



## The effect of hydroalcoholic extract of *Pistacia vera* on pentylenetetrazole-induced kindling in rat

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### Abstract

**Background and objectives:** Most antiepileptic drugs that are commonly being used in the clinic have a wide range of unwanted side effects; while some species of pistachios have been used in the traditional medicine to treat epilepsy. The aim of the present study was to investigate the anticonvulsant effects of the hydroalcoholic extract of *Pistacia vera* L. in pentylenetetrazole (PTZ)-induced chemical kindling. **Methods:** this study was carried out on 40 male Wistar rats. Chemical kindling was induced by intraperitoneal administration of PTZ (40 mg/kg) on every alternate day (30 days). The hydroalcoholic extract of *P. vera* (50 and 100 mg/kg) were administered orally every day (30 days). In days which animals received both PTZ and extract, PTZ was injected 30 min after extract administration. Convulsive behavior was observed for 30 min after PTZ injection and scored according to Racine scale. Diazepam was used as the reference anticonvulsant drug. **Results:** Pretreatment with 50 and 100 mg/kg of *P. vera* extract decreased seizure scores, stage 4 latency and stage 5 duration compared to the control group. The anti-epileptic effects of *P. vera* extract were comparable to diazepam. **Conclusion:** The present findings demonstrated that the hydroalcoholic extract of *P. vera* may inhibit the development of seizure behavior following chronic PTZ-induced model of epilepsy in rats.

**Keywords:** pentylenetetrazole, *Pistacia vera*, rat, seizure

### Introduction

Epilepsy is one of the most common and serious diseases of the central nervous system (CNS) that affects about 1% of people worldwide [1]. Despite the new developments in understanding of epilepsy, the exact pathogenic mechanisms of epilepsy are still unclear [2]. It is found that repeated and prolonged seizures can produce

cognitive and emotional impairments [3]. Epilepsy reduces the patients' quality of life and greatly increases the risk of injury and even mortality [4]. Approximately 30% of epileptic patients are considered to be pharmacoresistant, despite the using the available treatment [5]. Chemical kindling models have often been used

for preclinical evaluation of antiepileptic drugs. In this model, the seizure is induced by repeated administration of subconvulsive doses of pentylenetetrazole (PTZ). These daily administrations of PTZ decrease the seizure threshold and a generalized seizure occurs [6]. Kindling is a valuable model for inducing complex partial epilepsy in animal [7].

There are some drugs that provide a symptomatic cure but sometimes have no efficacy, serious side effects, and chronic toxicity. It is needed to explore about new natural medicines for the development of alternative and complementary treatment of epilepsy [8]. There are several reports that support the use plant extracts for the treatment of epilepsy and show promising antiepileptic activities in different animal models [3,4]. *Pistacia vera* (Anacardiaceae) is native of arid zones of Central and West Asia and its fruits (pistachio) is used in traditional herbal medicine [9]. Experimental studies have provided evidence demonstrating various pharmacological effects of pistachio such as antioxidant [10], antinociceptive, anti-inflammatory [11] and hepatoprotective activities [12]. It has been shown that some species of *Pistacia* have CNS-depressant activity. For example, *P. integerrima* extracts have anticonvulsant activity in PTZ-induced seizures [13]. Also, *P. lentiscus* has been traditionally used as an *antiepileptic agent*. However, there is no evidence to confirm its effectiveness in epilepsy [14].

According to our investigations, the antiepileptic effect of *P. vera* has not been studied so far. The present study was designed to examine the effects of hydroalcoholic extract of *P. vera* on PTZ-kindled seizures in male rats.

## Experimental

### Chemicals

PTZ and Dimethyl sulfoxide (DMSO) was purchased from Sigma Chemical Company (Poole, UK). Diazepam was obtained from Chemidarou Company (Tehran, Iran).

### Plant material

Dried *P. vera* fruits (pistachio) were purchased from a herbal market in Rafsanjan, Iran and

authenticated by Hamid Alipour, (Pistachio Research Institute, Rafsanjan, Iran).

### Extraction

Dried and finely powdered fruits (100 g) were macerated in 1 L of ethanol (80%) for 72 h to obtain the total extract using maceration method. The solvent was evaporated under low pressure in a rotary evaporator (Rotavap, England). The pistachio extract (PE) was frozen and stored at -20 °C. For administration, the extract was dissolved freshly in dimethyl sulfoxide 10% (DMSO, Sigma-Aldrich, Germany).

### Animals

Forty male Wistar rats (250-300 g) were obtained from the animal house of School of Medicine, Rafsanjan University of Medical Sciences, Rafsanjan, Iran. The temperature of the animal house was  $24 \pm 2$  °C with a 12-hour-light/12-hour-dark cycle (lights on at 07:00). The animals were kept in cages with free access to food and water. The ethical guidelines for using experimental animals were followed in all tests in accordance with ethical committee acts of Rafsanjan University of Medical Sciences and the European Communities Council Directive 24 November 1986 (86/609/EEC). All experiments were done at the same time in the morning (10-12 AM) to avoid a circadian rhythm bias.

### Kindling model

Animals were injected intraperitoneally with subconvulsive doses of PTZ (40 mg/kg) in normal saline every other day by a total of 15 injections. After each injection, the convulsive behavior was observed for 30 min and scored as follows (racine scale): no response, stage 0; hyperactivity, vibrissae twitching, stage 1; head nodding, head clonus and myoclonic jerks, stage 2; unilateral forelimb clonus, stage 3; rearing and bilateral forelimb clonus, stage 4; generalized tonic-clonic seizure and loss of writing reflex, stage 5 [15].

### Treatment groups

Rats were randomly assigned to four experimental groups (10 animals in each group)

as follows: (1) Control group (rats received 1mL of 10 % DMSO as vehicle orally); (2) Extract 50 group (rats received 50 mg/kg PE orally); (3) Extract 100 group (rats received 100 mg/kg PE orally); (4) Dazepam group (rats received diazepam as reference drug, 1 mg/kg intraperitoneally). Drugs and vehicle were administered every day for 30 days. In days which animals received both PTZ and drugs/vehicle, PTZ was injected 30 min after drugs/vehicle administration.

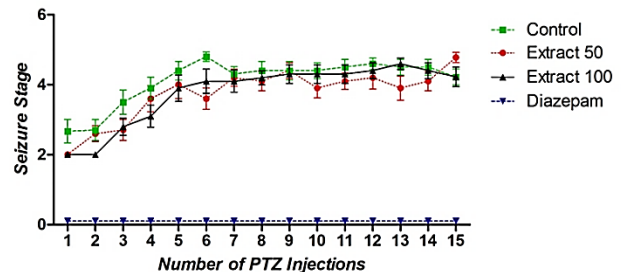
### Statistical Analysis

The results were reported as mean±standard error of the mean (SEM). The statistical analyses were performed using one-way analysis of variance (ANOVA) by SPSS (v.20). The comparison between experimental groups was performed with complementary post-hoc Tukey test and *p* values less than 0.05 were considered as significant differences.

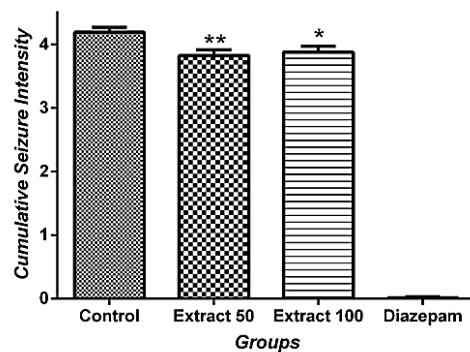
### Results and Discussion

Figure 1 illustrates the effect of PE in the PTZ model of kindling. The results showed that repeated administration of PTZ that was injected on every other day for 15 sessions (control group) gradually decreased seizure threshold, which was manifested as increased seizure scores. Moreover, administration of different doses of PE (50 and 100 mg/kg) did not elicit significant changes between rats that received DMSO and those received different doses of PE. Diazepam as control drug at the dose of 1 mg/kg completely protected animals from PTZ-induced seizures. The effects of PE on PTZ-induced seizure intensity have been presented in figure 2. In animals treated with PE (50 and 100 mg/kg), the seizure intensity was significantly decreased compared to the control group ( $p<0.01$  and  $p<0.05$ , respectively). As shown in figure 3, administration of PE at doses of 50 and 100 mg/kg produced a significant decrease in S5D time compared to the control group (all  $p<0.001$ ). The PE did not decrease seizure stage in PTZ-induced kindling. However, at the dose of 50 and 100 mg/kg produced a significant increase in S4L ( $p<0.001$ ) (figure 4). Treatment with diazepam (1

mg/kg) exhibited 100% protection from seizures in the PTZ-induced convulsion model (the measured indices have not been shown).

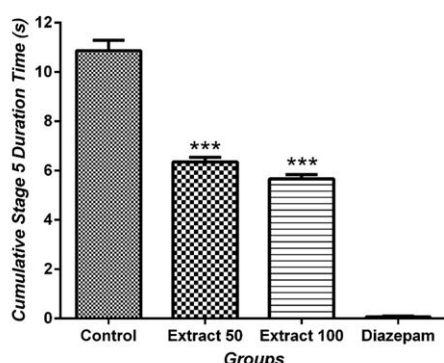


**Figure 1.** Effect of pistachio extract at the doses of 50 and 100 mg/kg on seizure intensity in seizures induced by PTZ. The extract was given orally 30 min prior to PTZ that was injected every other day (n=10 rats). Values are mean±SEM.

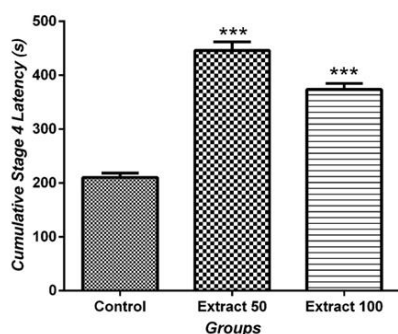


**Figure 2.** Effect of pistachio extract at the doses of 50 and 100 mg/kg on cumulative seizure intensity in seizures induced by PTZ. The extract was given orally 30 min prior to PTZ that was injected every other day and the convulsive behavior was observed for 30 min (n=10 rats). Values are mean±SEM. \* $p<0.05$  and \*\* $p<0.01$  compared with control group.

PTZ is a known chemoconvulsant model for studying the pathogenesis of refractory epilepsy and evaluation of the antiepileptic drugs. One well-accepted mechanism by which PTZ induces convulsions is by inhibiting gamma-aminobutyric acid (GABA) neurotransmission via binding to picrotoxin site of the GABA type A (GABA<sub>A</sub>) receptor and reduces chloride conductance [16]. Drugs that excite or inhibit GABA<sub>A</sub> receptor can attenuate and enhance seizures, respectively [17].



**Figure 3.** Effect of pistachio extract at the doses of 50 and 100 mg/kg on cumulative state 5 duration time in seizures induced by PTZ. The extract was given orally 30 min prior to PTZ that was injected every other day and the convulsive behavior was observed for 30 min (n=10 rats). Values are mean±SEM. \*\*\* $p$ <0.001 compared with control group.



**Figure 4.** Effect of pistachio extract at the doses of 50 and 100 mg/kg on the cumulative latency of stage 4 in seizures induced by PTZ. The extract was given orally 30 min prior to PTZ that was injected every other day and the convulsive behavior was observed for 30 min (n=10 rats). Diazepam could protect the animal from seizures in the PTZ-induced convulsion model therefore the measured indices were not shown. Values are mean±SEM. \*\*\* $p$ <0.001 compared with control group.

Drugs like diazepam and phenobarbital can prevent seizure by enhancing GABA<sub>A</sub> receptor [18]. Previous studies have used herbal extract for treating seizures, for example, *Valeriana officinalis* and *Rosa damascena* have been shown to elicit anticonvulsant effects [19,20]. It has also been reported that some species of *Pistacia* such as *P. integerrima* extracts have anticonvulsant

activity by modulation of the GABA<sub>A</sub> receptor or blocking sodium channels [13]. In the present study, we demonstrated that the pistachio extract exhibited anticonvulsant activity in PTZ-induced seizures in rats. Since PTZ-induced convulsions are related to suppression of GABA neurotransmission, pistachio extract may possibly show anticonvulsant activity by enhancing the activation of GABA<sub>A</sub> receptors or interfering with GABAergic mechanisms and facilitating the GABA-mediated opening of chloride channels. Ziaee and Hosseinzadeh reported that *P. vera* hydroalcoholic extract has antianxiolytic, muscle relaxant and hypnotic effects that may be explained by enhancing GABAergic neurotransmission. This further supports the hypothesis that PE may affect GABAergic mechanism(s) to exert its anticonvulsant activity in PTZ seizure model [21]. According to the previous study, the plant methanol extract was relatively safe (non-toxic) in mice at the doses used in this study [13]. Because the anticonvulsant activity of PE was accompanied by a decrease in S5D and an increase in S4L, it could be concluded that both partial and generalized seizure stages might have been affected by the extract [22].

The role of oxidative stress in pathophysiology of epilepsy has been well characterized. Animal studies have demonstrated that during PTZ-induced seizures, lipid peroxidation (LPO) increases, while antioxidant enzyme activities decrease. It has been reported that GABA<sub>A</sub> receptors are highly sensitive to oxidative stress and free radicals could reduce GABA<sub>A</sub> neurotransmission [23]. Some studies have shown the protective effects of antioxidants like ascorbic acid and alphanatocopherol against seizure. These suggest that free radicals may be implicated in epilepsy [24]. Also, it has been shown that free radicals can induce seizures directly by inactivating glutamate decarboxylase, thereby causing an abnormal accumulation of this excitatory neurotransmitter in the brain. Glutamate opens the NMDA receptors and permits Ca<sup>++</sup>-influx and Ca<sup>++</sup> increases nitric oxide (NO) formation via activating calmodulin [25]. Previous reports indicated that NO plays an

important role in PTZ-induced seizures [26]. Diazepam as a reference anticonvulsant drug exerts its anticonvulsant effects by enhancing GABAergic neurotransmission and inhibiting NO formation [27]. It has been well established in some studies that pistachio elicits significant antioxidant activity similar to the synthetic antioxidants [28]. A recent study in humans has shown that pistachio significantly improves oxidative status and reduced circulating inflammatory biomarkers in inflammatory bowel diseases [29]. Accordingly, it is a possibility that pistachio can decrease the seizure score through inhibition of oxidative stress.

The main active constituents of pistachio are naringenin, eriodictyol, daizein, genistein, quercetin, kaempferol, apigenin, and luteolin [30]. Some of these natural chemicals have significant protective effects in animal models of seizure. For example, in a recent study, it has been demonstrated that apigenin had anticonvulsant effects via antioxidative properties [31]. Another study revealed that terpinen-4-ol has a depressant effect on the central nervous system and significant anticonvulsant activity through the GABAergic system and decreasing the sodium current [32]. So it is inferred that apigenin and terpinen-4-ol in pistachio could be responsible for the anticonvulsant properties exhibited by this plant.

In the present study, the hydroalcoholic extract of pistachio showed anticonvulsant activity. Oral administration of hydroalcoholic extract of *P. vera* significantly reduced the seizure intensity as well as the duration of S5D and significantly increased the S4L produced by PTZ in rats. Diazepam as the reference anticonvulsant drug completely abolished the seizures and protected all the animals against PTZ-induced seizures. To the best of our knowledge, this is the first scientific report on the anticonvulsant activity of pistachio in literature. However, further studies are required to clarify the mechanism(s) and the active compound(s) involved in this effect.

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#### Declaration of interest

The authors declare that there is no conflict of interest. The authors alone are responsible for the content of the paper.

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