Original Article

Effect of *Ocimum basilicum* hydro-alcoholic extract on oxidative damage of brain tissue following seizures induced by pentylenetetrazole in mice

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Abstract

Introduction: A relationship between epileptic seizures and brain tissue oxidative damage has been suggested. *Ocimum basilicum* (*O. basilicum*) has been shown to have beneficial effects including hypnotic and protective against tissue oxidative damage. The present study was designed to evaluate the effects of *O. basilicum* hydro-alcoholic extract on oxidative damage of brain tissue following seizures induced by pentylenetetrazole (PTZ) in mice.

Methods: The animals were grouped and treated as follows: 1- control group which received saline; 2- PTZ group (90 mg/kg, ip); 3 to 5- three groups which received 25, 50 or 100 mg/kg of a hydro-ethanolic extract of *O. basilicum* before PTZ. First minimal clonic seizure (MCS) and the first generalized tonic-clonic seizure (GTCS) latencies were analyzed. The brains of the animals were then collected and stored to use for biochemical evaluation.

Results: The plant extract in 50 and 100 mg/kg doses, significantly postponed the MCS and GTCS seizures onsets (\(P<0.05\)-\(P<0.01\)) when administered before PTZ. PTZ - induced seizures also increased lipid-peroxidation in the brain tissue which was presented by a high level of malondialdehyde (MDA) in the brain tissue compared to the control group (\(P<0.001\)). *O. basilicum* extract attenuated MDA levels in the brain (\(P<0.05\)-\(P<0.001\)). PTZ - induced seizures also decreased brain tissue total thiols compared to the control group (\(P<0.001\)). Pretreatment with all doses of *O. basilicum* extract improved thiol content in the brain tissue (\(P<0.05\)).

Conclusion: The current study revealed that hydro-ethanolic extract of *O. basilicum* possesses significant antioxidant and anticonvulsant activities.

Keywords: *Ocimum basilicum*; Hydro-alcoholic extract; Pentylenetetrazole; Seizures; Mice; Oxidative damage; Brain

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Introduction

Epilepsy as an important neurological disorder has been reported to occur in nearly 1% of the population (Sander, 2003). A significant effect on learning, memory and cognition has been reported to occur in epileptic patients (Meador, 2002). During seizure attacks, a high level of free radicals are produced which might be followed by oxidative damage to
proteins, lipids and DNA (Kudin et al., 2002). Brain tissue has been reported to be containing high levels of lipids which makes them vulnerable to oxidative stress. Brain tissue oxidative damage has been reported to be a main contributor in pathogenesis on central nervous system (CNS) diseases. It has been reported that some of complications of seizures may be related to oxidative damage to the brain tissue (Mehla et al., 2010). There are also some reports that an increased production of reactive oxygen species (ROS) may take part in convulsant and neurotoxicity of pentylenetetrazol (PTZ) (Hosseini et al., 2013). The results of human and animal studies imply that epilepsy and seizures lead to the brain tissue oxidative damage especially in the cortical and hippocampal regions which are accompanied with cognitive functions impairments especially learning and memory deficits (Kudin et al., 2002; Mehla et al., 2010; Rosche et al., 2010). Furthermore, some of well-known anti-oxidants are reported to have anti-convulsant effects (Gupta and Brijal, 2006). We also previously introduced some of the plant extracts with anti-convulsant effects which were accompanied with protective effects against the brain tissue oxidative damage (Hosseini et al., 2013).

The medicinal plants have secondary metabolites and essential oils with a therapeutic importance. Their advantages are includes being safe, economic, effective, easily available and having less side effects (Ramesh and Padmavathi, 2010). Recently, researchers interested to study on possible use of medicinal plants because of having anxiolytic, analgesic, antidepressant and antiepileptic effects. Interestingly, a focus seems to be on antioxidative and anticonvulsant effects of the plant extracts (Singh et al., 2009).

*Ocimum basilicum* Linn (O. basilicum), is popularly known as “sweet basil” (Muralidharan and Dhananjayan, 2004). It belongs to basilicum species and *Ocimum* genus and Lamiaceae family (Bilal et al., 2012). The word basil that means king, is coming from Greek (basileus), because of its royal fragrance (Dashputre and Naikwade, 2010). The origin of Sweet basil is Persia and Sindh and lower hills of Punjab in India (Bilal et al., 2012). The plants of genus *Ocimum* are very useful for their therapeutic potentials and are rich in phenolic compounds (Ramesh and Padmavathi, 2010). Leaves and flowering parts of *O. basilicum* have been traditionally used for their antispasmodic, aromatic and digestive effects (Kaya et al., 2008).

Several *Ocimum* species (Lamiaceae) are used to treat CNS disorders in various parts of the world (Agrawal et al., 2009). A decoction extract of root of the plant has been used by Brazilian natives as a sedative for children (Di Stasi et al., 2002). In an experimental study, an acute *O. basilicum* leaf essential oil administration increased the hypnosis induced by sodium thiopental and prevented the convulsions induced by pentylenetetrazole (PTZ) (Oliveira et al., 2009). In another experimental study, a reduction in ischemia-induced oxidative stress was seen in the brain after administration of ethyl acetate fraction of *O. basilicum* (Bora et al., 2011). The most components of essential oil of *O. basilicum* are reported to be linalool, 1,8-cineole and geraniol (Oliveira et al., 2009). Also, linalool has been able to increase glutathione content while, it decreased acrylamide-induced lipid peroxidation in the brain tissue of rats (Mehri et al., 2015).

The present study was designed to evaluate the effects of *O. basilicum* hydro-alcoholic extract on oxidative damage of brain tissue following seizures induced by PTZ in mice.

**Materials and methods**

**Animals and drugs**

In this study, 40 virgin male mice, 25 ± 5 g in weight were used. The animals were maintained at the animal house under controlled conditions including 12 h light and dark cycle, 22-24 °C temperature and appropriate humidity with laboratory chow and water provided *ad libitum*.

The mice were grouped (*n* = 8) and treated as: 1-control group which received saline; 2- PTZ (Sigma-Aldrich Company, St. Louis, USA) group (90 mg/kg, intraperitoneal, ip) with saline and a drop of tween; 3 to 5- three groups including (Ext 25-PTZ, Ext 50-PTZ and Ext 100-PTZ) which received 25, 50 or 100 mg/kg of a hydro-ethanolic extract (ip) of *O. basilicum* (dissolved in tween and diluted by saline) before PTZ (Askari et al., 2016).

The mice of groups 2-5, were injected ip by vehicle or three doses of the plant extract since 3 days before starting the experiments. The animals of these groups were also continued to be treated by vehicle
or the extract 30 min before ip injection of a single dose (90 mg/kg) of PTZ. It was previously shown that PTZ in this dose induces generalized tonic-clonic seizures in rats (Ebrahimzadeh Bideskan et al., 2011; Farrokhi et al., 2014; Hosseini et al., 2013). In the second group, vehicle was administered instead of the plant extract. The brains were then removed for biochemical measurements. The mice of the control group were injected by saline instead of both PTZ and the extract and the brain tissue were then removed without inducing the seizures. The animals were maintained in a good general health, in accordance with the European Communities Council Directive (2010/63/UE) and under supervision of Mashhad University of Medical Sciences, Ethical Committee (Ethic number: IR.MUMS.REC.1396.57). All behavioral tests were carried out between 10:00 and 14:00.

**Preparation of the extract**

*O. basilicum* aerial parts were gathered from Razavi Khorasan, Iran. The plant was identified and confirmed at School of Pharmacy, Mashhad University of Medical Sciences, Mashhad, Iran. To prepare a hydroalcoholic extract, the dried plant materials (100 g) were mixed with 300 ml of ethanol (70%). The extraction was carried using a Soxhalet instrument. The solvent was then removed to prepare a relatively dried extract and kept at −20°C until being used (Beheshti et al., 2017).

**Induction of seizures by PTZ**

The animals were injected by PTZ and were located inside a Plexiglas box (30 cm × 30 cm × 30 cm). The behaviors of the animals were recorded for period of 60 min after PTZ (90 mg/ kg) injection (Ebrahimzadeh Bideskan et al., 2011; Farrokhi et al., 2014; Hosseini et al., 2011; Hosseini et al., 2013; Hosseini et al., 2014). The latency to the onset of the first minimal clonic seizure (MCS) and the latency to the first generalized tonic-clonic seizures (GTCS) were noted and compared between the groups (Hosseini et al., 2009).

**Biochemical assessment**

After behavioral study, the mice were quickly decapitated under deep sodium pentobarbital anesthesia, their whole brains were removed and conserved for biochemical measurements. The animals were killed by a competent person with a minimum pain, suffering and distress. The brains were homogenized in a cold phosphate-buffered saline to provide a 10% (w/v) solution. To measure total thiol content, a 2, 2'-dinitro- 5, 5'-dithiobisbenzoic acid (DTNB) reagent was used which reacts with the thiols and produces a solution with a yellow color. Briefly, 50 μl of the homogenates were added to 1 ml of tris-EDTA buffer (pH = 8.6) and absorbance was read at 412 nm and recorded as \( A_1 \). Then 20 μl of DTNB reagent was added and the mixture was stored in room temperature and for 15 min. The absorbance was recorded again \( A_2 \). Absorbance of DTNB solution was also recorded as a blank (B). The following formula was used to calculate total thiol concentration (Hosseini et al., 2014; Pourganji et al., 2014):

\[
\text{Total thiol concentration (mM)} = (A_2-A_1-B) \times 1.07/0.05 \times 13.6
\]

As an index of lipid peroxidation, the brain tissue malondialdehyde (MDA) level was measured (Sakina et al., 1990). MDA reacts with thiobarbituric acid (TBA) as a thiobarbituric acid reactive substance (TBARS) and produces a complex with a red color (Sakina et al., 1990). A complex reagent containing TBA/ trichloroacetic acid / hydrochloric acid was mixed with the tissue homogenates. The provided solution was boiled using a water bath for 40 min. The solution was allowed to reach the room temperature and was then centrifuged (1000 g / 10 min). The absorbance was recorded at 535 nm (Hosseini et al., 2013; Hosseini et al., 2014; Vafaee et al., 2014; Pourganji et al., 2014). The following formula was used to calculate MDA: \[ C(M)= \frac{\text{Absorbance}}{(1.65 \times 10^3)} \]

**Statistical analysis**

The data were provided as mean ± SEM. ANOVA test followed by Tukey's post hoc was used to analyze the data. The difference was considered to be statistically significant when \( P \) values were less than 0.05.

**Results**

**Effect of the extract on behaviors of the rats**

The behavioral result sowed that PTZ injection induced MCS and GTCS in the animals of all groups. Additionally, all three doses including 25, 50 and 100
mg/kg of the extract increased MCS latencies compared to PTZ group ($P<0.05$-$P<0.01$). There was no significant difference between three doses of the extract (Fig. 1a). Pretreatment by both 50 and 100 mg/kg of the extract postponed GTCS onsets ($P<0.05$) while, 25 mg/kg was not able to change the GTCS latency. There was no significant difference between three doses of the extract (Fig. 1b). The results showed that the extract didn’t affect mortality rate (Table 1).

**Effect of the extract on oxidative damage in the brain tissue**

The results showed that induction of seizures by PTZ...
increased the brain tissue MDA levels compared to the control animals ($P<0.001$). Pretreatment with all doses of the extract prevented from elevation of the brain tissue MDA compared to PTZ ($P<0.05$ and $P<0.001$ as compared to PTZ group). Interestingly, the highest dose of the plant extract was more effective to prevent from elevation of the brain tissue MDA than that of both the medium and the lowest doses ($P<0.001$, Fig. 2).

The results showed that induction of seizures by PTZ decreased total thiol contents in the samples of the brain ($P<0.001$). Compared to PTZ group,
administration of all doses of the plant extract before PTZ, prevented from decreasing of brain tissue total thiol content (P<0.05, Fig. 3). The results also showed that the brain tissue thiol contents in all Ext 25-PTZ, Ext 50-PTZ and Ext 100-PTZ groups were in a lower level compared to the control group (P<0.001). Additionally, no significant difference was observed between the three extract treated groups (Fig. 3).

**Discussion**

Oxidative damage has been suggested as a main cause of nervous system diseases. Oxidative stress has also been previously considered to have an important role in the etiology of epilepsy and seizure (Costello and Delanty, 2004; Kudin et al., 2002; Patel, 2004). It has also been well documented that oxidative stress has an important role in the brain damage due to epilepsy (Hosseini et al., 2013; Zhen et al., 2014). Brain tissue oxidative damage is suggested to contribute in the complications of seizures and epilepsy including cognitive impairments and learning and memory deficits (Meador, 2002; Rosche et al., 2010). PTZ has been well known that bind to the GABA_A receptor to inhibit chloride channels. As a well-known convulsant chemical agent, PTZ is used in rodents to examine the possible natural or synthetic antiepileptic agents (Hosseinzadeh and Sadeghnia, 2007; Porter, 1983). When is used in a high dose, PTZ induces a continuing seizure attacks. At first, the seizures are presented with myoclonic jerks in face and forelimbs but righting reflex is kept. These attacks are known to be minimal clonic seizure or MCS. Some of clonic seizures in the limbs are then presented which are accompanying with losing of righting reflex. In this stage the animals show some tonic extensions in both their hind and forelimbs. The later attacks are known to be generalized tonic-clonic seizures or GTCS (Loscher et al., 1991). We also previously showed that injection of PTZ induced the seizures which were accompanied with brain tissue oxidative damage (Hosseini et al., 2013). ROS production has been suggested to have an important role in the neurotoxication due to PTZ- induced seizures (Liu et al., 2012; Xie et al., 2012). Also, in the current study, an increased level of MDA concentration was observed while, total thiol contents decreased in the brains of the animals of PTZ injected group. Similar to our results an increased level of ROS, such as hydroxyl radicals, superoxide anions and hydrogen peroxide have been well documented to occur in the brains of the animals subjected to seizures (Rosche et al., 2010; Sudha et al., 2001). On the other hand, brain tissue oxidative damage by free radicals is suggested to have an important role in psychiatric or cognitive issues for example, depression, anxiety and memory impairment (Costello and Delanty, 2004; Reilly et al., 2011). Additionally, a reduction in the life span observed in the epileptic persons has been suggested to be due to brain tissue oxidative damage (Maldonado et al., 2010). Oxidative stress has also been considered to be able to link aging to epilepsy (Liang et al., 2007). In keeping with these observations, several natural antioxidants have been considered for their anticonvulsant effects. We also previously showed that some of the plant extracts including Coriandrum sativum, Achillea wilhelmsii and Nigella sativa showed anticonvulsant effects which was accompanied with a protective effect against the brain tissue oxidative damage (Hosseini et al., 2013; Vafaei et al., 2014).

From ancient times, some of the plants were commercially used both as foods and medicinal substances (Burdock and Carabin, 2009). Ocimum genus is containing of about fifty species which are mainly growing in tropical and warm temperate regions (Ghasemi Pirbalouti et al., 2013). *O. basilicum* also known as basil or sweet basil is reported to be the most famous plant from this class (Ghasemi Pirbalouti et al., 2013). *O. basilicum* is generally known as Rehan in Egypt and Iran. It is known to have a wide range of pharmacological actions, so it is widely used in traditional remedies to treat several diseases. The plant has been reported to have other treatment properties such as antimicrobial, anti-inflammatory, analgesic or antiseptic properties (Bakkali et al., 2008), in addition to antifungal and insect repellent properties (Oxenham et al., 2005). *O. basilicum* has also been suggested to use externally to treat insect stings, snake bites, acne and skin infections (Farag, 2013). Some of volatile compounds, essential oils and flavonoids are reported to be present in all *Ocimum* genuses. These compounds have been reported to be responsible for some flavoring as well as medicinal effects of the plant (Chiang et al., 2005).
The extract of *O. basilicum* has been experimentally examined for some of its pharmacological properties (Mehla et al., 2010). Additionally, several *Ocimum* species have been reported to have anti-inflammatory, anticonvulsant and analgesic effects (Quintans-Junior et al., 2013). In present study, all doses of the extract increased the MCS latency. Additionally, both 50 and 100 mg/kg of the plant extract significantly increased the GTCS. Considering the results of present study, it seems the higher doses of the plant extract were more effective than the lower doses however, it is impossible to judge about a dose dependent effect of the extract and further studies using higher doses is suggested to be done in the further studies. Similarly, *O. basilicum* extract has also been reported to have anticonvulsant effects in animal models of seizures (Sakurada et al., 2009). Consistently, an extract of leaves of *Ocimum gratissimum*, another genu of Lamiaceae family, was reported to postpone onsets of tonic and tonic-clonic seizures induced by PTZ and also protected the animals against mortality (Okoli et al., 2010). The results of present study showed that *O. basilicum* extract was able to prolong convulsions induced by PTZ however, it was better to compare the effects of the plant extract with a standard anticonvulsant drug such as diazepam. We previously showed 3 mg/kg of diazepam was able convulsive effects of PTZ in a dose which was used in the present study (Hosseini et al., 2011).

It has also been reported that *O. basilicum* extract improved the anti-oxidant enzymes including glutathione, superoxide dismutase and catalase (Muscat and Willner, 1992) while, decreased TBARS levels in the serum of the rats exposed to gamma radiation rats (Farag, 2013). In this study, we measured MDA concentrations in the brains to examine the effects of the plant extract on lipid peroxidation. MDA has been well known to increase as an indicator of lipid peroxidation in brain tissue in the animal models of seizures and epilepsy (Golechha et al., 2010; Xie et al., 2012). In our experiment, an increased level of MDA was observed in the brain tissue following seizures which was prevented by the *O. basilicum* extract. Total thiol groups are also well- known to be very sensitive to oxidative stress and are depleted following an oxidative insult (Soszynski and Bartosz, 1997). Therefore, we studied the effect of the extract on total thiol concentrations in brain tissue after seizures. Similar to other studies, thiol groups were decreased in the brains following a seizure which was prevented by the extract. The antioxidant impact of the plant has also been well demonstrated in other studies (Jayasinghe et al., 2003; Politeo et al., 2007). It seems the higher doses of the plant extract were more effective than the lower doses however they were not significant. Dose-dependent effects can be seen at higher doses.

Considering the results of present study and because of having antioxidant effects, it seems that *O. basilicum* might be able to prevent from complications of epilepsy; however, it needs to be more investigated in the future. It is mentionable that oxidative stress has been considered to have an important role in both the etiology and complications of epilepsy and seizure (Costello and Delanty, 2004; Meador, 2002; Patel, 2004).

The component(s) responsible for the beneficial effects of the plant extract was not determined in the present study. *O. basilicum* has also been shown to be containing a large amount of α-terpineol (59.78%), β-caryophyllene (10.54%) and estragole (22.6%) (Bayala et al., 2014; Oliveira et al., 2009). Other compounds including rosmarinic acid, eugenol, apigenin, cirsimartin, cirsilineol and isothymusin which have been isolated from the leaves of *Ocimum sanctum* (Khanna and Bhatia, 2003). Each of these agents may play a role in beneficial effects of the plant extract which was shown in the present study. Consistently, linalool has also been reported to modulate glutamate neurotransmitter and receptors by which affects PTZ kindling (Elisabetsky et al., 1999).

**Conclusion**

In conclusion, the present data demonstrated that the hydro-alcoholic extract of *O. basilicum* aerial parts was able to prolong convulsions induced by PTZ in this study, suggesting an anticonvulsive activity to this species. This activity was accompanied by an antioxidant effect in the brain tissue. However, additional studies using other more precise methods are needed to confirm this thinking. Additionally, further studies are needed to be done to determine the responsible component(s).
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Conflict of interest
The authors have no conflict of interests to declare.

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