

# Anticonvulsant Effects of the Hydroalcoholic Extract of *Alpinia officinarum* Rhizomes in Mice: Involvement of Benzodiazepine and Opioid Receptors

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## Original Article

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**Background and Purpose:** Epilepsy is one of the most common serious neurological conditions. The current therapeutic treatment of epilepsy with modern antiepileptic drugs is associated with side effects, dose-related and chronic toxicity, and teratogenic effects and in approximately 30% of the patients is ineffective. *Alpinia officinarum* is used in Iranian traditional medicine for treatment of different diseases like back pain and seizure.

**Methods:** In this study, anticonvulsant effects of hydroalcoholic extract of *Alpinia officinarum* rhizomes were examined by using pentylentetrazole (PTZ) model in mice. *Alpinia officinarum* rhizomes extract (200, 400 and 600 mg/kg), diazepam (1 mg/kg) and normal saline (10 mL/kg) were injected (ip) 30 minutes before PTZ (90 mg/kg, ip). The time taken before the onset of clonic convulsions, the duration of clonic convulsions, and the percentage of seizure and mortality protection were recorded. For further clarification of the mechanism of action for *Alpinia officinarum*, flumazenil (2 mg/kg, ip) and naloxone (5 mg/kg, ip) were also injected 5 minutes before *Alpinia officinarum* extract.

**Results:** *Alpinia officinarum* extract at the doses of 200 and 400 mg/kg prolonged the time of onset of seizure and decreased the duration of seizures compared to control (saline) group ( $p < 0.05$ ). At the dose of 600 mg/kg, percentage of seizure protection was 16.66%. Naloxone and flumazenil could suppress anticonvulsant effects of *Alpinia officinarum*.

**Conclusions:** It seems that *Alpinia officinarum* could be a good candidate and be useful for seizure control and treatment, and in these effects, opioid and benzodiazepine receptors might probably be involved.

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**Key words:** *Alpinia officinarum*, Seizure, Hydroalcoholic extract, Naloxone, Flumazenil

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## Introduction

Epilepsy is one of the most common neurological disorders characterized by spontaneous and recurrent seizures. Seizures are caused by abnormal and excessive discharges produced by a group of cerebral neurons.<sup>1</sup> It is demonstrated that repeated and prolonged seizures produce mental (cognitive and behavioral) impairments via induction of neuronal death.<sup>2</sup> Epilepsy decreases the quality of life and increases the risk of injury and even mortality.<sup>3</sup> Despite new developments in antiepileptic drugs, there are difficulties in cure of the different types of epilepsy.<sup>4</sup> In addition, about one-third of patients are

considered to be pharmaco-resistant and do not respond to these medications.<sup>5</sup> Therefore, it is necessary to explore new pharmacological treatments; especially those originated from plants and other natural sources.<sup>6-8</sup>

*Alpinia officinarum* (Zingiberaceae), commonly known as galangal, is one of the medicinal plants recommended for treatment of seizure in Iranian traditional medicine.<sup>9</sup> Some other pharmacological activities also have been mentioned in traditional medicine for various conditions such as abdominal pain, emesis, diarrhea, impotence, renal diseases, and dysentery.<sup>10</sup> Recent studies have demonstrated that *Alpinia officinarum* extracts possess a wide variety of biological activ-

ities including antioxidant,<sup>11</sup> anti-inflammatory,<sup>12</sup> immunostimulatory,<sup>13</sup> and anticancer<sup>14</sup> activities. Several bioactive components of *Alpinia officinarum* have been reported in the literature such as diaryl heptanoids, flavonoids and essential oils.<sup>15</sup>

According to our knowledge, some traditional uses of *Alpinia officinarum* have been confirmed in modern scientific studies. But the antiepileptic effect of *Alpinia officinarum* has not been studied so far. Following our previous studies on antiepileptic effect of Iranian medicinal plant,<sup>7</sup> in the present study, anticonvulsant activity and possible mechanisms of action of the rhizomes of *Alpinia officinarum* hydroalcoholic extract (AOE) were studied in pentylenetetrazole induced seizures in mice for the first time.

## Methods

### Preparation of the hydroalcoholic extract of *Alpinia officinarum* rhizome

*Alpinia officinarum* rhizomes were purchased from a local herbal market in Tehran, Iran and its authenticity was approved by Dr. Golamreza Amin (School of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran). The voucher specimen number PMP-234 was deposited in the school of pharmacy. Hydroalcoholic extract (70:30 alcohol:water) of *Alpinia officinarum* rhizome was prepared by maceration method (3 times) and the extract was dried by rotary evaporator and stored at -20°C until tested. The extract was dissolved in normal saline when used in the animal study.

### Animals

In this study, male albino mice weighing 25-35 g (animal house of Iran University of Medical Sciences, Tehran, Iran) were used. The animals were housed in polycarbonate cages under 24 ± 2°C with a 12-hour light/dark cycles. All animals were similarly handled in the animal house and had free access to food and tap water. All possible steps were taken to avoid animal suffering at each stage of the experiment. The ethical guidelines for using experimental animals were followed in all tests in accordance with ethical committee acts of Iran University of Medical Sciences and the European Communities Council Directive 24 November 1986 (86/609/EEC).

### Chemicals

Drugs used in this study are as follows: pentylenetetrazole (PTZ) (Sigma, USA), flumazenil (Sigma, USA), diazepam (Sigma, USA), naloxone (Sigma, USA). All drugs were dissolved in sterile physiological saline.

## Experimental studies

### Anticonvulsant activity

PTZ (60 mg/kg, i.p.) was used to induce convulsions in the mice.<sup>16</sup> The animals were divided randomly into 5 groups (n = 6) as follows: 1) control group, received normal saline (10 mL/kg, i.p.) 30 min before administrating PTZ; 2) diazepam group, received diazepam (1 mg/kg, i.p.) 30 min before administrating PTZ; 3-5) AOE groups, received different doses of AOE dissolved in water (200, 400 and 600 mg/kg, i.p.) 30 min before administrating PTZ. Following induction of convulsions in mice, the seizure parameters were immediately documented. Each animal was placed into an individual plastic cage and monitored for 30 min for signs of neurological deficits, particularly hindlimb tonic extensions. The seizure latency, seizure duration and percentage of seizure protection were recorded and the animals were observed for mortality for 24 h after PTZ injection.<sup>7</sup>

### Evaluation of the effect of flumazenil on the anticonvulsant activity of AOE

To investigate the possible involvement of benzodiazepine receptors, we examined the effects of flumazenil (benzodiazepine receptor antagonist) on the anticonvulsant activity of AOE.<sup>17</sup> The animals were divided randomly into 6 groups (n = 6) as follows: 1) control group, received normal saline (10 mL/kg, i.p.) 30 min before administrating PTZ; 2) flumazenil group, received flumazenil (2 mg/kg, i.p.) 20 min before administrating PTZ; 3) diazepam group, received diazepam (1 mg/kg, i.p.) 30 min before administrating PTZ; 4) Diazepam + Flumazenil group, received flumazenil (2 mg/kg, i.p.) 5 min before the injection of diazepam (1 mg/kg, i.p.) and 20 min before the injection of PTZ; 5) AOE group, received AOE (600 mg/kg, i.p.) 30 min before administrating PTZ; 6) AOE 600 + flumazenil group, received flumazenil (2 mg/kg, i.p.) 5 min before the injection of AOE (600 mg/kg, i.p.) and 20 min before the injection of PTZ.<sup>17</sup> Then seizure latency, seizure duration and percentage of seizure protection in 30 min after injection of PTZ, and also mortality rate during the first 24 h were recorded. The anticonvulsant activity of AOE and diazepam in mice pretreated with flumazenil was assessed and compared with mice in control, flumazenil, diazepam and AOE (600 mg/kg, i.p.) groups.

### Evaluation of the effect of naloxone on the anticonvulsant activity of AOE

To investigate the probable modulatory effect of opioid receptors, we examine the effects of naloxone (opioid receptor antagonist) on

the anticonvulsant activity of AOE.<sup>18</sup> The animals were divided randomly into 4 groups (n = 6) as follows: 1) control group, received normal saline (10 mL/kg, i.p.) 30 min before administrating PTZ; 2) naloxone group, received naloxone (5 mg/kg, i.p.) 20 min before administrating PTZ; 3) AOE group, received AOE (600 mg/kg, i.p.) 30 min before administrating PTZ; 4) AOE 600 + naloxone group, received naloxone (5 mg/kg, i.p.) 5 min before the injection of AOE (600 mg/kg, i.p.) and 20 min before the injection of PTZ.<sup>18</sup> Then seizure latency, seizure duration and percentage of seizure protection in 30 min after injection of PTZ, and also mortality rate during the first 24 h were recorded. The anticonvulsant activity of AOE in mice pre-treated with naloxone was assessed and compared with mice in control, naloxone and AOE (600 mg/kg, i.p.) groups.

### Statistical analysis

The results of the study are reported as mean  $\pm$  S.E.M. The differences among the groups were evaluated by one-way analysis of variance followed by the Tukey test by using GraphPad Prism 5.0 (GraphPad Software Inc., La Jolla, CA, USA) software. For all tests, results with  $p < 0.05$  were considered as statistically significant.

## Results

### Anticonvulsant activity of AOE on PTZ-induced seizure

Injection of the AOE (400 and 600 mg/kg) prolonged significantly the onset of seizure ( $p < 0.05$  and  $p < 0.001$ , respectively) and decreased the duration of seizures ( $p < 0.05$  and  $p < 0.01$ , respectively) compared with the control group. In addition, AOE (600 mg/kg) exhibited protection against seizure in 100% of animals and reduced mortality compared with the control group. Diazepam protected 100% of mice from PTZ-induced seizure and mortality (Table 1).

### The effect of flumazenil on the anticonvulsant activity of AOE

Administration of flumazenil 5 min before AOE (600 mg/kg) and 20 min before the injection of PTZ, resulted in inhibition of prolonged latency produced by AOE ( $p < 0.001$ ); but had no significant effect on duration of seizure. However, the difference between the latency and the duration of seizure in mice that had received AOE (600 mg/kg) pretreated with flumazenil and the control group (all  $p < 0.01$ ) was statistically significant. Also, Flumazenil reduced seizure protection (%) in AOE (600 mg/kg) from 100% to 16.66%. So, flumazenil reversed the anticonvulsant action of AOE to some extent. In addition, flumazenil inhibited the anticonvulsant activity of diazepam (Table 2).

**Table 1.** Effect of AOE in PTZ-induced seizures in mice

Treatment group	% Protection (30 min)	Seizure latency (s)	Seizure duration (s)	Mortality (24 h)
Control	0	87.80 $\pm$ 8.434	58.00 $\pm$ 15.14	4/6
Diazepam	100	$\infty$ ***	0.00 $\pm$ 0.00**	0/6
AOE 200	0	147.3 $\pm$ 62.34	23.00 $\pm$ 5.508	2/6
AOE 400	0	751.3 $\pm$ 319.7*	18.67 $\pm$ 8.353*	0/6
AOE 600	100	$\infty$ ***	0.00 $\pm$ 0.00**	0/6

The data are expressed as mean  $\pm$  S.E.M. (n = 6).

AOE, *Alpinia officinarum* extrac; PTZ, pentylenetetrazole.

\* $p < 0.05$ ; \*\* $p < 0.01$ ; \*\*\* $p < 0.001$  compared to the control group.

**Table 2.** Effect of flumazenil on the anticonvulsant activity of AOE and diazepam in PTZ-induced convulsion in mice

Treatment group	% Protection (30 min)	Seizure latency (s)	Seizure duration (s)	Mortality (24 h)
Control	0	87.80 $\pm$ 8.434	58.00 $\pm$ 15.14	4/6
Flumazenil	0	77.33 $\pm$ 3.844	52.00 $\pm$ 6.083	3/6
Diazepam	100	$\infty$ ***	0.00 $\pm$ 0.00**	0/6
Diazepam + Flumazenil	0	113.7 $\pm$ 10.34	54.33 $\pm$ 7.535	2/6
AOE 600	100	$\infty$ ***	0.00 $\pm$ 0.00**	0/6
AOE 600 + Flumazenil	16.66	739.5 $\pm$ 58.7***##	11.75 $\pm$ 4.973**	1/6

The data are expressed as mean  $\pm$  S.E.M. (n = 6). Abbreviations: PTZ, pentylenetetrazole; AOE, *Alpinia officinarum* extract.

\* $p < 0.05$ ; \*\* $p < 0.01$ ; \*\*\* $p < 0.001$  compared to the control group; ## $p < 0.001$  compared to the AOE 600 group.

**Table 3.** Effect of naloxone on the anticonvulsant activity of AOE in in PTZ-induced convulsion in mice

Treatment group	% Protection (30 min)	Seizure latency (s)	Seizure duration (s)	Mortality (24 h)
Control	0	87.80 ± 8.434	58.00 ± 15.14	4/6
Naloxone	0	53.40 ± 3.763	50.20 ± 4.259	4/6
AOE 600	100	∞***	0.00 ± 0.00**	0/6
AOE 600 + naloxone	16.66	630.8 ± 394.8 <sup>#</sup>	20.50 ± 9.251*	1/6

The data are expressed as mean ± S.E.M. (n = 6).

PTZ, pentylenetetrazole; AOE, *Alpinia officinarum* extract.

\* $p < 0.05$ ; \*\* $p < 0.01$ ; \*\*\* $p < 0.001$  compared to the control group; <sup>#</sup> $p < 0.01$  compared to the AOE 600 group.

### The effect of naloxone on the anticonvulsant activity of AOE

Administration of naloxone 5 min before AOE (600 mg/kg) and 20 min before the injection of PTZ, resulted in inhibition of prolonged latency produced by AOE ( $p < 0.01$ ); but had no significant effect on duration of seizure. However, the difference between the duration of seizure in mice that had received AOE (600 mg/kg) pretreated with naloxone and the control group ( $p < 0.05$ ) was statistically significant. Also, Naloxone reduced seizure protection (%) in AOE (600 mg/kg) from 100% to 16.66%. So, naloxone reversed the anticonvulsant action of AOE to some extent (Table 3).

### Discussion

The results of our study indicated that AOE significantly reduces the seizures duration and increases latency period of seizures induced by PTZ in mice in a dose-dependent manner. We also found that these effects may be related to its effect on opioid and benzodiazepines receptors since the effect was highly diminished by opioid and benzodiazepine receptor antagonists.

*Alpinia officinarum* is originally from China and it has been reported to have an array of biological activities including antioxidant, anti-inflammatory, immunostimulatory, and anticancer clinical applications.<sup>19</sup> Although *Alpinia officinarum* has been used traditionally for many centuries, a few studies were undertaken for evaluation of its safety and the systematic toxicity. In a recent study, Honmore et al.,<sup>20</sup> reported that oral administration of methanolic extract of *Alpinia officinarum* with 2000 mg/kg did not produce any behavioral abnormalities and mortality.

PTZ is a known chemoconvulsant model for studying epilepsy which causes the seizure similar to petit mal epilepsy. It is well-demonstrated that PTZ induces convulsions by inhibiting and/or attenuation of GABAergic neurotransmission.<sup>21</sup> Anticonvulsant drugs like diazepam and phenobarbital treat seizure by inhibiting GABA<sub>A</sub> receptor.<sup>22</sup> Several studies in human and animal have demonstrated

the important role of oxidative stress in the pathophysiology of epilepsy.<sup>23</sup> These studies showed that during seizure, lipid peroxidation and nitric oxide (NO) formation do increase, while antioxidant enzyme activities decrease.<sup>24</sup> Moreover, it has been demonstrated that free radicals reduce GABAergic neurotransmission and GABA<sub>A</sub> receptor is highly sensitive to oxidative stress.<sup>25</sup> The anticonvulsant effects of antioxidant agents suggesting that oxidative stress may be implicated in epilepsy.<sup>26</sup> Furthermore, it has been demonstrated that diazepam (as a prototype for anticonvulsant drugs) exert its anticonvulsant effects by direct enhancement of GABA<sub>A</sub> receptor and decreasing NO formation.<sup>27</sup> In the present study, we found that AOE (400 and 600 mg/kg) injection prolonged the onset of seizure and decreased the duration of seizures compared with the control group. Our results also indicated that administration of flumazenil before AOE (600 mg/kg) antagonizes the effect of AOE on latency time seizures in the PTZ model. The results of the present study suggested that anticonvulsant effects of AOE might have been mediated directly and/or indirectly by acting on GABA complex, since benzodiazepines have receptor on GABA complex and we have shown that pretreatment with benzodiazepine antagonist, flumazenil, could prevent the effect of AOE on seizure. In addition, antioxidant effects of *Alpinia officinarum* are reported in previous studies.<sup>20,28</sup> So, there is also a possibility that AOE exert its antiseizure effect via antioxidant activity. This needs further investigation.

It is well established that central opioidergic system possesses seizure-modulating properties.<sup>29</sup> Several investigations suggest that low doses of morphine ( $\mu$ -opioid receptor agonist) have an anticonvulsive effect, while in high doses, it increase the seizure susceptibility induced by chemoconvulsant drugs such as picrotoxin and PTZ.<sup>30</sup> The relation between GABAergic system and central opioidergic system in seizure modulation has been shown previously.<sup>31</sup> Accordingly, naloxone (a nonselective opioid receptor antagonist) inhibits these effects of morphine.<sup>32</sup> We found that administration of naloxone before AOE (600 mg/kg) decreased the prolongation of seizure latency induced by AOE in the PTZ model. Hence, it seems

that the anticonvulsive effects of AOE could have been mediated by the opioid system.

It is well established that the brain or peripheral tissue inflammation reduces the threshold for seizures.<sup>33</sup> Various studies consistently demonstrated the important involvement of inflammatory mediators in pathogenesis of epilepsy.<sup>34</sup> They showed that epilepsy increases the level of proinflammatory cytokines like NO, TNF- $\alpha$ , IL-6 and IL-8.<sup>35</sup> In addition, some studies showed the important role of cyclooxygenase isoenzymes in the epilepsy. They have tested COX-inhibitors with promising results in the treatment of epilepsy.<sup>36</sup> Several lines of evidence support the contention that AOE has profound anti-inflammatory effects. Mirzaei et al.,<sup>37</sup> showed that hydroalcoholic extract of *Alpinia officinarum* have anti-inflammatory effect via inhibition of NF- $\kappa$ B signaling. NF- $\kappa$ B regulates the transcription of a large number of genes, particularly those involved in the inflammatory responses such as cyclooxygenase-2 (COX-2), inducible nitric-oxide synthase (iNOS), TNF- $\alpha$ , IL-6 and IL-8.<sup>38</sup> Moreover, Honmore et al., demonstrated that *Alpinia officinarum* had anti-inflammatory effects through the COX-inhibitory effects and was useful in treatment of inflammatory disorders.<sup>20</sup> Accordingly, the anti-inflammatory effects of AOE may possibly inhibit the seizures induced by PTZ in mice.

Several constituents of *Alpinia officinarum* are known namely: methylcinnamate,  $\alpha$ -cadinene, apigenin, kaempferide, alpinin, galangol, and some diarylheptanoids like curcumin. Some of these active components have significant protective effect in different animal models of seizure.<sup>19</sup> For example, in a recent study, Han et al.,<sup>39</sup> demonstrated that apigenin have anticonvulsant activity in kainate-induced seizure via antioxidative properties. The anti-epileptic action of curcumin was demonstrated in various studies.<sup>40,41</sup> Accordingly, it might suggest that apigenin and curcumin present in in AOE could have been responsible for the anticonvulsant properties exhibited by hydroalcoholic extract of this plant.

In conclusion, results of our study showed the anticonvulsant activity of hydroalcoholic extract of *Alpinia officinarum* rhizomes. Also, our results suggest that benzodiazepines or/and opioidergic system could have been partially involved in the anticonvulsant activity of *Alpinia officinarum*. These results support the traditional use of the plant in the treatment of convulsion. However, further research is required to find out the underlying mechanism(s) and the active component(s) responsible for the observed activity.

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