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Effects of Hydro-alcoholic Extract of Anethum Graveolens Seed on Pentylenetetrazol-induced Seizure in Adult Male Mice

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ABSTRACT

Introduction: Regarding chronic nature of epilepsy and its side effects and to access the effective treatment procedures, herbal medicine has received remarkable interest. The aim of this study was to determine the anticonvulsant effects of hydro-alcoholic extract of Anethum graveolens seed on pentylenetetrazol (PTZ) -induced seizure in male mice.

Methods: Fifty-six albino male mice were divided randomly into seven groups including the negative control (saline), positive control (Phenobarbital) and treatment groups using different doses of hydro-alcoholic extract of Anethum graveolens seed (50, 100, 300, 500 and 1000 mg/kg). To provoke convulsion, PTZ was injected to all groups and initiation time of myoclonic and tonic-clonic seizures as well as surveillance after 24 h were measured.

Results: The results indicated that hydro-alcoholic extract of Anethum graveolens seed (AGS) delayed the initiation time of myoclonic and tonic-clonic seizures in comparison with saline group. The latency was considerable for myoclonic and tonic-clonic seizures at all above mentioned doses of AGS extract except for the lowest one. Moreover, the protective effect of AGS extract against mortality was statistically significant at all doses except for 50 mg/kg.

Discussion: As the hydro-alcoholic extract of AGS showed an appropriate response in experimental model of convulsion, it might be considered as an adjuvant therapy with other traditional antiepileptic medications.

1. Introduction

Epilepsy is a chronic neurological disorder of the brain that affects about 70 million people worldwide (Katchanov & Birbeck, 2012). It is characterized by recurrent involuntary movement accompanied by loss of consciousness which briefly termed seizures. The episodes are a consequence of excessive electrical discharges in a group of brain cells at different regions. Seizures can vary from the briefest lapses of attention, to severe and prolonged convulsions. Although, standard therapy permits control of seizures in about 80% of these patients, millions (500,000 people in the USA alone) have uncontrolled epilepsy (Roger, Porter, Brian, & Meldrum, 2009).

Regarding chronic nature of epilepsy, resistance to chemical drugs, and lack of access to more effective treatment procedures, herbal medicine have received remarkable interest. Anethum graveolens is an annual herb of umbelliferae family which has been used traditionally to

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treat convulsion, stomach cramps and insomnia (Zargari, 1991). It's antimicrobial, anti-hyperlipidemic and antipyretic effects have also been reported (Hajhashemin & Abbasi, 2008; Mansouri et al., 2012). Additionally, the spasmolytic effect of Anethum graveolens seed (AGS) on rat uterus and ileum has been described (Gharib Naseri, Mard, & Farbod, 2005). Recently, some constituents of Anethum graveolens seed such as d-carvon, d- limonene, α- and β- pinene, linalool, and myrecene have been analyzed (Singh, Maurya, De Lampasona, & Catalan, 2005; Taher, Ghannadi, & Karimiyan, 2007). Concerning several properties of dill on human body, we studied the effects of hydro- alcoholic extract of Anethum graveolens seed on PTZ – induced seizure in male adult mice.

2. Methods

2.1. Chemicals and Plant Extraction

Pentylenetetrazol (PTZ) and Phenobarbital were purchased from Sigma-Aldrich (St. Louis, MO, USA). The seeds of Anethum graveolens were purchased from a local medicinal plants market and identified by Dr. Ali Sonboli. A voucher specimen was deposited in the herbarium of medicinal plants and drugs research institute, Shahid Beheshti University, Tehran, Iran (no. MPH-1966). After cleaning and drying the plant seeds in room temperature, the seed powder (100 g) was macerated in ethanol 80% for 24 h at dark. Then, the mixture was filtered and concentrated under reduced pressure at 40 ºC by rotary evaporator. The yield of hydro-alcoholic extract was 6.4% (g/g).

2.2. Animals

Fifty-six albino male mice weighing 20-25 g were obtained from the animal house of School of Medicine, Gilan University of Medical Sciences, Gilan, Iran. The animals were housed in the standard cages with free access to food and water. The temperature of animal house was 22 ± 2 ºc with a 12 hr light/dark cycle. The ethical guidelines for using experimental animals were followed in all tests in accordance with ethical committee acts of Gilan University of Medical Sciences. The animals were randomly divided into seven groups (n=8). All groups were injected intraperitoneally (i.p). Negative control group received normal saline (10 ml/kg). Positive control group received Phenobarbital (40 mg/kg). Experimental groups received hydro- alcoholic extract of AGS at doses of 50, 100, 300, 500 and 1000 mg/kg. All groups were injected 45 min before administration of PTZ (Dhir & Kulkarni, 2006).

2.3. Pentylenetetrazol (PTZ) -Induced Seizure

In order to induce experimental model of epilepsy, PTZ (80 mg/kg) was dissolved in normal saline (0.9%) and injected intraperitoneally 45 min after administration of saline, phenobarbital and different amounts of hydro-alcoholic extract of AGS (50, 100, 300, 500 and 1000 mg/kg). The animals were controlled after PTZ injection for 30 min. Then, the initiation time of myo-clonic and tonic-clonic seizures and the percent of mortality within 24 h were evaluated.

2.4. Statistical Analysis

Data are expressed as mean ± SEM. Statistical analysis was performed using one way analysis of variance (ANOVA) followed by Tukey's test (t-test) for multiple comparisons. In addition, Dunnett t-tests treat one group as a control, and compare all other groups with it. Protective effects of hydro-alcoholic extract of AGS against mortality after 24 h were evaluated by the Fisher's Exact test. $P < 0.05$ was statistically significant.

Table 1. Effect of saline, phenobarbital and different doses of hydro-alcoholic extract of Anethum graveolens seed on percent of mortality within 24 h in eight rats in each group

Group	Dose	Mortality within 24 h %
Saline (negative control group)	**10 (ml/kg)**	**100**
Phenobarbital (positive control group)	40 (mg/kg)	0
Anethum graveolens seeds extract	1000 (mg/kg)	25**
Anethum graveolens seed extract	500 (mg/kg)	25**
Anethum graveolens seed extract	300 (mg/kg)	25**
Anethum graveolens seed extract	100 (mg/kg)	37.5*
Anethum graveolens seed extract	50 (mg/kg)	75

*P < 0.05; ** P < 0.01 compared to control group.

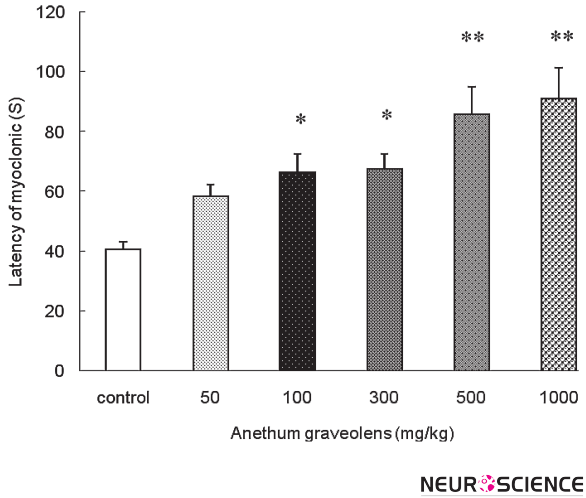

Figure 1. The effect of different doses of hydro-alcoholic extract of Anethum graveolens seed on latency of myo-clonic seizure compared to control group (saline); values are mean + SEM of eight mice in each group.

* P < 0.05 ; ** P < 0.01 compared to control group.

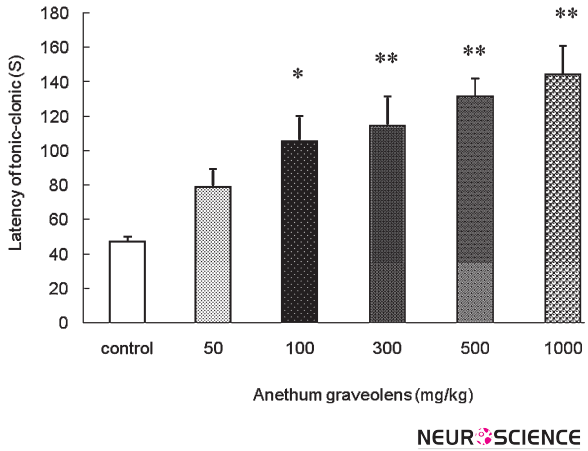

Figure 2. The effect of different doses of hydro-alcoholic extract of Anethum graveolens seed on latency of tonic-clonic seizure compared to control group (saline); values are mean + SEM of eight mice in each group

* P < 0.05; ** P < 0.01 compared to control group

3. Results

3.1. Effect of Hydro-alcoholic Extract of AGS on Latency of Myo-clonic Seizure

All animals in negative and treatment groups were shown seizure after PTZ administration. The results showed that all concentrations of extracts increased the initiation time of myo–clonic seizures in comparison with control group (normal saline). This increase was significant at doses of 1000 and 500 mg/kg ($P < 0.01$) as well as 300 and 100 mg/kg ($P < 0.05$) (Fig.1).

3.2. Effect of Hydro-alcoholic Extract of AGS on Latency of Tonic-clonic Seizure

As shown in Fig.2, all amounts of extract in comparison with saline group increased the initiation time of tonic–clonic seizures. The increment in time was significant at doses of 1000, 500 and 300 mg/kg ($P < 0.01$) and 100 mg/kg ($P < 0.05$).

3.3. Protective Effect of Hydro-alcoholic Extract of AGS against Mortality after PTZ -induced Seizure

Hydro-alcoholic extract of AGS decreased the percent of mortality within 24 h compared to control group. The protective effect against mortality (convulsion survivors/animals tested) of hydro-alcoholic fraction was 75% at a doses of 300 - 1000 mg/kg, 62.5% at a dose of 100 mg/kg and 25% at a dose of 50 mg/kg. According to table 1, this reduction was significant at doses of 300, 500 and 1000 mg/kg ($P < 0.01$) as well as 100 mg/kg ($P< 0.05$). Phenobarbital completely inhibited PTZ-induced seizures; therefore, the percent of survival of animals after 24 h was %100.

4. Discussion

In the present study, it was indicated that the hydro-alcoholic extract of AGS is effective on reduction of PTZ -induced seizure. Our finding is in agreement with Akaberi et al., study, in which they showed that aqueous extract of Anethum graveolens leaves had antagonistic effects on seizure induced by PTZ in mice (Akaberi, Mohammad-Zadeh, Mirmoosavi, Tazari, & Abarashi, 2013). Hence, the results of this study may underlie its traditional use in treatment of convulsive disorder (Zargari, 1991).

Components that influence on experimental model of tonic–clonic seizures are considered effective in controlling myo-clonic and absence seizures in humans (Nisar, Khan, Simjee, Gilani, & Obaidullah, 2008). In fact, substances that inhibit convulsions or increase the latency of PTZ-induced seizures are suggested as having anticonvulsant activity (Haruna, 2000). Therefore, demonstration of AGS activity on seizure models suggests that this herb possesses anticonvulsant potential.

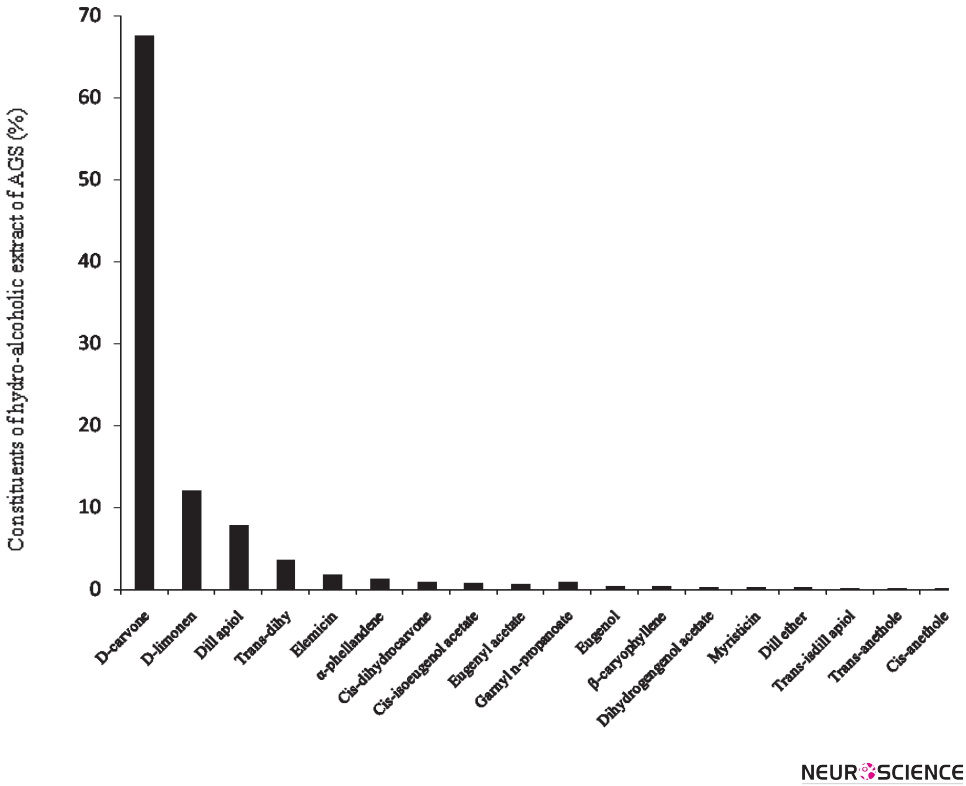

Figure 3. The constituents of hydro-alcoholic extract of Anethum graveolens seed based on GC/MS analysis (Adopted from Taher et al. 2007)

Here, we used hydro-alcoholic extract of dill to obtain more amounts of active components compared to water extract in accordance with Jana et al. results (Jana & Shekhawat, 2010). To achieve enough time for peritoneal absorption, extract administrated 45 min before induction of chemical convulsions (Dhir & Kulkarni, 2006). According to our findings, AGS antagonized PTZ effect in a dose response manner based on the increase of latency of myo-clonic and tonic-clonic seizures in comparison with control group.

According to phytochemical screening depicted in Fig.3, various components such as monoterpenes have been isolated from Anethum graveolens (Taher, Ghannadi & Karimiyan, 2007). Monoterpenes such as carvone and limonene have protective effect against PTZ-induced convulsion (Sayyah, Moaied, & Kamalinejad, 2005; De Sousa, De Faras Nobrega, & De Almedia, 2007). Flavonoids and their derivatives have been found in AGS (Gebhardt et al., 2005; Moehle, Heller, & Wellmann, 1985). Flavonoids as an important class of natural compounds exert anti-oxidant properties (Dirscherl K et al., 2010). Since, chemical seizure induced by PTZ responds to antioxidant compound; flavonoids in AGS extract may be assumed potentially involved in anti-convulsant outcome (Pages et al., 2010).

Flavonoids have several neuropharmacological activities. Some of these effects are related to γ-aminobutyric acid type A (GABA)A receptors in the central nervous system (Abbasi, Nassiri-Asl, Shafeei, & Sheikhi, 2012). As, PTZ induces convulsion by antagonizing (GABA)A receptor chloride channel complex, manipulation of GABAA receptors by flavonoids, in turn, affect on CNS activity (Naseer, Shupeng, & Kim, 2009). Linalool as a further constituent of Anethum graveolens has an inhibitory effect on glutamate binding in the rat's cerebral cortex (Elisabetsky, Marschner, & Souza, 1995). Since N-methyl-D-aspartate (NMDA) subtypes of glutamate receptors are involved in epileptic activity, blockade of this receptor may also explain anticonvulsant effects of AGS extract (Da Silva, Witter, Boeijinga, & Lohman, 1990). Hence, one of the probable explanations of dill anti-convulsant effect could be described by the existence of all above mentioned components in Anethum graveolens extract.

There is some evidence that show inhibitory effect on calcium L- type channels which are involved in convulsion (Gharib Naseri, Mard, & Farbod, 2005; Zamponi, Lory, & Prez-Reyes, 2010). This antagonistic effect may partially be explained by decreasing the seizure initiation time obtained in this study.

Regarding the protective effect of Anethum graveolens against mortality after PTZ -induced seizure, our findings indicate that all concentrations exert appropriate protection except for the attenuated one. Although, at least partly of these consequences is due to the presence of AGS components which induce suppression of nuclear transcriptional factor, but other plausible mechanisms can not be anticipated at this step (Kim, Shin, Lee, & Kim, 2012).

5. Conclusion

Here, hydro-alcoholic extract of AGS demonstrated anticonvulsant activity against PTZ-induced seizure. It might be considered as an adjuvant therapy with other traditional antiepileptic medications. Nevertheless, further studies are necessary for elucidation the involvement of probable neurotransmitter which mediated the functional mechanisms of whole extract.

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