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The role for nitric oxide on the effects of hydroalcoholic extract *of Achillea wilhelmsii* on seizure

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Abstract

Objective: Nitric oxide (NO) plays an important role both as a consequence and as a cause of epileptic seizures. Regarding the central nervous system depressant effects of *Achillea wilhelmsii (A. wilhelmsii)*, as well the effects of the plant on NO, this study was aimed to elucidate the possible role for nitric oxide on the effects of hydroalcoholic extract of *A. wilhelmsii* on pentylenetetrazole (PTZ)-induced seizures.

Materials and Methods: Fifty-six male Wistar rats were divided into 7 groups (n=8 in each group) and treated with (1) normal saline, (2) normal saline before pentylenetetrazole (PTZ, 90 mg/kg), (3-7) *A. wilhelmsii* extract (100, 200, 400, 800, and 1200 mg/kg) before PTZ. Latency to first minimal colonic seizure (MCS) and the first generalized tonic-clonic seizures (GTCS) as well as the mortality rate were recorded. The brain tissues were then removed for biochemical measurements. Fisher's exact probability test as well as analysis of variance (ANOVA), followed by Tukey's test were used for statistical evaluation.

Results: Treatment with 100- 1200 mg/kg of the extract did not affect MCS latencies. 400 mg/kg of the extract prolonged GTCS latency ($p<0.001$), however, the lower and higher doses were not effective. Nitric oxide metabolites concentrations in the hippocampal tissues of the animals treated with 100, 200, and 400 mg/kg of the extract were increased compared with saline ($p<0.05$-$p<0.01$).

Conclusion: The present study showed that hydroalcoholic extract of *A. wilhelmsii* affects NO metabolites in brain tissues as well the severity of seizures in PTZ-induced seizure model.

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Introduction

Recurring seizures or convulsions are important neurological manifestations of a brain disorder, epilepsy, which afflict about 0.5–1% of the people in the world (Dhir et al., 2006; Hachinski, 1998). One of the

most commonly used means to study seizure, is administration of pentylenetetrazole (PTZ) to rats or mice which its epileptic effects appear in high doses (more than 40 mg/kg) (Itoh et al., 2004, Jiang et al., 2004; Kliqueva et al., 2001).

These effects consist of two types of motor seizures: 1) minimal and 2) major. The latter is generalized tonic-clonic responses with muscle contractions of the whole body often followed by a cramped tonic state. However, the first one is restricted to forelimbs, and mostly clonic (Kliqueva et al., 2001).

The cause of increased seizure susceptibility has long been known to be an irregularity in neurotransmitter release in the brain (Coitinho et al., 2001) or an imbalance in excitatory and inhibitory functions (Peeters et al., 1989). There are plenty of genetic studies proving that mutation in voltage-gated sodium and potassium channels and nicotinic acetylcholine receptors can be possible responsible mechanisms in this disorder as well as disruption of gamma-aminobutyric acid-ergic (GABAergic) and glutamatergic systems (Baulac et al., 2001, Coitinho et al., 2001; Emanuelli et al., 2000).

NO (Nitric Oxide) has recently drawn a rather increasing attention to itself as an important cellular signaling molecule involved in many physiological and pathological processes. There are three isozymes of nitric oxide synthase (NOS) in the body (De Luca et al., 2006; Moezi et al., 2012) whose activity can result in production of NO from its precursor, arginine (Lesani et al., 2010), amongst which the imbalance of nNOS (neural NOS) is of great importance in neural disorders. NO functions as a vasodilator through stimulating guanylyl cyclase, increasing cyclic guanosine monophosphate (cGMP) production, and regulating the activity of dopaminergic, glutaminergic, and GABAergic systems (Jayakumar et al., 1999; Moezi et al., 2012; Paul and Subramanian, 2002). Some other studies also suggest its role in hormone secretion (Ceccatelli, 1997) and cell death in human nervous system (Kamoshima et al., 1997; Nowicki et al., 1991). It has also been well documented that this gaseous messenger has an important role in pain and analgesia (Hosseini et al., 2011a; Hosseini et al., 2011b; Karami et al., 2011). Putting all these together, NO's probable role in convulsion is inferred. Supporting this claim, several investigations were carried out confirming the anticonvulsive influence of NO in convulsions or seizures induced by C-methyl-D-aspartate (Buisson et al., 1993), penicillin (Marangoz et al., 1994), kainic acid (KA) (Penix et al., 1994), picrotoxin (Jayakumar et al., 1999; Paul and Subramanian, 2002), and pentylenetetrazole (Lesani et al., 2010; Moezi et al., 2012; Shafaroodi et al., 2012). Other studies in this field have revealed an enhanced expression of type II nitric oxide synthase mRNA in rat brains as a result of consumption of anticonvulsant drugs (Suzuki et al., 2002). While these studies confirm the anticonvulsive impact of NO, some others pile up evidence against this theory.

Some investigators demonstrated that a decline in NO production by application of NOS inhibitors or administration of NO precursors leads to inhibition of convulsions evoked by PTZ (Bashkatova et al., 2003; De Luca et al., 2006; Itoh et al., 2004; Osonoe et al., 1994). As reported, anticonvulsive and proconvulsive effects of NO vary depending upon several factors such as model of seizures, dose of the substance used for evoking seizure, pretreatment time (De Luca et al., 2006; Paul and Subramanian, 2002), brain structure and age of animals (De Luca et al., 2006), source of nitric oxide, and finally, other neurotransmitter systems involvement (Itoh and Watanabe, 2009; Moezi et al., 2012).

Achillea is a plant belonging to the family of Compositae (Nemeth and Bernath 2008). Many pharmacological properties have been reported for Achillea genus

including antiulcer (Cavalcanti et al., 2006), hepatoprotective (Yaeesh et al., 2006), anti-inflammatory (Benedek et al., 2008), antitumor (Csupor-Loffler et al., 2009; Tozyo et al., 1994), antispasmotic (Lemmens-Gruber et al., 2006; Yaeesh et al., 2006), and choleretic (Benedek et al., 2006). *Achillea Wilhelmsii* (A. Wilhelmsii), the most important species of Achillea, grows in some countries such as Iran (Asgary et al., 2000), Egypt, and Turkey (Azadbakht et al., 2003).

A. Wilhelmsii is called "boomadaran" in Iran (Lavander cotton) and is found in many areas of the country (Khan and Rezazadeh, 2010). It has chemical components including borneol, linalol, caryophyllene, 1,8-Cineol, semithujone, flavonoids (rutin), glycoalkaloids, carvacrol, chrysanthenol acetate, and camphor (Afsharypuor et al., 1996; Azadbakht et al., 2003; Javidnia et al., 2004). Some studies have indicated that Achillea species such as A. santolina (Ardestani and Yazdanparast, 2007), A. ligustica (Tuberoso et al., 2005), and A. clavennae (Stojanovic et al., 2005) have antioxidative activity which can reduce free radicals. Moreover, it has been shown that Achillea contains aromatic bitter substances and tannins which have important effects on the nervous system and neurological diseases such as neurasthenia, epilepsy, and seizures (Azadbakht et al., 2003; Kabuto et al., 1992).

Regarding the facts that of NO probably has a role in seizure and considering the possible effects of *A. wilhelmsii* on both seizure and NO, this study aimed to elucidate the possible role for nitric oxide on the effects of hydroalcoholic extract of *A. wilhelmsii* on seizure.

Materials and methods

Animals and grouping

This experimental research was done according to ethics committee guidelines and all the protocols of animal experiments have been approved by the Institution's Animal Care Committee. In this study, 56 virgin male Wistar rats, 250±20 g were used. The animals were maintained in the animal house under controlled conditions including 12/12 h light and dark cycle, 22-24^{o}C temperature and 50% relative humidity with laboratory chow and water provided *ad libitum*.

The animals were divided into 7 groups randomly (n=8 in each group) and were treated with (1) Normal saline, (2) Normal saline before PTZ, (3-7) *Achillea wilhelmsii* extract (100, 200, 400, 800, and 1200 mg/kg) before PTZ. After PTZ (Sigma aldrich St. Louis, USA) (90 mg/kg body weight, i.p.) injection, the animals were observed for 60 min and the behavioral responses were recorded (Ebrahimzadeh Bideskan et al., 2011; Hosseini et al., 2011; Hosseini et al., 2009). Behavioral responses of the animals to PTZ administration were evaluated using these criteria: latency to first minimal clonic seizure (MCS), incidence of MCS, latency to the first generalized tonic–clonic seizures (GTCS), incidence of GTCS, protection percentage against GTCS, and protection percentage against mortality (Ebrahimzadeh Bideskan et al., 2011; Hosseini et al., 2011; Hosseini et al., 2009). The brain tissues were then removed and submitted to biochemical measurements.

Extracts preparation

A. wilhelmsii was collected from Nishabour city, Khorasan Razavi Province, Iran and identified by botanists in Ferdowsi University of Mashhad, Iran and a voucher number was deposited (4-2012-142). The plants were then dried at room temperature. To prepare hydroalcoholic extract, 50 g of the chopped and dried aerial parts of plant were soaked in ethanol (50%) for 48 h and paper filter was used to filter the solute after mixing. The solvent of the extracts was then removed to dryness with a rotary vacuum evaporator (Rakhshandah and Hosseini, 2006). The output of the extract was 9%. The extracts were dissolved in normal saline.

Biochemical assessment

After behavioral study, the animals were sacrificed, the hippocampi were removed and dissected on an ice-cold surface and submitted to NO metabolite measurements in the tissue. The Griess reaction was adapted to assay nitrates as previously described (Azizi-Malekabadi et al., 2012; Sadeghian et al., 2012). Briefly, standard curves for nitrates (Sigma, St. Louis, Missouri, USA) were prepared and samples (50 µl serum and 100 µl tissue suspension) were added to the Griess reagent. The proteins were subsequently precipitated by adding 50 µl of 10% trichloroacetic acid (Sigma). The contents were then vortex-mixed and centrifuged and the supernatants were transferred to a 96-well flat-bottomed microplate. Absorbance was read at 520 nm using a microplate reader and final values were calculated from standard calibration plots (Azizi-Malekabadi et al., 2012; Hosseini et al., 2010; Sadeghian et al., 2012).

Statistical analysis

Data expressed as mean±SEM. Fisher's exact probability test, as well as analysis of variance (ANOVA), followed by Tukey's test, were used for statistical evaluation. p-values less than 0.05 were considered to be statistically significant.

Results

All the animals in different treatment groups (except for the control group which did not receive PTZ) showed MCS and GTCS following PTZ administration (90 mg/kg). Treatment by 100-1200 mg/kg of the extract didn't affect MCS latencies (Table 1). 400 mg/kg of the extract prolonged GTCS latency ($p<0.001$), however, the lower and higher doses were not effective (Table 1). Mortality rate in the animals treated with 200 and 400 mg/kg of the extract was lower than that of PTZ group ($p<0.001$). There were no significant differences in mortality rate between treated groups by lower and higher doses of the extract compared with PTZ group.

Table1. Latencies to minimal clonic seizures (MCS) and generalized tonic–clonic seizures (GTCS) onsets in PTZ and *Achillea wilhelmsii* extract (Ex) -treated animals. The animals were treated with normal saline or extract (100, 200, 400, 800, or 1200 mg/kg) before a single injection (90 mg/kg) of PTZ. *** $p<0.001$ as compared with PTZ group

	MCS latency (Sec)	GTCS latency (Sec)	Mortality
PTZ	62.2±3.26	102.36±11.14	8/8
Ext 100	54.37±4.53	124.5±8.07	8/8
Ext 200	50.5±4.31	134.63±33.27	4/8*
Ext 400	58.62±13.65	298.63±46.23***	4/8*
Ext 800	63.87±4.99	186.88±43.38	5/8
Ext 1200	64.37±4.63	196.13±26.37	5/8

There was no significant difference between NO2 or NO3 concentrations in the hippocampal tissues of PTZ-treated group compared with saline. Nitric oxide metabolites concentrations in the hippocampal tissues of the animals treated by 100, 200 and 400 mg/kg of the extract increased compared with sham treated ($p<0.05$, $p<0.01$, and $p<0.05$, respectively). Treatment of the animals with other doses of the extract did not affect the NO metabolites compared with PTZ and saline (Figure. 1).

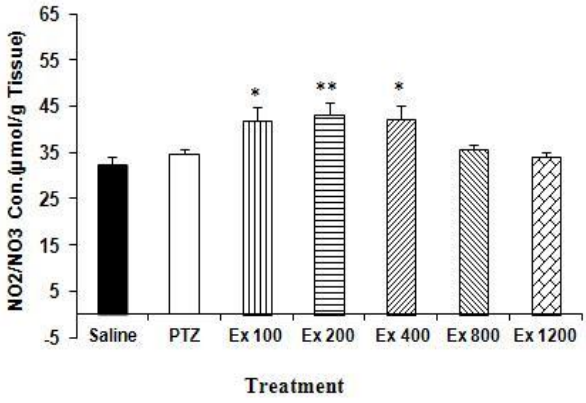

Figure 1. The concentrations of nitric oxide (NO) metabolites (NO2-NO3) in hippocampal tissue in PTZ and *Achillea wilhelmsii* extract (Ex) -treated animals. The animals were treated with normal saline or extract (100, 200, 400, 800, or 1200 mg/kg) before a single injection (90 mg/kg) of PTZ. * $p<0.05$ and ** $p<0.01$ as compared to PTZ group.

Discussion

In the present study, Nitric oxide metabolites concentrations in the hippocampal tissue of the animals treated with different doses of hydroalcoholic extract *of A. wilhelmsii* were increased compared with normal saline treated ones. In the brain, NO acts as a neuronal messenger and a modulator of neurotransmission (Moncada et al., 1991). It has been documented that NOS substrates, NO donors, and NOS inhibitors exert various anticonvulsant (Buisson et al., 1993; Starr and Starr, 1993) or proconvulsant (Mulsch et al., 1994, Nidhi et al., 1999) effects in different seizure models. Bosnak et al. (2007) showed that systemic administration of L-arginine significantly decreased the frequency of epileptiform electrocorticographical (ECoG) activity on penicillin-induced seizures in male rats while it did not modulate anti-seizures activity of pyridoxine and clonazepam (Bosnak et al., 2007; Gupta et al., 2000). However, a proconvulsant activity for L-arginine has also been reported (Mulsch et al., 1994). Noyan et al.,. (2007) showed that central administration of L-NAME had no effects on the latency and severity of seizures following pillocarpine injection (Noyan et al., 2007). It has also been reported that while systemic administration of L-NAME (non-specific NOS inhibitor) had no effects on penicillin-induced seizures in male rats, but 7-nitroindazole (7-NI, a nNOS inhibitor) significantly decreased epileptiform ECoG activity (Bosnak et al., 2007).

Another research showed that N omega-nitro-L-arginine (NNA), an inhibitor of NOS, aggravated KA-induced seizures (Penix and Davis, 1994). A functional relationship between the NO cGMP signaling pathway and the anticonvulsant activities of adenosine and pyridoxine has also been suggested (Akula et al., 2008; Bosnak et al., 2007). The results of our previous study showed that NO has a role in seizures susceptibility following PTZ administration and this effect was different in the presence or absence of ovarian hormones (Hosseini et al., 2009). In the present study, the concentrations of NO metabolites in hippocampal tissues were not different between convoluted rats by PTZ and control groups.

On the other hand, the animals pretreated with hydroalcoholic extract of *A. wilhelmsii* extract showed an elevation in NO metabolites concentrations. It has been reported that *A. wilhelmsii* extract has a strong antioxidant activity (Khan and Rezazadeh 2010; Souri et al., 2010). In contrast to this finding, it was shown that *A. millefolium* administration resulted in a decrease in plasma nitrite and nitrate concentrations in patients with chronic kidney disease (Vahid et al., 2012). It was also shown that *Achillea santolina* reduced the plasma NO increased in diabetic rats (Yazdanparast et al., 2007). The extract of *Achillea fragrantissima*, prevented the nitric oxide overproduction induced by lipopolysaccharide in glial cells (Elmann et al., 2011).

The present study showed that hydroalcoholic extract of *A. wilhelmsii* affects NO metabolites in brain tissues as well the severity of seizures in PTZ-induced seizure model.

Refrences

Afsharypuor S, Asgary S, Lockwood G. 1996. Constituents of the Essential Oil of Achillea wilheimsii from Iran. Planta med, 62: 77-78.

Akula KK, Dhir A, Kulkarni SK. 2008. Nitric oxide signaling pathway in the anti-convulsant effect of adenosine against pentylenetetrazol-induced seizure threshold in mice. Eur J Pharmacol, 587: 129-134.

Ardestani A, Yazdanparast R. 2007. Antioxidant and free radical scavenging potential of Achillea santolina extracts. Food chem, 104: 21-29.

Asgary S, Naderi GH, Sarrafzadegan N, Mohammadifard N, Mostafavi S, Vakili R. 2000. Antihypertensive and antihyperlipidemic effects of Achillea

wilhelmsii. Drugs under Experimental and Clinical Research Drugs Exp Clin Res, 26: 89- 93.

Azadbakht M, Semnani M, Khansari N. 2003. The essential oil omposition of Achillea wilhelmsii leaves and flowers. J Med Plants, 2: 55-59.

Azizi-Malekabadi H, Hosseini M, Soukhtanloo M, Sadeghian R, Fereidoni M, Khodabandehloo F. 2012. Different effects of scopolamine on learning, memory, and nitric oxide metabolite levels in hippocampal tissues of ovariectomized and Sham-operated rats. Arq Neuropsiquiatr, 70: 447-452.

Bashkatova V, Narkevich V, Vitskova G, Vanin A. 2003. The influence of anticonvulsant and antioxidant drugs on nitric oxide level and lipid peroxidation in the rat brain during penthylenetetrazole-induced epileptiform model seizures. Prog Neuropsychopharmacol Biol Psychiatry, 27: 487-492.

Baulac S, Huberfeld G, Gourfinkel-An I, Mitropoulou G, Beranger A, Prud'homme J-F, Baulac M, Brice A, Bruzzone R, LeGuern E. 2001. First genetic evidence of GABAA receptor dysfunction in epilepsy: a mutation in the [gamma]2-subunit gene. Nat Genet, 28: 46-48.

Benedek B, Geisz N, Jager W, Thalhammer T, Kopp B. 2006. Choleretic effects of yarrow (Achillea millefolium s.l.) in the isolated perfused rat liver. Phytomedicine, 24: 702-706.

Benedek B, Rothwangl-Wiltschnigg K, Rozema E, Gjoncaj N, Reznicek G, Jurenitsch J, Kopp B, Glasl S. 2008. Yarrow (Achillea millefolium L. s.l.): pharmaceutical quality of commercial samples. Die Pharmazie, 63: 23-26.

Bosnak M, Ayyildiz M, Yildirim M, Agar E. 2007. The role of nitric oxide in the anticonvulsant effects of pyridoxine on penicillin-induced epileptiform activity in rats. Epilepsy Res, 76: 49-59.

Buisson A, Lakhmeche N, Verrecchia C, Plotkine M, Boulu RG. 1993. Nitric oxide: an endogenous anticonvulsant substance. Neuroreport, 4: 444-446.

Cavalcanti AM, Baggio CH, Freitas CS, Rieck L, de Sousa RS, Da Silva-Santos JE, Mesia-Vela S, Marques MC. 2006. Safety and antiulcer efficacy studies of Achillea millefolium L. after chronic treatment in Wistar rats. J Ethnopharmacol, 107: 277-284.

Ceccatelli S. 1997. Expression and Plasticity of NO Synthase in the Neuroendocrine System. Brain Res Bull, 44: 533-538.

Coitinho AS, de Mello CF, Lima TTF, de Bastiani J, Fighera MR, Wajner M. 2001. Pharmacological evidence that α-ketoisovaleric acid induces convulsions through GABAergic and glutamatergic mechanisms in rats. Brain Res, 894: 68-73.

Csupor-Loffler B, Hajdu Z, Zupko I, Rethy B, Falkay G, Forgo P, Hohmann J. 2009. Antiproliferative effect of flavonoids and sesquiterpenoids from Achillea millefolium s.l. on cultured human tumour cell lines. Phytother Res, 23: 672-676.

De Luca G, Di Giorgio RM, Macaione S, Calpona PR, Di Paola ED, Costa N, Cuzzocrea S, Citraro R, Russo E, De Sarro G. 2006. Amino acid levels in some brain areas of inducible nitric oxide synthase knock out mouse (iNOS−/−) before and after pentylenetetrazole kindling. Pharmacol Biochem Behav , 85: 804-812.

Dhir A, Naidu PS, Kulkarni SK. 2006. Effect of cyclooxygenase inhibitors on pentylenetetrazol (PTZ)-induced convulsions: Possible mechanism of action. Prog Neuropsychopharmacol Biol Psychiatry., 30: 1478-1485.

Ebrahimzadeh Bideskan AR, Hosseini M, Mohammadpour T, Karami R, Khodamoradi M, Nemati Karimooy H, Alavi H.2011. Effects of soy extract on pentylenetetrazol-induced seizures in ovariectomized rats. Zhong Xi Yi Jie He Xue Bao, 9: 611-618.

Elmann A, Mordechay S, Erlank H, Telerman A, Rindner M, Ofir R.2011. Anti-neuroinflammatory effects of the extract of Achillea fragrantissima. BMC Complement Altern Med, 11: 98.

Emanuelli T, Prauchner CA, Dacanal J, Zeni A, Reis EC, de Mello CF, de Souza DO. 2000. Intrastriatal administration of 5-aminolevulinic acid induces convulsions and body asymmetry through glutamatergic mechanisms. Brain Res, 868: 88-94.

Gupta N, Bhargava VK, Pandhi P. 2000. Tolerance and withdrawal to anticonvulsant action of clonazepam: role of nitric oxide. Methods Find Exp Clin Pharmacol, 22: 229-235.

Hachinski V. 1998. New antiepileptic drugs: the cost of innovation. Arch Neurol, 55: 1142.

Hosseini M, Ghasemzadeh Rahbardar M, Sadeghnia HR, Rakhshandeh H.2011. Effects of different extracts of Rosa damascena on pentylenetetrazol-induced seizures in mice. Zhong Xi Yi Jie He Xue Bao, 9: 1118-1124.

Hosseini M, Taiarani Z, Karami R, Abad AA.2011. The effect of chronic administration of L-arginine and L-NAME on morphine-induced antinociception in ovariectomized rats. Indian J Pharmacol, 43: 541-545.

Hosseini M, Sadeghnia HR, Salehabadi S, Alavi H, Gorji A. 2009. The effect of L-arginine and L-NAME on pentylenetetrazole induced seizures in ovariectomized rats, an in vivo study. Seizure, 18: 695-698.

Hosseini M, Taiarani Z, Hadjzadeh MA, Salehabadi S, Tehranipour M, Alaei HA.2011. Different responses of nitric oxide synthase inhibition on morphine-induced antinociception in male and female rats. Pathophysiology, 18: 143-149.

Hosseini M, Dastghaib SS, Rafatpanah H, Hadjzadeh MA, Nahrevanian H, Farrokhi I. 2010. Nitric oxide contributes to learning and memory deficits observed in hypothyroid rats during neonatal and juvenile growth. Clinics (Sao Paulo), 65: 1175-1181.

Itoh K, Watanabe M. 2009. Paradoxical facilitation of pentylenetetrazole-induced convulsion susceptibility in mice lacking neuronal nitric oxide synthase. Neuroscience, 159: 735-743.

Itoh K, Watanabe M, Yoshikawa K, Kanaho Y, Berliner LJ, Fujii H. 2004. Magnetic resonance and biochemical studies during pentylenetetrazole-kindling development: The relationship between nitric oxide, neuronal nitric oxide synthase and seizures. Neuroscience, 129: 757-766.

Javidnia K, Miri R, Sadeghpour H. 2004. Composition of the volatile oil of Achillea wilhelmsii C. Koch from Iran. DARU, 12: 63- 66.

Jayakumar AR, Sujatha R, Paul V, Puviarasan K, Jayakumar R. 1999. Involvement of nitric oxide and nitric oxide synthase activity in anticonvulsive action. Brain Res Bull, 48: 387-394.

Jiang W, Xiao L, Wang J-C, Huang Y-G, Zhang X. 2004. Effects of nitric oxide on dentate gyrus cell proliferation after seizures induced by pentylenetrazol in the adult rat brain. Neurosci Lett, 367: 344-348.

Kabuto H, Yokoi I, Mori A.1992. Monoamine metabolites, iron induced seizures, and the anticonvulsant effect of tannins. Neurochem Res. 17: 585-590.

Kamoshima W, Kitamura Y, Nomura Y, Taniguchi T. 1997. Possible involvement of ADP-ribosylation of particular enzymes in cell death induced by nitric oxide-donors in human neuroblastoma cells. Neurochem Int, 30: 305-311.

Karami R, Hosseini M, Khodabandehloo F, Khatami L, Taiarani Z.2011. Different effects of L-arginine on morphine tolerance in sham and ovariectomized female mice. J Zhejiang Univ Sci B, 12: 1016-1023.

Khan AM, Rezazadeh SH. 2010.Review on Iranian medicinal plants with antioxidant properties. J Med Plants, 9: 19-32s.

Klioueva IA, van Luijtelaar ELJM, Chepurnova NE, Chepurnov SA. 2001. PTZ-induced seizures in rats: effects of age and strain. Physiol Behav, 72: 421-426.

Lemmens-Gruber R, Marchart E, Rawnduzi P, Engel N, Benedek B, Kopp B. 2006. Investigation of the spasmolytic activity of the flavonoid fraction of Achillea millefolium s.l. on isolated guinea-pig ilea. Arzneimittelforschung, 56: 582- 588.

Lesani A, Javadi-Paydar M, Khodadad TK, Asghari-Roodsari A, Shirkhodaei M, Norouzi A, Dehpour AR. 2010. Involvement of the nitric oxide pathway in the anticonvulsant effect of tramadol on pentylenetetrazole-induced seizures in mice. Epilepsy Behav, 19: 290-295.

Marangoz C, Ayyildiz M, Agar E. 1994. Evidence that sodium nitroprusside possesses anticonvulsant effects mediated through nitric oxide. Neuroreport, 5: 2454-2456.

Moezi L, Shafaroodi H, Hassanipour M, Fakhrzad A, Hassanpour S, Dehpour AR. 2012. Chronic administration of atorvastatin induced anti-convulsant effects in mice: The role of nitric oxide. Epilepsy Behav, 23: 399-404.

Moncada S, Palmer RM, Higgs EA. 1991. Nitric oxide: physiology, pathophysiology, and pharmacology. Pharmacol Rev, 43: 109-142.

Mulsch A, Busse R, Mordvintcev PI, Vanin AF, Nielsen EO, Scheel-Kruger J, Olesen SP. 1994. Nitric oxide promotes seizure activity in kainate-treated rats. Neuroreport, 5: 2325-2328.

Nahrevanian H, Dascombe MJ. 2001. Nitric oxide and reactive nitrogen intermediates during lethal and nonlethal strains of murine malaria. Parasite Immunol, 23: 491-501.

Nahrevanian H, Najafzadeh M, Hajihosseini R, Nazem H, Farahmand M, Zamani Z. 2009. Anti-leishmanial effects of trinitroglycerin in BALB/C mice infected with Leishmania major via nitric oxide pathway. Korean J Parasitol, 47: 109-115.

Nemeth E, Bernath J. 2008. Biological activities of yarrow species (Achillea spp.). Curr Pharm Des, 14: 3151-3167.

Nidhi G, Balakrishnan S, Pandhi P. 1999. Role of nitric oxide in electroshock and pentylenetetrazole seizure threshold in rats. Methods Find Exp Clin Pharmacol, 21: 609-612.

Nowicki JP, Duval D, Poignet H, Scatton B. 1991. Nitric oxide mediates neuronal death after focal cerebral ischemia in the mouse. Eur J Pharmacol, 204: 339-340.

Noyan B, Jensen MS, Danscher G. 2007. The lack of effects of zinc and nitric oxide in initial state of pilocarpine-induced seizures. Seizure, 16: 410-416.

Osonoe K, Mori N, Suzuki K, Osonoe M. 1994. Antiepileptic effects of inhibitors of nitric oxide synthase examined in pentylenetetrazol-induced seizures in rats. Brain Res, 663: 338-340.

Paul V, Subramanian EH. 2002. Evidence for an involvement of nitric oxide and gamma aminobutyric acid in the anticonvulsant action of l-arginine on picrotoxin-induced convulsions in rats. Pharmacol Biochem Behav, 72: 515-519.

Peeters BW, van Rijn CM, Vossen JM, Coenen AM. 1989. Effects of GABA-ergic agents on spontaneous non-convulsive epilepsy, EEG and behaviour, in the WAG/RIJ inbred strain of rats. Life Sci, 45: 1171-1176.

Penix LP, Davis W, Subramaniam S. 1994. Inhibition of NO synthase increases the severity of kainic acid-induced seizures in rodents. Epilepsy Res, 18: 177-184.

Rakhshandah H, Hosseini M. 2006. Potentiation of pentobarbital hypnosis by Rosa damascena in mice. Indian J Exp Biol, 44: 910-912.

Sadeghian R, Fereidoni M, Soukhtanloo M, Azizi-Malekabadi H, Hosseini M.2012. Decreased nitric oxide levels in the hippocampus may play a role in learning and memory deficits in ovariectomized rats treated by a high dose of estradiol. Arq Neuropsiquiatr, 70: 874-879.

Shafaroodi H, Moezi L, Ghorbani H, Zaeri M, Hassanpour S, Hassanipour M, Dehpour AR. 2012. Sub-chronic treatment with pioglitazone exerts anti-convulsant effects in pentylenetetrazole-induced seizures of mice: The role of nitric oxide. Brain Res Bull, 87: 544-550.

Souri E, Amin G, Dehmobed-Sharifabadi A, Nazifi A, Farsam H. 2010.Antioxidative activity of sixty plants from Iran. Iran J Pharm Res, 3: 55-59.

Starr MS, Starr BS. 1993. Paradoxical facilitation of pilocarpine-induced seizures in the mouse by MK-801 and the nitric oxide synthesis inhibitor L-NAME. Pharmacol Biochem Behav, 45: 321-325.

Stojanovic G, Radulovic N, Hashimoto T, Palic R. 2005. In vitro antimicrobial activity of extracts of four Achillea species: the composition of Achillea clavennae L. (Asteraceae) extrac. J Ethnopharmacol, 101: 185-190.

Suzuki E, Nakaki T, Shintani F, Kanba S, Miyaoka H. 2002. Antipsychotic, antidepressant, anxiolytic, and anticonvulsant drugs induce type II nitric oxide synthase mRNA in rat brain. Neurochem Int, 333: 217-219.

Tozyo T, Yoshimura Y, Sakurai K, Uchida N, Takeda Y, Nakai H, Novel IH. 1994. antitumor sesquiterpenoids in Achillea millefolium. Chem Pharm Bull (Tokyo), 42: 1096-1100.

Tuberoso CIG, Kowalczyk A, Coroneo V, Russo MT, Dessی S, Cabras P. 2005. Chemical composition and antioxidant, antimicrobial, and antifungal activities of the essential oil of Achillea ligustica All. J Agric Food Chem, 53: 10148-10153.

Vahid S, Dashti-Khavidaki S, Ahmadi F, Amini M, Salehi Surmaghi MH.2012. Effect of herbal medicine achillea millefolium on plasma nitrite and nitrate levels in patients with chronic kidney disease: a preliminary study. Iran J Kidney Dis, 6: 350-354.

Yaeesh S, Jamal Q, Khan AU, Gilani AH. 2006. Studies on hepatoprotective, antispasmodic and calcium antagonist

activities of the aqueous-methanol extract of Achillea millefolium. Phytother Res, 20: 546-551.

Yazdanparast R, Ardestani A, Jamshidi S. 2007. Experimental diabetes treated with Achillea santolina: effect on pancreatic oxidative parameters. J Ethnopharmacol, 112: 13-18.