups (P < 0.05). GTCS latency in 50, 100, 250 and 500 mg/kg of aqueous extract of clove groups was significantly increased as compared with the saline group (P < 0.01). See Table 1.

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>MCS latency (Mean±s.d)</th>
<th>GTCS latency (Mean±s.d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>10</td>
<td>42.40±0.95</td>
<td>50.11±0.87</td>
</tr>
<tr>
<td>Aqueous extract of clove (50 mg/kg, i.p.)</td>
<td>10</td>
<td>54.00±2.96*</td>
<td>91.00±10.95**</td>
</tr>
<tr>
<td>Aqueous extract of clove (100 mg/kg, i.p.)</td>
<td>10</td>
<td>71.00±8.4*</td>
<td>127.67±17.78**</td>
</tr>
<tr>
<td>Aqueous extract of clove (250 mg/kg, i.p.)</td>
<td>10</td>
<td>76.77±12.48*</td>
<td>152.89±29.35**</td>
</tr>
<tr>
<td>Aqueous extract of clove (500 mg/kg, i.p.)</td>
<td>10</td>
<td>56.30±5.14*</td>
<td>99.70±15.33**</td>
</tr>
</tbody>
</table>

\* P < 0.05, \** P < 0.01, vs saline group. MSC: minimal clonic seizure; GTCS: generalized tonic-clonic seizure; PTZ: pentylenetetrazole; i.p.: intraperitoneal injection.

2.2 Effects of ethanolic extract of clove on MSC and GTCS latency MCS latency in mice treated with 50, 100 and 250 mg/kg of the ethanolic extract of clove was significantly increased when compared with that of mice treated with saline plus Tween (P < 0.05). The effect of 500 mg/kg of the ethanolic extract of clove on MCS latency remained non-significant. GTCS latency in mice treated with 50, 100, 250 and 500 mg/kg of ethanolic extract of clove was significantly increased when compared with that of mice treated with saline plus Tween (P < 0.05 or P < 0.01). See Table 2.

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>MCS latency (Mean±s.d)</th>
<th>GTCS latency (Mean±s.d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline plus 1% of Tween 80</td>
<td>10</td>
<td>47.18±3.13</td>
<td>63.60±6.94</td>
</tr>
<tr>
<td>Ethanolic extract of clove (50 mg/kg, i.p.)</td>
<td>10</td>
<td>61.40±4.84</td>
<td>107.40±12.48</td>
</tr>
<tr>
<td>Ethanolic extract of clove (100 mg/kg, i.p.)</td>
<td>10</td>
<td>58.83±3.10</td>
<td>124.50±12.34</td>
</tr>
<tr>
<td>Ethanolic extract of clove (250 mg/kg, i.p.)</td>
<td>10</td>
<td>59.87±4.50</td>
<td>96.00±10.63</td>
</tr>
<tr>
<td>Ethanolic extract of clove (500 mg/kg, i.p.)</td>
<td>10</td>
<td>53.14±2.26</td>
<td>86.83±5.71</td>
</tr>
</tbody>
</table>

\( \Delta P < 0.05, \quad \Delta \Delta P < 0.01, \quad \Delta \Delta \Delta \) saline plus 1% of Tween 80 group. MSC: minimal clonic seizure; GTCS: generalized tonic-clonic seizure; PTZ: pentylenetetrazole; i.p.: intraperitoneal injection.

2.3 Effects of chloroformic extract of clove on MSC and GTCS latency There were no significant differences regarding MCS and GTCS latency between different chloroformic extract of clove and saline plus DMSO groups. There were no significant differences in mortality rate following PTZ administration between different groups.

3 Discussion

In the present study, it was demonstrated that clove induces the anticonvulsant effect by increasing the MCS and GTCS latency upon PTZ administration. Therefore, the present study supports traditional use of this plant as an antiepileptic agent.

To obtain better insight into the nature of compounds responsible for the antiepileptic effect of clove, three different extracts were prepared: the aqueous extract which contains polar constituents, the ethanolic extract which contains polar and medium-to low-polarity compounds, and the chloroformic one which mostly contains lipophilic agents.\textsuperscript{29} Chloroformic extract of clove did not affect MCS and GTCS parameters indicating that lipophilic compounds such as alkaldes, fatty acids and steroles are not responsible for the anticonvulsant effect of clove. Also, the aqueous extract was found to be more effective than the ethanolic one. Therefore, it is reasonable to assume that polar or medium-polarity constituents of clove are responsible for its anticonvulsant activity. However, there are some reports that eugenol, an aromatic molecule derived from essential oil of clove, exerts the same activity.\textsuperscript{30} Therefore, the nature of components responsible for the anticonvulsant effect of this plant is still an open question.

Agents affecting the PTZ-induced convulsion...
can inhibit absence seizures. Thus, regardless of the exact nature of compounds involved, the clove may have beneficial effect on this type of seizure. The precise molecular mechanisms behind this protective function are still unclear. Clove and its effective constituent, eugenol, have been reported to have antistress, analgesic and even anesthetic effects on the nervous system\(^\text{[17,23,31]}\). Therefore, it seems that clove has a sedative effect which may contribute to the anticonvulsant effect. It has been previously reported that essential oil of *Eugenia caryophyllata* increased the threshold of clonic seizures induced by PTZ. Furthermore, clove oil possesses anticonvulsant activity against maximal electroshock-induced tonic seizures\(^\text{[32]}\). It also causes motor impairment which was attributed to eugenol and carvacrol which are present in the essential oil of this plant\(^\text{[32]}\).

The anticonvulsant effect as well as muscle relaxant and antistress activities of these compounds have been well documented, previously\(^\text{[32,33,34]}\). It has been also shown that carvacrol had anxiolytic effects when acutely administered in rodents which were reversible by flumazenil. However it did not show any sedative or muscle-relaxant properties\(^\text{[32]}\). Furthermore, the anticonvulsant activity of several agents with antioxidant effects such as melatonin, vitamin e, trans-resveratrol and α-lipoic acid has been shown\(^\text{[36,37]}\). In keeping with these observations, there are some reports that reactive oxygen species may underlie the convulsant and neurotoxic effects of PTZ\(^\text{[38]}\). Therefore, the antioxidant effects of different clove components may also be another explanation for the anticonvulsant effects of the extract which was observed in the present study\(^\text{[39,40]}\).

It has been reported that eugenol inhibits epileptiform neuronal activity and spreading depression in hippocampal and neocortical tissues\(^\text{[30]}\). Furthermore, eugenol has been shown to inhibit N-methyl-D-aspartate-induced neurotoxicity and potentiate gamma-aminobutyric acid, receptor responses, *in vitro*. Both of these receptors have important roles in seizure induction and spreading and therefore, these properties may also contribute to the anticonvulsant activity of clove bud extract which was seen in the present study\(^\text{[41,42]}\). The suppressing effect on Na\(^+\) currents has also been considered as an possible mechanism for modulation of neuronal excitability by eugenol, for example, in seizures\(^\text{[43]}\). Regarding the results of present study it might be suggested that besides of eugenol, there are also other polar compounds in buds of clove which have anticonvulsant effects, but it needs to be investigated.

In conclusion, the aqueous and ethanolic extracts of clove inhibit PTZ-induced convolution and this plant has the potential to be used as a new agent for control of seizure. The exact mechanism and the active compounds responsible for the anticonvulsant effect need to be clarified in future studies.

4 Acknowledgements

The authors would like to thank the Vice-Chancellor of Research Affairs of Mashhad University of Medical Sciences for financial supports.

5 Conflict of interests

The authors declare that they have no competing interests.

REFERENCES


36 Gupta YK, Briyal S. Protective effect of vinoatrol against kaic acid induced seizures, oxidative stress and on the expression of heat shock proteins in rats. Eur Neuro-
psychopharmacol. 2006; 16(2): 85-91.

不同丁香提取物对戊四唑诱导的小鼠癫痫发作的影响

Mahmoud Hosseini1, Taha Jafarianheris1, Navid Seddighi1, Mohammad Parvaneh1, Ahmad Ghorbani1, Hamid Reza Sadeghnia2, Hassan Rahshandeh1
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, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran
3. Pharmacological Research Center of Medicinal Plants, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran

目的:研究不同丁香提取物对戊四唑引发小鼠癫痫的作用。
方法:实验小鼠被随机分为生理盐水对照组、不同剂量(50、100, 250 和 500 mg/kg)丁香水提取物组、不同剂量(50, 100, 250 和 500 mg/kg)丁香乙醇提取物组、不同剂量(500, 100, 250 和 500 mg/kg)丁香三氯甲烷提取物组。给予戊四唑前 60 min 为各组小鼠注射药物。记录并比较各组小鼠阵发性强直性抽搐潜伏期（generalized tonic-clonic seizure, GTCS）、最小阵发性痉挛潜伏期（minimal clonic seizure, MCS）及死亡率。
结果:低、中、高三种剂量的丁香水提取物能有效延长 MSC 和 GTCS 的潜伏期（P<0.05）。50, 100 和 250 mg/kg 的丁香乙醇提取物显著增加了小鼠 MCS 潜伏时间（P<0.05）。与盐水治疗组相比，惊厥小鼠模型经 50, 100, 250 和 500 mg/kg 丁香的乙醇提取物治疗后，其 GTCS 的潜伏期显著高于盐水治疗组，但丁香的三氯甲烷提取物对惊厥小鼠的 GTCS 和 MCS 潜伏期没有显著的影响。
结论:丁香乙醇和水提取物能抑制由戊四唑诱发的小鼠惊厥，但其确切的作用机制和有效的抗惊厥活性物质还有待进一步的研究。
关键词:丁香、戊四唑、癫痫发作、小鼠