

Anticonvulsant Activity of Hydroalcoholic *Phoenix dactylifera* Fruit Extract and *Pimpinella anisum* Oil in Mice

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Article history

Received: 15-03-2019

Revised: 25-04-2019

Accepted: 25-05-2019

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Abstract: Epilepsy is a group of long-term neurological disorders characterized by epileptic seizures. The present study sought to investigate the anticonvulsant and neuroprotective Activity of Hydroalcoholic *Phoenix dactylifera* fruit extract (HAPD) and *Pimpinella Anisum* Oil (PAO) against Pentylentetrazole (PTZ) and Maximal Electric Shock (MES) induced a seizure in mice. Mice groups were treated with HAPD1000 mg/kg, PAO 4 ml/kg. The onset of a seizure and generalized seizure were measured, followed by Gamma-Aminobutyric Acid (GABA) and antioxidant enzymes assessments. The potential of these substances to induce any neurological toxicity was also evaluated by using rotarod, forced swim test and horizontal screen test. The current study demonstrates that HAPD and PAO delay onset of the seizure and generalized seizure, also elevated GABA and antioxidant enzymes level and haven't any neurological toxicity. The current study suggests that hydroalcoholic *Phoenix dactylifera* extract and *Pimpinella anisum* oil have anticonvulsant, neuroprotective and antioxidant activity and can increase brain GABA level.

Keywords: Seizure, GABA, Pentylentetrazole, Maximal Electric Shock, *Phoenix dactylifera* Fruit Extract, *Pimpinella anisum* oil

Introduction

Epilepsy is not a concept of a single disease, but is a common endpoint of many forms of acquired brain pathology (such as tumors, infection, stroke and traumatic brain injury), or mutation of a single gene (genetic epilepsy) and it can be one component of a neurodevelopmental disorder (Mulley and Mefford, 2011).

A seizure occurrence is the main symptom of epilepsy disease that generally characterized by behavioral changes and physical features which are followed by episodes of abnormal brain electrical activity (Fisher *et al.*, 2014).

One of the key molecules that maintain the inhibitory tone and counterbalances neuronal excitation is the neurotransmitter GABA. When this balance is perturbed, seizures may arise (Treiman, 2001). The hyperexcitability of neurons and excessive production of free radicals result in oxidative damage to brain tissue which has been implicated in the pathogenesis of epilepsy (Parfenova *et al.*, 2012). A community-based study in southern France estimated that up to 22.5% of Patients with epilepsy have drug-resistant epilepsy (Picot *et al.*, 2008). Moreover,

dose-related neurotoxicity, cognitive impairment and systemic side effects are the major problems caused by Antiepileptic Drugs (AEDs) (Reynolds and Trimble, 1985). Even in drug-responsive cases, AEDs only suppress seizures without interfering with the epileptogenic disease process that converts a healthy brain into an epileptic one (Loeb, 2011). Accordingly, the discovery of a new anticonvulsant with better efficacy and fewer side effects is of paramount importance. Several plant extracts and products may be useful for the treatment of convulsions or seizures giving a promising source of new antiepileptic drugs (Sucher and Carles, 2015).

Medicinal plants are being regarded as an easily available and potent source of antioxidants as they contain a mixture of different compounds that may act individually or synergistically to cure disease and improve health (Miguel, 2010). *Phoenix dactylifera* L is one of the oldest crops native to the Middle East and North Africa. It is a monocotyledon woody lasting tree belonging to the Arecaceae family which consists of about 3000 species and 200 genera (Hazzouri *et al.*, 2015). Many studies discussed pharmacological properties of *Phoenix*

dactylifera which includes antioxidant activity (Borochove-Neori *et al.*, 2015), antitumor (Al-Sayyed *et al.*, 2014), hepatoprotective (Mallhi *et al.*, 2014), immunomodulatory (Karasawa *et al.*, 2011), antidiabetic (Khan *et al.*, 2016) and neuroprotective activity (Majid *et al.*, 2008) and anti-inflammatory effect (Boghdadi *et al.*, 2012).

Pimpinella anisum L or anise belongs to Umbelliferae family. It was first cultivated in the eastern Mediterranean region and south-west Asia (Shojaii and Fard, 2012), but was brought to Europe for its medicinal value. There are plenty of published researches describing medicinal and pharmacological activities of *Pimpinella anisum* such as antibacterial (Al-Bayati, 2008), antioxidant (Iyer and Andallu, 2015), analgesic/anti-inflammatory (Tas *et al.*, 2006), muscle relaxant (Tirapelli *et al.*, 2007), nephroprotective (Aiswarya *et al.*, 2018), hepatoprotective (Jamshidzadeh *et al.*, 2015) and spasmolytic effect (Saini *et al.*, 2014).

The Antiepileptic Drugs (AEDs) associated with undesirable side effects which often render treatment difficult; in addition to at least 22.5% of patients are drug resistant (Picot *et al.*, 2008). So that searching for new anticonvulsants is vital. Medicinal and aromatic plants have many constituents which have anticonvulsant action and neuroprotective effects without side effects of chemical drugs. Therefore, the current experiments were conducted to investigate the anticonvulsant and neuroprotective effects of *Phoenix dactylifera* extract and *Pimpinella anisum* against experimentally induced convulsions in mice.

Materials and Methods

Materials

Phoenix dactylifera L. dried fruit was collected in October from a local market in Sharkia province, Egypt. The fruit was separated from seeds, cut into small pieces and ground. Hydroalcoholic *Phoenix dactylifera* (HAPD) extract was prepared by macerating 500 gm. of *Phoenix dactylifera* fruit in 2 L of ethanol-water (70/30, v/v) for 48hr at room temperature with frequent shaking, then filtered and the extract was concentrated under reduced pressure on Rotary evaporator until a semisolid residue was obtained (Ragab *et al.*, 2013). Sterilized distilled water was used as a solvent to 100 mg/mL concentration.

Pimpinella Anisum Oil (PAO) the oil was purchased from a commercial source, Cairo, Egypt; the oil was pale yellow liquid with licorice odor. By High-Performance Liquid Chromatography (HPLC) analysis of *Pimpinella anisum* oil revealed that trans-anethole (90%) was the main compound of the oil, γ -himachalene (2-4%), p-anisaldehyde (1%), methyl chavicol (0.9-1.5%),

cispseudoisoeugenyl-2-methyl butyrate (3%) and t pseudoisoeugenyl-2-methyl butyrate (1.3%).

The plant materials identified and authenticated by the department of pharmacognocny, faculty of pharmacy, Zagazig University.

Drugs and Chemicals

- Pentylenetetrazole (PTZ) was purchased from (Sigma-Aldrich co. R07AB03) was dissolved in sterilized distilled water to 8.5mg/ml
- Sodium valproate (Depakine 200 mg®) was purchased from (Sanofi co. N03AG01) dissolved in sterilized distilled water to 20 mg/ml
- Phenytoin sodium (Phenylin 100 mg®) was purchased from (Nile co. N03AB02) dissolved in sterilized distilled water to 10 mg/ml

All other chemicals were purchased from standard commercial suppliers and were of analytical grade. All solutions were prepared immediately before use.

Experimental Animals

Seventy male albino mice of 20-30 gm average body weight of each were obtained from the faculty of Veterinary Medicine farm; Zagazig University. The animals were randomly housed in appropriate cages at 22±2°C on a 12 h light/dark cycle with adequate food and water ad-labium.

The experimental protocol was carried out according to the guidelines approved with the Ethical Committee of Zagazig University (No. ZU-IACUC/2/S/18/2018)

Experimental Protocols

Anticonvulsant Activity of *Phoenix dactylifera* and *Pimpinella anisum* oil against Pentylenetetrazole induced convulsion in mice

For pentylenetetrazole (PTZ) induced convulsions all protective treatments given for 7days and at the end of treatment, PTZ (85 mg/kg) (Al-Taher, 2008), injected I/p one shot. 25 mice were divided into 5 equal groups.

Negative control Group (1): administered distilled water 0.2 ml per os for 7 days. Positive control Group (2): administered distilled water 0.2 ml per os for 7 days + PTZ (85 mg/kg). *Phoenix dactylifera* Group (3): mice administered *Phoenix dactylifera* hydro alcoholic extract 1000 mg/kg per os for 7 days (Agbon *et al.*, 2014) + PTZ. *Pimpinella anisum* oil Group (3): mice administered *Pimpinella anisum* oil (PAO) 4 ml/kg per os + PTZ (Karimzadeh *et al.*, 2012). Sodium valproate Group (4): mice administered Sodium Valproate (SV) 100 mg/kg per os for 7 days (Ilhan *et al.*, 2005)+PTZ.

The mice were observed for 1 hour after PTZ injection and onset of the seizure (sec) and generalized seizure (sec) were recorded. After 1 hour of PTZ injection, mice were sacrificed by decapitation and the brains were removed and dissected on an ice-cold surface.

Tissue preparation for biochemical measurements: the brain was taken and washed out in Phosphate Buffer Saline (PBS), pH 7.4. One gram of the brain was homogenated in 5-10 ml of cold buffer (50 mM Tris-HCl).

The homogenate was centrifuged at 10000 rpm or 15 min at 4°C. The supernatant was removed for biochemical assay.

Biochemical Measurements

GABA measurement by using a double antibody sandwich ELISA test according to the published report (Ben-Ari *et al.*, 2007). Glutathione Peroxidase (GPX) was assayed as reported (Paglia and Valentine, 1967). In this assay oxidized glutathione (GSSG), produced upon reduction of an organic peroxide by GPx, is recycled to its reduced state by the enzyme Glutathione Reductase (GR). The oxidation of reduced Nicotinamide Adenine Dinucleotide Phosphate (NADPH) to Nicotinamide Adenine Dinucleotide Phosphate (NADP⁺) is accompanied by a decrease in absorbance at 340 nm.

Superoxide Dismutase (SOD) was determined according to method (Nishikimi *et al.*, 1972) and the results were expressed as USOD/mg of protein. One unit of SOD is defined as the amount of enzyme that inhibits the rate of adrenochrome formation in 50%. Malondialdehyde (MDA) as lipid peroxidation marker was assessed as reported (Ohkawa *et al.*, 1979), which is based on the reaction with thiobarbituric acid reactive species (TBARS) during an acid-heating reaction. The absorbance of the product is measured at 534 nm.

Histopathological examination: Parts of the brains were kept in 10% formalin for at least 72 h and then processed for histopathological studies.

Anticonvulsant Activity of Phoenix dactylifera Extract and Pimpinella anisum oil against Maximal Electric Shock in Mice

About 20 mice were divided into 4 equal groups.

Group (1): mice received distilled water 0.2 ml per os for 7 days. Group (2): mice received *Phoenix dactylifera* hydroalcoholic extract 1000 mg/kg per os for 7 days. Group (3): mice received *Pimpinella Anisum* Oil (PAO) 4 ml/kg per os. Group (4): mice received phenytoin sodium 25 mg/kg orally for 7 days per os (Jaykare *et al.*, 2013).

At the 7th day, mice received an electrical stimulus with intensity (150 mA, 50-60 Hz, 0.2-second duration) after administration of all drugs by 1 hour through mouth electrode to induce hind limb tonic extension. Mice that didn't exhibit hind limb tonic extension considered protected (Toman *et al.*, 1946).

Neurotoxicity Tests Application (Rotarod Test, Forced Swim Test, Horizontal Screen Test)

There were 25 mice divided equally into 5 groups.

Group (1): mice received distilled water 0.2 ml p. o for 7 days. Group (2): mice received *Phoenix dactylifera* Hydro alcoholic extract 1000 mg/kg per os for 7 days. Group (3): mice received *Pimpinella anisum* oil 4 ml/kg per os. Group (4): received sodium valproate 100 mg/kg per os for 7 days. Group (5): received phenytoin sodium 25 mg /kg per os for 7 days. At the 7th-day neurotoxicity tests applied:

- *Rotarod test*: For the evaluation of motor coordination rotarod test was performed. Mice were walked on accelerating rotarod, which revolves 5-10 rpm for a period of 120 s. The diameter of the rod was 3.2 cm. Normal mice are able to maintain balance for many minutes, but mice with deficits in motor coordination fall more quickly (Dunham and Miya, 1957).
- *Forced swim test*: According to the method of (Porsolt *et al.*, 1977), forced swim test was performed. Mice were placed in a chamber (Diameter: 45 cm: Height: 20 cm) containing water with a height of 15 cm at 25±2°C. The immobility time through 5 min of the test was measured. Immobility was allocated when no additional activity was observed further than that required to keep the mouse's head over the water.
- *Horizontal screen test*: Neural impairment was also assessed using horizontal screen test. A 13×14 cm square wire screens apparatus mounted horizontally on a steel rod was used.

Mice were held individually on the top of wire screen when the screen inverted through an arc of 180°C, mice unable to climb to an upright position within 2 min were rated as failures (Coughenour *et al.*, 1977).

Statistical Analysis

Data analysis was done using SPSS and the data were expressed as mean ± SE. One way ANOVA test was used for comparison between different groups for parametric data. The p-value ≤0.05 was considered statistically significant.

Results

The Effect of the Hydro-Alcoholic Extract of Phoenix dactylifera and Pimpinella anisum Oil on PTZ-Induced Seizures

Administration of 85 mg/kg of PTZ induced a tonic-clonic seizure in mice. For PTZ group the onset of convulsions was (59.5±0.65 sec). Administration of HAPD extract, PAO and sodium valproate orally significantly

delayed the time of onset of convulsions 98 ± 1.1 , 122.5 ± 1.04 and 119.5 ± 0.64 sec, respectively compared with 59.5 ± 0.65 sec for PTZ group.

In addition, to delay generalized convulsion, onset HAPD extract, PAO and sodium valproate were 253.5 ± 2.02 , 306.5 ± 1.26 and 302 ± 0.91 sec, respectively. These findings were significant if compared with PTZ group for 174.75 ± 2.02 sec. PAO was significantly better than sodium valproate in delaying convulsions (Fig. 1).

Effect on Gamma-Aminobutyric Acid (GABA) in the Brain

Administration of PTZ showed significant ($p < 0.05$) decrease in GABA level in the brain (172.3 ± 10.71 $\mu\text{g/g}$) compared with control group (577.28 ± 47.1 $\mu\text{g/g}$).

Pre-treatment with HAPD extract, PAO and sodium valproate for 1 week revealed significant ($p < 0.05$) increase in GABA level 291.13 ± 16.26 , 413.81 ± 9.37 and 430.19 ± 40.51 $\mu\text{g/g}$, respectively if compared with 172.3 ± 10.71 $\mu\text{g/g}$ for PTZ treated group. PAO significantly elevated GABA level similar to sodium valproate (Fig. 2a).

Effect on Brain Malondialdehyde (MDA) as a Biomarker for Lipid Peroxidation

Administration of PTZ showed significant ($p < 0.05$) increase in brain MDA activity (39.35 ± 0.22 n mol/g) compared with (6.5 ± 0.36 n mol/g) for the control group.

Administration of HAPD extract, PAO and sodium valproate orally for 1 week revealed significant decrease in MDA activity 17.62 ± 0.39 , 14.49 ± 0.26 , 26.84 ± 0.39 n mol/g, respectively, if compared to 39.35 ± 0.39 n mol/g for PTZ group (Fig. 2b).

Effect on Brain Superoxide Dismutase (SOD)

Administration of PTZ (85 mg/kg I/p) Showed Significant Decrease in SOD brain level (4.33 ± 0.19 U/mg) compared with (23.49 ± 0.66 U/mg) for control group. Administration of HAPD extract, PAO orally and sodium valproate orally for 1 week revealed significant increase in SOD brain level 8.96 ± 0.16 , 10.21 ± 0.18 , 5.05 ± 0.04 U/mg, respectively, if compared to 4.33 ± 0.34 U/mg for PTZ group (Fig. 2b).

Effect on Brain Glutathione Peroxidase (GPx) Level

Administration of PTZ showed significant decrease in GPX brain level (10.97 ± 0.27 U/g) compared with (33.23 ± 0.66 U/g) for control group. Administration of HAPD extract, PAO orally and sodium valproate orally for 1 week revealed significant increase in GPX level in brain 19.25 ± 0.21 , 20.71 ± 0.46 , 14.01 ± 0.37 U/g, respectively, if compared with 10.97 ± 0.27 U/g for PTZ group (Fig. 2b).

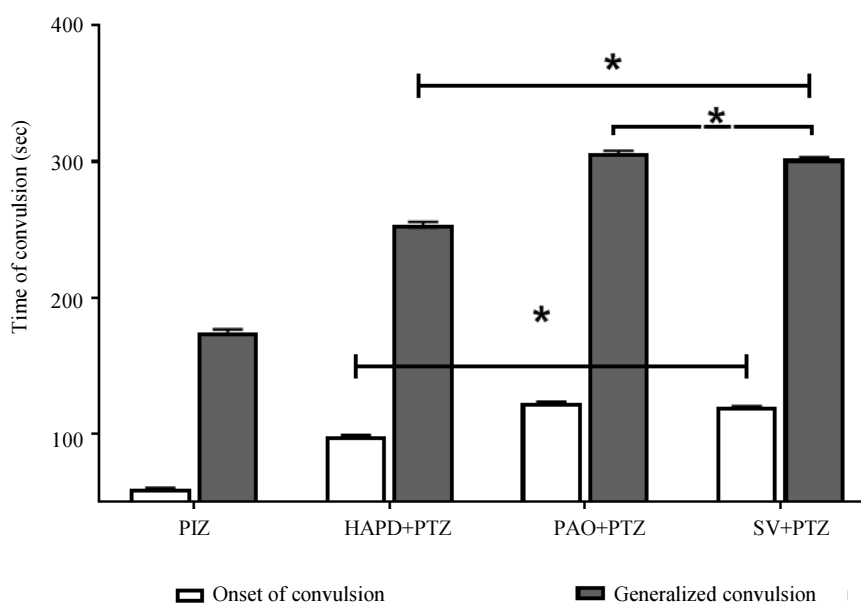


Fig. 1: Effect of HAPD (1000mg/kg), PAO (4ml/kg) orally on delaying onset time of convulsion and generalized time of convulsion measured by seconds after PTZ (85 mg/kg i/p) using sodium valproate (100 mg/kg) orally as standard (Mean \pm SE)(n=5). The values represent the average from five independent experiments. HAPD extract, PAO and sodium valproate treated group were significantly delayed the time of onset of convulsions and generalized convulsions compared with PTZ group ($*p < 0.05$). PAO was significantly effective than sodium valproate in delaying convulsions ($*p < 0.05$)

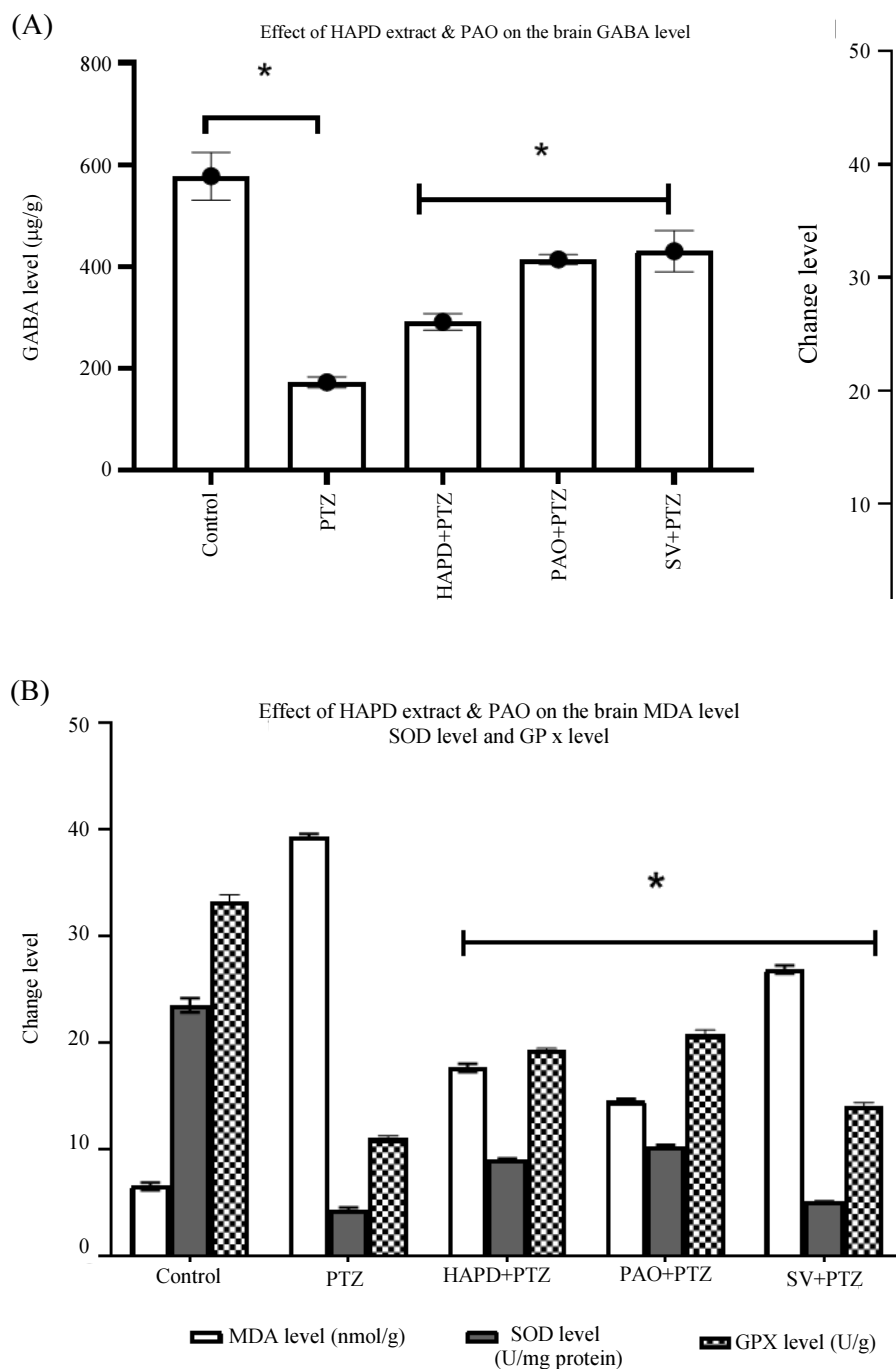


Fig. 2: Effect of HAPD extract(1000 mg/kg) and PAO(4 ml/kg) orally on brain GABA level (A),and MDA level, SOD level, GPx level (B) after administration of PTZ(85 mg/kg i/p) using SV 100 mg/kg orally as standard (Mean ± SE) (n=5) The values represent the average from five independent experiments. (a) PTZ treated group significant decrease in GABA level in the brain compared with control group (*p<0.05). Pre-treatment with HAPD extract, PAO and sodium valproate treated group revealed significant increase in GABA level compared with PTZ treated group(*p<0.05). (b) Administration of HAPD extract, PAO and sodium valproate revealed significant difference in MDA activity, SOD brain level and GPx brain level compared with PTZ group (*p<0.05)

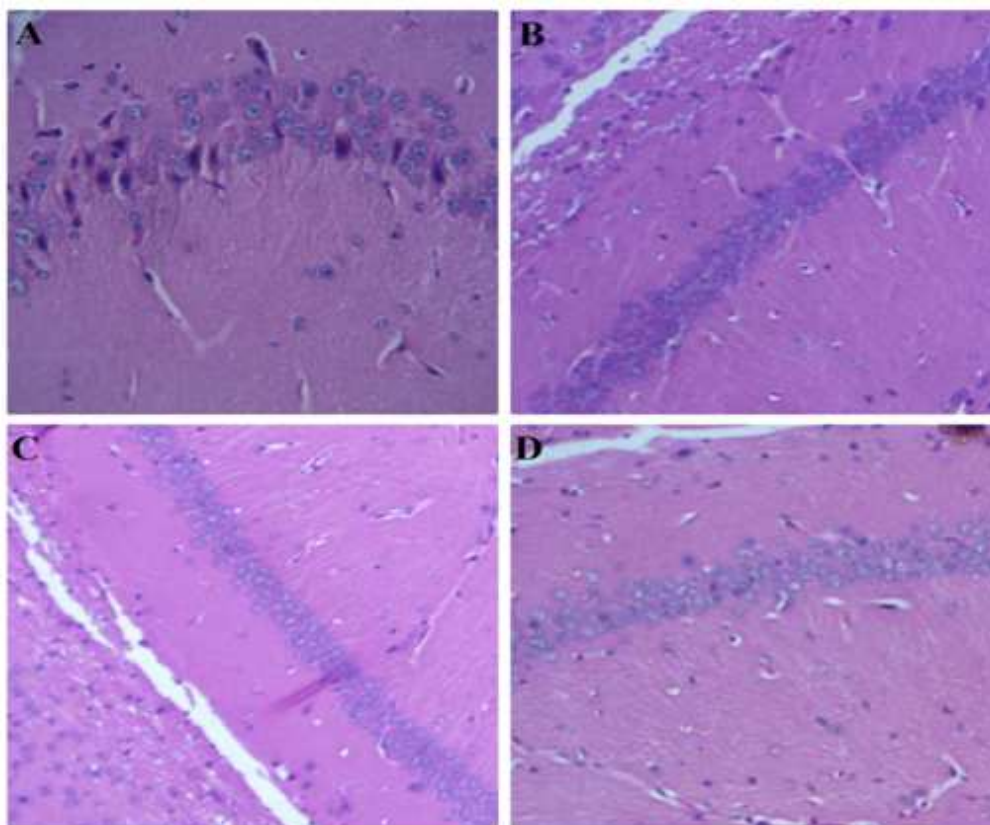


Fig. 3: Histopathological examination of the brain tissues showing the protective effect of *Phoenix dactylifera* *Pimpinella anisum* against degenerative or necrotic changes A) H&E stained mice brain (hippocampus slices); pentylenetetrazole group showed dark neurons among the hippocampus cells represented about 15-20%. B) *Phoenix dactylifera* group showed normal hippocampus. (C) *Pimpinella anisum* group showed few number of hippocampus cells was degenerated about 0.1%. D) Sodium valproate group apparently revealed normal hippocampus.

Histopathological Picture

They were displayed in (Fig. 3A). The seizures induced by injection of PTZ increased the numerical density of dark neurons which were peculiar among the hippocampus cells and represented about 15-20%. Pretreatment with *Phoenix dactylifera* protected hippocampal cells from any degenerative or necrotic changes could be observed (Fig. 3B).

It was observed that a few numbers of hippocampus cells have degenerated (darkly appeared) 0.1% in *Pimpinella anisum* oil treated group (Fig. 3C). Differently, sodium valproate group revealed normal hippocampus cells apparently (Fig. 3D).

Anticonvulsant Activity of *Phoenix dactylifera* Extract and *Pimpinella anisum* oil against Maximal Electric Shock in Mice

After the administration of hydroalcoholic *Phoenix dactylifera* extract 1000 mg/kg p. o, or *Pimpinella anisum*

oil 4 ml/kg p. o or phenytoin sodium 25 mg/kg p. o, the maximal electric shock applied to mice. The animals that didn't exhibit hind limb tonic extension were considered protected. The results of HAPD extract was similar to phenytoin sodium in the protection of mice from maximal electric shock by 80% of mice (Fig. 4).

PAO wasn't able to protect all mice from maximal electric shock as phenytoin sodium and protected only 40% of mice (Fig. 4)

Neurotoxicity Tests Application (Rotarod Test, Forced Swim Test, Horizontal Screen Test)

Rotarod test: Administration of HAPD (1000 mg/kg), PAO (4 ml/kg) and sodium valproate (100 mg/kg bwt) orally for 1 week caused no significant effect on the latency to fall off the rotarod compared to the control 175.25±0.85, 174.5±1.04 and 166.25±0.85 sec, respectively. Control gave a value of 175±2.04sec (Fig. 5a).

Phenytoin sodium 25 mg/kg significantly decreased the latency to fall off the rotating rod 61.5±1.55 sec compared with control one 175±2.04 sec (Fig. 5a).

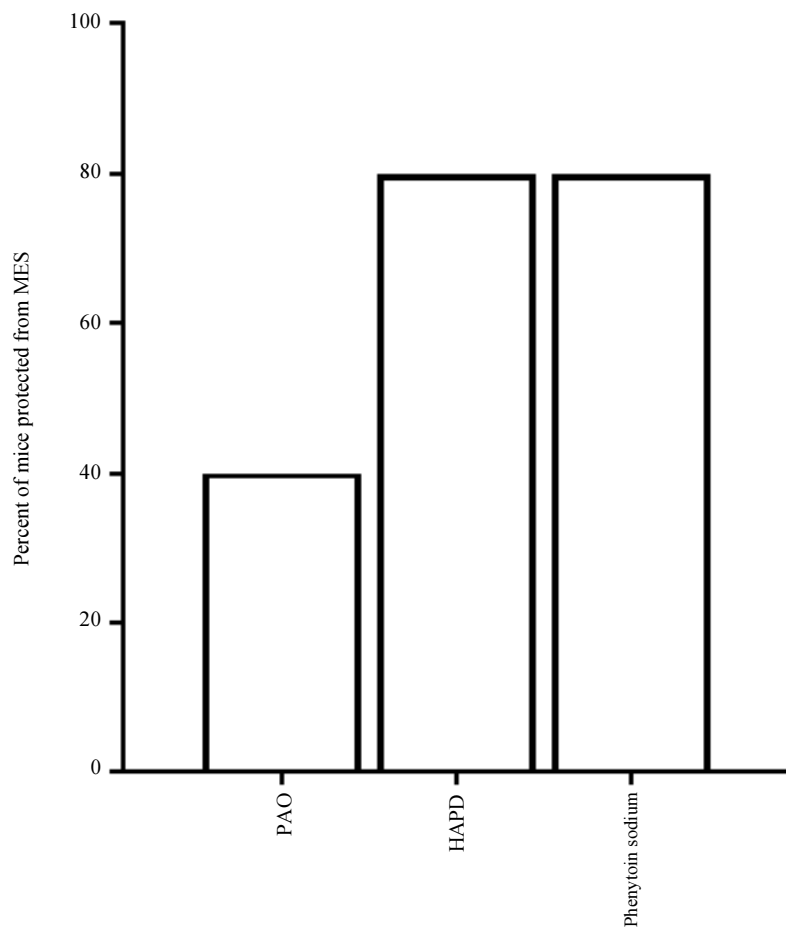
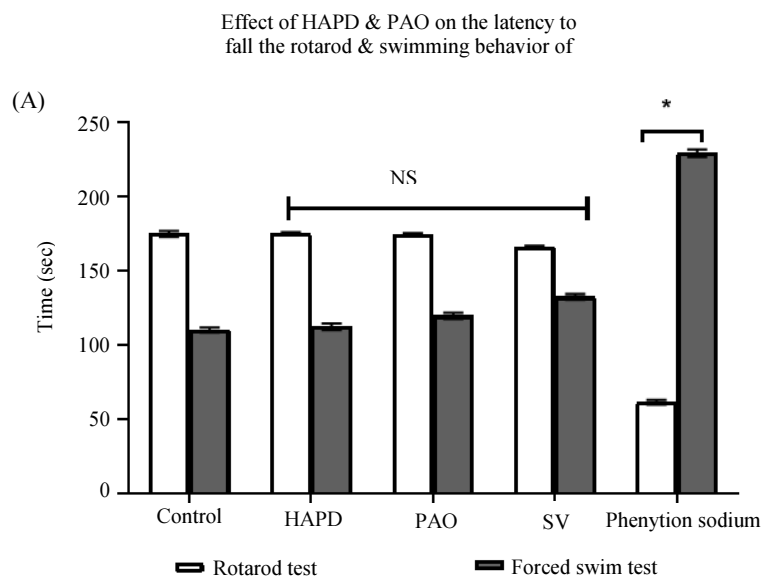


Fig. 4: Effect of HAPD extract 1000 mg/kg bwt per os and PAO 4ml/kg bwt per os for one week. Then maximal electric shock applied. Phenytoin sodium 25 mg/kg bwt per os was used as standard (n=5)



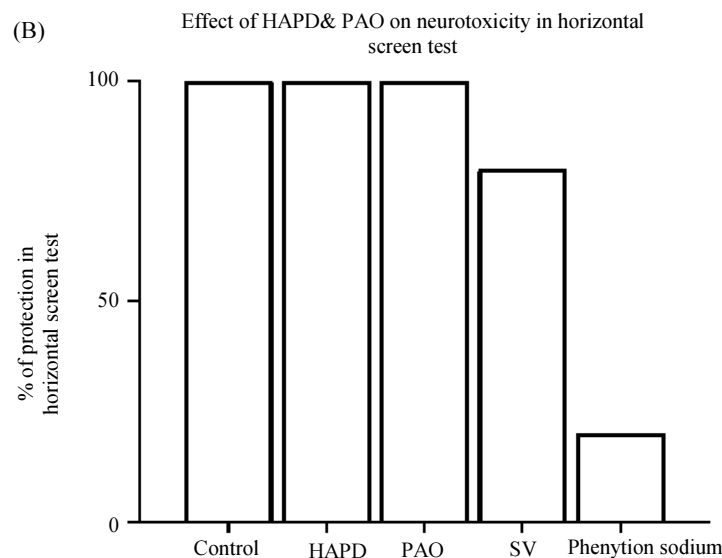


Fig. 5: Administration of HAPD and PAO showing neglected neurotoxicity effect on mice. (A) Effect of HAPD (1000 mg/kg), PAO (4ml/kg), sodium valproate 100 mg/kg, phenytoin sodium 25 mg/kg orally for one week on rotarod test and forced swim test. The values represent the average from five independent experiments (n=5). Administration of HAPD, PAO and sodium valproate caused no significant difference on the latency to fall off the rotarod and swimming behavior of mice compared to the control group (NS= non significant = $p>0.05$). Phenyton sodium significantly decreased the latency to fall off the rotating rod and swimming activity in mice compared with control group ($*p<0.05$). (B) Effect of HAPD (1000 mg/kg), PAO (4ml/kg), sodium valproate 100 mg/kg, phenytoin sodium 25 mg/kg orally for one week on horizontal screen test the values represent the average from five independent experiments (n=5)

Forced swim test: Administration of HAPD extract (1000 mg/kg bwt), PAO (4 ml/kg bwt) and sodium valproate (100 mg/kg bwt) orally for one week caused no significant effect on swimming behavior of mice and didn't affect the immobility time of mice 112.5 ± 2.17 , 119.75 ± 2.17 , 132.5 ± 1.96 sec, respectively, if compared to control 110.25 ± 1.65 sec.

Phenyton sodium 25mg/kg bwt significantly decreased swimming activity in mice and decreased immobility time of 229.75 ± 2.48 sec compared with 110.25 ± 1.65 sec for the control group (Fig. 5a).

Horizontal screen test: Pretreatment with HAPD extract (1000 mg/kg bwt), PAO (4 ml/kg) and sodium valproate (100 mg/kg) orally for 7days haven't any neurotoxicity effect on mice.

Phenyton sodium 25 mg/kg, revealed its neurotoxic effect on mice and all mice can't pass the horizontal screen test and fallen down compared to other groups (Fig. 5b).

Discussion

Epilepsy is a prevalent and serious neurological disorder which involves the occurrence of at least one or more epileptic seizures. Worldwide, it affects not less than 50 million people (Behr *et al.*, 2016).

The present study showed that PTZ (85mg/kg) induced onset of convulsion within less than 1minute and strong

myoclonic convulsions in line with Deyn and Macdonald (1989) PTZ causes neuron depolarization by increase calcium and sodium influx which produces muscle contraction (Papp *et al.*, 1987).

The present study showed that pretreatment with HAPD extract (1000 mg/kg) delayed onset time of convulsion, delayed time of generalized convulsions and decreased the strength of myoclonic convulsions. These effects could be due to the involvement of the GAB Aergic pathways (as mechanism of action of *Phoenix dactylifera* effect) as anticonvulsant as reported (Al-Taher, 2008).

The present study revealed that administration of PAO (4 ml/kg) significantly increased the delay of the onset of a seizure in mice and prevented tonic convulsions induced by I.p. injection of PTZ to 20% of mice. An earlier study (Pourgholami *et al.*, 1999) found that *Pimpinella Anisum* oil I/p block tonic convulsions induced by PTZ thanks to the enhancement of the activity of the Na^+-K^+ AT Pase Pump.

The natural defense system provided by several enzymes such as Superoxide Dismutase (SOD) and Glutathione Peroxidase (GPX) play a vital role for detoxification of free radicals. Many studies demonstrated that oxidative stress plays an important role in the pathogenesis of epileptic seizures (Golechha *et al.*, 2010). Many reports showed that animal models of

epilepsy have a high level of MDA and low SOD and GPX levels in brain tissues due to lipid peroxidation (Xie *et al.*, 2012). The results of the present study showed that PTZ (85 mg/kg I/p) caused a significant decrease in the activities of brain Glutathione Peroxidase (GPX) and Superoxide Dismutase (SOD) as well as significant increase in the activities of brain MDA as an end product of lipid peroxidation.

Phoenix dactylifera fruit showed high antioxidant activity, reducing power, free radical scavenging activity which was attributed to its phytoconstituents (flavonoids, phenolics, carotenoids, vitamins) (Vyawahare *et al.*, 2009). The findings of our study demonstrated that HAPD has antioxidant action significant raised GPX and SOD levels and decreased MDA levels compared with PTZ and sodium valproate group.

Pimpinella anisum oil is strong antioxidant according to study (Singh *et al.*, 2008). The results of this study appeared to increase levels of GPX and SOD and to decrease MDA levels regarding PTZ and sodium valproate groups.

Low GABA level in the brain leads to neurons firing and generation of convulsions (Watanabe *et al.*, 2002). In the present study, GABA levels were significantly inhibited by PTZ which is a non-competitive GABA antagonist of the gamma-aminobutyric acid (GABA) A receptor complex (Ramanjaneyulu and Ticku, 1984).

HAPD 1000 mg/kg for 1week significantly elevated GABA compared with PTZ group, PTZ elicited a significant decrease in GABA brain level compared with the control group. Another study revealed that daily oral administration of pits of date palm evoked a significant decrease in the side effect of methylprednisolone on some neurotransmitter content in the brain and a significant increase in neurotransmitter contents Norepinephrine (NE), Dopamine (DA) and GABA (Bawazir and Saddiq, 2010).

Indeed, results indicated that *Pimpinella anisum* oil 4 ml/kg administered orally for 1week elicited a significant increase in GABA level.

The same result was obtained with sodium valproate. This finding is in line with data (Sahraei *et al.*, 2002) who reported that anise oil exerts its effect on opioid receptors via activation of GABAA receptors in mice.

It has also been evidenced that recurrent and even a single convulsive seizure induced by such as pentylene-tetrazole (gamma-aminobutyric acid inhibitor) lead to neuronal damage and cell death in form of appearance of dark neurons in different areas of the brain including the pyramidal layer of cortex, reticular formation, hippocampus and limbic system (Bideskan *et al.*, 2015).

The present research found that pentylene-tetrazole (85 mg/kg I/p) induced dark neuron formations which were peculiar among the hippocampus cells and represented about 15-20%. Protection with HAPD (1000

mg/kg) prevented dark neuron generation in the hippocampus. The neuroprotective effect of *Phoenix dactylifera* was earlier reported (Majid *et al.*, 2008), it showing its tendency to reduce brain damage and prevent neurological deficits.

Pimpinella anisum oil (4 ml/kg) gave protection for the brain and hippocampal cells against degeneration and formation of dark neurons except for a few numbers of hippocampus cells (0.1%). This finding was supported (Karimzadeh *et al.*, 2012) that PAO prevented the production of dark neurons and acting as a neuroprotective substance. Mice received an electrical stimulus of sufficient intensity to induce maximal seizures of their hind limbs, with tonic extension as the endpoint of the test (Holmes, 2007). Our results clearly demonstrated the ability of HAPD1000mg/kg to prevent hind limb extension due to MES probably by prolonging the inactivation of sodium channels (Holmes, 2007).

Pimpinella anisum oil 4ml/kg was able to prevent hind limb extension for only 40% for mice compared with 80% for *Phoenix dactylifera* and phenytoin sodium groups. Neurotoxicity from anticonvulsant compounds in mice manifested by signs of neurological deficits such as sedation, hypo locomotion, ataxia, abnormal gait and cognitive deficits (Loscher and Schmidt, 1988).

Phoenix dactylifera hydroalcoholic extract had no neurotoxic effect on mice and didn't induce any disturbance in motor coordination. No changes in swimming behavior were observed in line with a former study (Vyawahare *et al.*, 2009) showing that *Phoenix dactylifera* extract did not demonstrate any effect on muscle coordination.

Pimpinella anisum oil had relaxant muscle effect and might cause motor impairment for mice (Tirapelli *et al.*, 2007), but the dose that used as anticonvulsant didn't cause any motor disturbance has appeared in the present study (Pourgholami *et al.*, 1999). In this study, we successfully identify *Phoenix dactylifera* and *Pimpinella anisum* oil has anticonvulsant and antioxidant activities. Both of them had no neurotoxic effect on mice and didn't induce any disturbance in motor coordination. Further studies should be considered to validate this strategy by fractionation of the *Phoenix dactylifera* and *Pimpinella anisum* oil to know the active constituents of these herbal extracts for rapid identification of exact mode of action. A combined effect of the *Phoenix dactylifera* and *Pimpinella anisum* oil should be included in the further studies to have the exact evidence of the synergism between these herbal extract in treatment of epilepsy

Conclusion

Phoenix dactylifera and *Pimpinella anisum* oil have anticonvulsant and antioxidant activity. Both of them have neural central properties to increase GABA level as well as neuroprotective effect.

Acknowledgment

The authors would like to thank Dr. Sayed Elattar for helping in the histopathological study and Dr. Wafaa Abdou Mohammed for helping in biochemical tests.

Funding Information

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Author's Contributions

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Conflict of Interest

The authors declare that they have no conflict of interests.

References

- Agbon, A.N., S.D. Ingbian and A.U. Dahiru, 2014. Preliminary histological and histochemical studies on the neuroprotective effect of aqueous fruit extract of *Phoenix dactylifera* L. (Date Palm) on atesunate-induced cerebellar damage in wistar rats. *Sub-Saharan African J. Medicine*, 1: 204. DOI: 10.4103/2384-5147.144744
- Aiswarya, N., V. Chandran, S. Teerthanath and K.B. Rakesh, 2018. Nephroprotective effect of aqueous extract of *pimpinella anisum* in gentamicin induced nephrotoxicity in wistar rats. *Pharmacognosy, Jo.* DOI: 10.105530/pj.2018.3.66
- Al-Bayati, A.F., 2008. Synergistic antibacterial activity between *thymus vulgaris* and *pimpinella anisum* essential oils and methanol extracts. *J. Ethnopharmacology*, 116: 403-406. DOI: 10.1016/j.jep.2007.12.003
- Al-Sayyed, H.F., H.R. Takruri and M.S. Shomaf, 2014. The effect of date palm fruit (*Phoenix dactylifera* L.) on 7, 12-Dimethylbenz (α) Anthracene (DMBA)-induced mammary cancer in rats. *Res. Opinions Anim. Vet. Sci.*, 4: 1-11.
- Al-Taher, Y.A., 2008. Anticonvulsant effects of 3, 4-Dimethoxy toluene, the major constituent of *Phoenix dactylifera* L Spathe in mice. *Scientific J. King Faisal University Basic Applied Sci.*, 9: 115-23.
- Bawazir, A.E and A.A. Saddiq, 2010. Antimicrobial activity of date palm (*Phoenix dactylifera*) pits extracts and its role in reducing the side effect of methyl prednisolone on some neurotransmitter content in the brain, hormone testosterone in adulthood. IV International Date Palm Conference, 882: 665-690. DOI: 10.17660/ActaHortic.2010.882.74
- Behr, C., M.A. Goltzene, G. Kosmalski, E. Hirsch and P. Rylvlin, 2016. Epidemiology of epilepsy. *Revue Neurologique*, 172: 27-36. DOI: 10.1016/j.neurol.2015.11.003
- Bideskan, A.R.E., M.L. Ataei, S. Mansouri and M. Hosseini, 2015. The effects of tamoxifen and soy on dark neuron production in hippocampal formation after pentylenetetrazole-induced repeated seizures in rats. *Pathophysiology*, 22: 125-135. DOI: 10.1016/j.pathophys.2015.04.001
- Boghdadi, G., A. Marei, A. Ali G. Lotfy and M. Abdulfattah, 2012. Immunological markers in allergic rhinitis patients treated with date palm immunotherapy. *Inflammation Res.*, 61: 719-724. DOI: 10.1007/s00011-012-0464-4
- Borochoy-Neori, H., S. Judeinstein, A. Greenberg, N. Volkova and M. Rosenblat, 2015. Antioxidant and antiatherogenic properties of phenolic acid and flavonol fractions of fruits of 'Amari' and 'Hallawi' date (*Phoenix dactylifera* L.) varieties. *J. Agric. and Food Chem.*, 63: 3189-3195. DOI: 10.1021/jf506094r
- Coughenour, L.L., J.R. McLean and R.B. Parker, 1977. A new device for the rapid measurement of impaired motor function in mice. *Pharmacology Biochemistry and Behavior*, 6: 351-353. DOI: 10.1016/0091-3057(77)90036-3
- Deyn, P.P.D and R.L. Macdonald, 1989. Effects of antiepileptic drugs on GABA responses and on reduction of GABA responses by PTZ and DMCM on mouse neurons in cell culture. *Epilepsia*, 30: 17-25. DOI:10.1111/j.1528-1157.1989.tb05275
- Dunham, N.W and T.S. Miya, 1957. A note on a simple apparatus for detecting neurological deficit in rats and mice. *J. Pharmaceutical Sci.*, 46: 208-209. DOI: 10.1002/jps.3030460322
- Fisher, R.S., C. Acevedo, A. Arzimanoglou, A. Bogacz and J.H. Cross, 2014. ILAE official report: A practical clinical definition of epilepsy. *Epilepsia*. DOI: 475-482 10.1111/epi.12550.
- Golechha, M., J. Bhatia and D.S. Arya, 2010. Hydroalcoholic extract of *emblica officinalis* Gaertn. Affords protection against PTZ-induced seizures, oxidative stress and cognitive impairment in rats. *Indian. J. Exp Biol.*, 48:474-478.

- Hazzouri, K.M., J. Flowers, H.J. Visser, H.S. Khierallah, U. Rosas and 2015. Whole genome re-sequencing of date palms yields insights into diversification of a fruit tree crop. *Nature Communication*. DOI: 10.1038/ncomms9824
- Holmes, G.L., 2007. Animal model studies application to human patients. *Neurology*, 69: S28-S32. DOI: 10.1212/01.WNL.0000302369.24230.C6
- Ilhan, A., A. Gurel, F. Armutcu, S. Kamisli and M. Iraz, 2005. Antiepileptogenic and antioxidant effects of nigella sativa oil against pentylenetetrazol-induced kindling in mice. *Neuropharmacology*, 49: 456-464. DOI: 10.1016/j.neuropharm.2005.04.004
- Iyer, S.R and B. Andallu, 2015. Radical scavenging and antiperoxidative efficacy of aniseeds (*Pimpinella anisum* L.): Identification of phytochemicals. *Pharmaceutical Bio. Eval.*, 2: 97-203
- Jamshidzadeh, A., R. Heidari, M. Razmjou, F. Karimi and M.R. Moein, 2015. An *in vivo* and *in vitro* investigation on hepatoprotective effects of *Pimpinella anisum* seed essential oil and extracts against carbon tetrachloride-induced toxicity. *Iranian J. Basic Medical Sci.*, 18: 205.
- Jaykare, S.C., V.M. Motghare, S.L. Padwal, V.S. Deshmukh and J.R. Patil, 2013. Evaluation of anticonvulsant activity of the seed oil extract of nigella sativa: An Experimental Study. *Hygeia J. Drugs Medicine*, 5: 21-26.
- Karasawa, K., Y. Uzuhashi, M. Hirota and H. Otani, 2011. Amatured fruit extract of date palm tree (*Phoenix dactylifera* L.) stimulates the cellular immune system in mice. *J. Agric. Chem.*, 59: 11287-11293. DOI: 10.1021/jf2029225
- Karimzadeh, F., M. Hosseini, D. Mangeng, H. Alavi and G.R. Hassanzadeh, 2012. Anticonvulsant and neuroprotective effects of *Pimpinella anisum* in rat brain. *BMC Complementary Alternative Medicine*, 12: 76. DOI: 10.1186/1472-6882-12-76
- Khan, S.A., A.R. Al Kiyumi, M.S. Al Sheidi, T.S. Al Khusaibi and N.M. Al Shehhi, 2016. In vitro inhibitory effects on α -glucosidase and α -amylase level and antioxidant potential of seeds of *Phoenix dactylifera* L. *Asian Pacific J. Tropical Biomedicine*, 6: 322-329.
- Loeb, J.A., 2011. Identifying targets for preventing epilepsy using systems biology. *Neuroscience Letters*, 497: 205-212. DOI: 10.1016/j.neulet.2011.02.041
- Lscher, W. and D. Schmidt, 1988. Which animal models should be used in the search for new antiepileptic drugs: A proposal based on experimental and clinical considerations. *Epilepsy Res.*, 2: 145-181. DOI: 10.1016/0920-1211(88)90054-X
- Majid, A.S., P. Marzieh, D. Shahriar, S.K. Zahed and K.T. Pari, 2008. Neuroprotective effects of aqueous date fruit extract on focal cerebral ischemia in rats. *Pak. J. Medicine Sci.*, 24: 661-65.
- Mallhi, T., M.I. Qadir, M. Ali, B. Ahmad and Y.H. Khan, 2014. Ajwa Date (*Phoenix dactylifera*): An Emerging Plant in Pharmacological Res. *Pak. J. Pharmaceutical Sci.*, 27: 607-616.
- Miguel, M.G., 2010. Antioxidant activity of medicinal and aromatic plants. A review. *Flavour Fragrance J.*, 25: 291-312. DOI: 10.1002/ffj.1961
- Mulley, J.C and H.C. Mefford, 2011. Epilepsy and the new cytogenetics. *Epilepsia*. 52: 423-432. DOI: 10.1111/j.1528-1167.2010.02932
- Nishikimi, M., N.A. Rao and K. Yagi, 1972. The occurrence of superoxide anion in the reaction of reduced phenazine methosulfate and molecular oxygen. *Biochemical biophysical res. Communi.*, 46: 849-854. DOI: 10.1016/s0006-291x(72)80218-3
- Ohkawa, H., N. Ohishi and K. Yagi, 1979. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Analytical Biochemistry*, 95: 351-358. DOI: 10.1016/0003-2697(79)90738-3
- Paglia, D.E. and W.N. Valentine, 1967. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. *J. Laboratory Clinical Medic.*, 70: 158-169.
- Papp, A., O. Feher and L. Erdelyi, 1987. The ionic mechanism of the pentylenetetrazol convulsions. *Acta Biologica Hungarica*, 38: 349-361.
- Parfenova, H., C.W. Leffler, S. Basuroy, J. Liu and A.L. Fedinec, 2012. Antioxidant roles of heme oxygenase, carbon monoxide and bilirubin in cerebral circulation during seizures. *J. Cerebral Blood Flow Metabolism*, 32: 1024-1034. DOI: 10.1038/JCBFM.2012.13
- Picot, M.C, M. Baldy-Moulinier, J.P. Daurès, P. Dujols and A. Crespel, 2008. The prevalence of epilepsy and pharmaco-resistant epilepsy in adults: A population-based study in a Western European country. *Epilepsia*, 49: 1230-8.
- Porsolt, R.D., A. Bertin and M. Jalfre, 1977. Behavioral despair in mice: A primary screening test for antidepressants. *Archives Int. Pharmacodynamie Therapie*, 229: 327-336.
- Pourgholami, M.H., S. Majzoob, M. Javadi, M. Kamalinejad and G.H.R. Fanaee, 1999. The fruit essential oil of *Pimpinella anisum* exerts anticonvulsant effects in mice. *J. Ethnopharmacology*, 66: 211-215. DOI: 10.1016/S0378-8741(98)00161-5
- Ragab, A.R., M.A. Elkablawy, B.Y. Sheik and H.N. Baraka, 2013. Antioxidant and tissue-protective studies on Ajwa extract: Dates from al-Madinah al-Monwarah, Saudi Arabia. *J Environ. Anal. Toxic*, 3 163. DOI: 10.4172/2161-0525.1000163

- Ramanjaneyulu, R. and M.K. Ticku, 1984. Interactions of pentamethylenetetrazole and tetrazole analogues with the picrotoxinin site of the benzodiazepine-GABA receptor-ionophore complex. *Eur. J. Pharmacology*, 98: 337-345.
DOI: 10.1016/0014-2999(84)90282-6
- Reynolds, E.H. and M.R. Trimble, 1985. Adverse neuropsychiatric effects of anticonvulsant drugs. *Drugs*, 29: 570-581.
- Sahraei, H., H. Ghoshooni, S.H. Salimi, A.M. Astani and B. Shafaghi, 2002. The effects of fruit essential oil of the *Pimpinella anisum* on acquisition and expression of morphine induced conditioned place preference in mice. *J. Ethnopharmacology*, 80: 43-47.
DOI: 10.1016/S0378-8741(02)00012-0
- Saini, N., G.K. Singh and B.P. Nagori, 2014. Spasmolytic potential of some medicinal plants belonging to family umbelliferae: A review. *Int. J. Res. Ayurveda Pharm*, 5: 74-83.
DOI: 10.7897/2277-4343.05116
- Shojaii, A. and M. Abdollahi Fard, 2012. Review of pharmacological properties and chemical constituents of *Pimpinella anisum*. *ISRN Pharmaceutics*,
DOI: 10.5402/2012/510795
- Singh, G., I.P.S. Kapoor, P. Singh, C.S. de Heluani and C.A.N. Catalan, 2008. Chemical composition and antioxidant potential of essential oil and oleoresins from anise seeds (*Pimpinella anisum* L.). *Int. J. Essential Oil Therapeutics*, 2: 122-130.
- Sucher, N.J. and M.C. Carles, 2015. A pharmacological basis of herbal medicines for epilepsy. *Epilepsy Behav.*, 52: 308-18.
DOI: 10.1016/j.yebeh.2015.05.012
- Tas, A., H. Ozbek, N. Atasoy, M. Enes Altug and E. Ceylan, 2006. Evaluation of analgesic and anti-inflammatory activity of *Pimpinella anisum* fixed oil extract. *Indian Vet. J.*, 83: 840-843.
- Tirapelli, C.R., C.R. de Andrade, A.O. Cassano, F.A. De Souza and S.R. Ambrosio, 2007. Antispasmodic and relaxant effects of the hidroalcoholic extract of *Pimpinella anisum* (Apiaceae) on rat anococcygeus smooth muscle. *J. Ethnopharmacology*, 110: 23-29.
- Toman, J.E., E.A. Swinyard and L.S. Goodman, 1946. Properties of maximal seizures and their alteration by anticonvulsant drugs and other agents. *J. Neurophysiology*, 9: 231-239.
DOI: 10.1152/JN.1946.9.3.231
- Treiman, D.M., 2001. GABAergic mechanisms in epilepsy. *Epilepsia*, 42: 8-12.
DOI: 10.1046/J.1528-1157.2001.042suppl.3008
- Vyawahare, N., R. Pujari, A. Khsirsagar, Ingawale, D., Patil, M., Kagathara, V., 2009. *Phoenix dactylifera*: An update of its indigenous uses, phytochemistry and pharmacology. *Internet J. Pharmacol.*, 7: 1-11.
- Vyawahare, N., R. Pujari, R. Rajendran, A. Khsirsagar and D. Ingawale, 2009. Neurobehavioral effects of *Phoenix dactylifera* in mice. *J. Pharmacists*, 1: 225.
DOI: 10.4103/0975-1483.57069
- Watanabe, M., K. Maemura, K. Kanbara, T. Tamayama and H. Hayasaki, 2002. GABA and GABA receptors in the central nervous system and other organs. *Int. Rev. Cytology*, 213: 1-47.
DOI: 10.1016/S0074-7696(02)13011-7
- Xie, T., W.P. Wang, Z.F. Mao, Z.Z. Qu and S.Q. Luan, 2012. Effects of epigallocatechin-3-gallate on pentylenetetrazole-induced kindling, cognitive impairment and oxidative stress in rats. *Neurosci. Lett.*, 516: 237-241. DOI: 10.1016/J.NEULET.2012.04.001