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Original Article

Antioxidant effect of *Achillea wilhelmsii* extract on pentylenetetrazole (seizure model)-induced oxidative brain damage in Wistar rats

Mahmoud Hosseini[1,2]*, Fatemeh Harandizadeh[3], Saeed Niazamand[2], Mohammad Soukhtanloo[4] and Maryam Mahmoudabady[2]

[1]Neurocognitive Research Center,
School of Medicine, Mashhad University of Medical Sciences, Iran
[2]Applied Physiology Research Center and Department of Physiology,
School of Medicine, Mashhad University of Medical Sciences, Iran
[3]Department of Biology, Faculty of Sciences,
Ferdowsi University of Mashhad, Mashhad, Iran
[4]Department of Biochemistry, School of Medicine,
Mashhad University of Medical Sciences

Abstract

An important role for oxidative stress both as a consequence and as a cause of epileptic seizures has been suggested**.** Since *Achillea wilhelmsii (A. wilhelmsii)* has been considered to have the antioxidant effects as well as central nervous system depressant properties, the anti-seizure effects of the plant extract in addition to its effects on brain tissues oxidative damage were investigated in pentylenetetrazole (PTZ)-induced seizures model. Male Wistar rats were divided into 5 groups: (1) Control, (2) PTZ, (3-5) *A. wilhelmsii* extract groups (AWE). The animals in groups 2-5 were treated with saline or AWE (100, 200 or 400 mg/kg) before single injection of PTZ (90 mg/kg). Latency to first minimal clonic seizure (MCS) and the first generalized tonic-clonic seizures (GTCS) were recorded. The brain tissues were then removed for biochemical measurements. MCS latencies in extract treated groups were not different from PTZ group. The animals treated by 200 mg/kg of AWE had a significant higher GTCS latency in comparison with PTZ group ($P<0.001$). The MDA levels in PTZ group were significantly higher and the total thiol concentrations were lower than control animals. Pretreatment with all 3 doses of the extract resulted in a significant reduction in the MDA levels ($P<0.05$, $P<0.01$ and $P<0.001$) and a significant elevation in total thiol concentration, as compared with PTZ group ($P<0.05$ and $P<0.01$). The present study showed that the hydroalcoholic extract of *A. wilhelmsii* possesses an antioxidant effect in the brain in PTZ induced seizure model.

INTRODUCTION

Epilepsy is a common and heterogeneous neurological disorder arising from biochemical and molecular events that are incompletely understood. To effectively manage epilepsies, it is important to understand the mechanisms underlying seizure-induced brain damage (1). However, the role of oxidative stress in epilepsies has only recently begun to be recognized (2). Neuronal hyperexcitability and excessive production of free radicals have been implicated in the pathogenesis of a considerable range of neurological disorders. The high rate of oxidative metabolism, coupled with the low antioxidant defenses and the richness in polyunsaturated fatty

***Corresponding author :**
Mahmoud Hosseini, Neurocognitive Research Center, School of Medicine, Mashhad University of Medical Sciences, Iran
E-mail : hosseinim@mums.ac.ir

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acids, makes the brain highly vulnerable to free radical damage. It has been found that prolonged seizures resulted in oxidative damage to lipids, DNA, and susceptible proteins (3). Also, increased amounts of free radicals have been reported during seizures (4). Regarding these facts, it seems that understanding of the role of oxidative stress in the pathophysiology of seizures is imoprtant (2). On the other hand, accumulating evidence also revealed the importance of oxidative stress as a consequence epileptic seizures (1, 5).

A limiting factor in long-term use of anti-epileptic drugs are adverse effects of these drugs such as teratogenic potential, dose-related and chronic toxicity (6). Several plants used for the treatment of epilepsy in different systems of traditional medicine have shown activity when tested in modern bioassays for the detection of anticonvulsant activity (7). *Achillea*, is one of the most important medicinal plants that belongs to the family of Compositae (8). Pharmacological effects of *Achillea* genus such as antiulcer (9), hepatoprotective (10) anti-inflammatory (11), antitumor (12), antispasmotic (10) and choleretic (13) have been reported.

Achillea Wilhelmsii (*A. Wilhelmsii*) is the major species of *Achillea* that wildly grows in different parts of Iran , Egypt and Turkey (14). *A. Wilhelmsii* locally known as "boomadaran" (Lavander cotton) is widely found in different part of Iran (15) has chemical components including borneol, linalol, caryophyllene, 1,8 Cineol, semithujone, flavonoids (rutin), glycoalkaloids, carvacrol, chrysanthenol acetate and camphor (14, 15). Some studies have indicated that *Achillea* species such as *A. santolina* (16), *A. ligustica* (17) and *A. clavennae* (18) have antioxidative activity which can reduce free radicals. Also it has been shown *Achillea* contains aromatic bitter substances and tannins which have important effects on the nervous system and neurological diseases such as neurasthenia, epilepsy and seizures (19). We therefore conducted this study to evaluate the effect of *A. Wilhelmsii* on seizures elicited by pentylenetetrazol (PTZ) in rats. The effects of the plant extract on brain tissues oxidative damage in PTZ- induced seizure model was also investigated.

Materials and Methods

Animals and grouping

This experimental research was done during 2012 in Mashhad University of Medical Sciences, Iran, 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, 40 virgin male Wistar rats, 250±20 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 50% relative humidity with laboratory chow and water provided *ad libitum*.

The animals were divided into 5 groups randomly (n=8 in each group) as follows: (1) Control, (2) Pentylenetetrazole (PTZ), (3-5) *Achillea wilhelmsii* extract (AWE). The animals in group 2 were treated with saline while, in groups 3-5 the animals were treated by *Achillea wilhelmsii* extract (100, 200 or 400 mg/kg) before injection of a single dose of PTZ (Sigma aldrich St. Louis, USA) (90 mg/kg body weight, i.p.). The animals were placed in a plexiglas arena and their behaviors were observed for 60 min (20, 21). The cortical tissues were then removed and submitted to biochemical measurements. In control group, saline was injected instead of PTZ and *Achillea wilhelmsii* extract, and the brain tissues were then removed without observing the behavior.

Plant extracts

Achillea wilhelmsii was collected from Nishabour city, Khorasan Province, Iran and identified by botanists in Ferdowsi University of Mashhad, Iran and a voucher number was deposited then the plants were dried at room temperature. To prepare hydroalcoholic extract, 50 g of the chopped and dried aerial parts of plant was 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.

PTZ-induced seizures

In order to observe ictal behavior, PTZ (90 mg/kg, i.p.) was injected and the animals were placed in plexiglas arena (30 cm × 30 cm × 30 cm) on the day of the experiment. The animals were observed during 60 min after PTZ administration. 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 (20, 21).

Biochemical assessment

After behavioral study, the animals were sacrificed, the brains were removed, the cortical tissues were separated and dissected on an ice-cold surface and conserved for biochemical measurements.

Total Sulfhydryl (SH) groups were measured using DTNB as the reagent. This reagent reacts with the SH groups to produce a yellow colored complex which has a peak absorbance at 412 nm (Ellman, 1959). Briefly, 1ml Tris-EDTA buffer (pH=8.6) was added to 50 µl brain homogenate in 1ml cuvettes and sample absorbance was read at 412 nm against Tris-EDTA buffer alone (A1). Then 20 µl DTNB reagents (10 mM in methanol) were added to the mixture and after 15 min (stored in laboratory temperature) the sample absorbance was read again (A2). The absorbance of DTNB reagent was also read as a blank (B). Total thiol concentration (mM) was calculated from the following equation (22, 23).

Total thiol concentration (mM) = (A2-A1-B) × 1.07/ 0.05 × 13.6.

Malondialdehyde (MDA) levels, as an index of lipid peroxidation, also were measured. MDA reacts with thiobarbituric acid (TBA) as a thiobarbituric acid reactive substance (TBARS) to produce a red colored complex which has peak absorbance at 535 nm. 2 ml from reagent of TBA/TCA/HCL was added to 1 ml of homogenate and the solution was heated in a water bath for 40 min. After cooling, the whole solutions were centrifuged within 1000 g for 10 min. The absorbance was measured at 535 nm. The MDA concentration was calculated as follows :

$$C(m) = \text{Absorbance}/(1.65 \times 10^5)(22\text{-}23).$$

Statistical analysis

The data were expressed as Mean±SEM and analyzed by using ANOVA followed by Tukey's post hoc comparison test. P values less than 0.05 were considered to be statistically significant.

Results

All the animals in different treatment groups (except the control group which did not receive PTZ) showed MCS and GTCS following a high dose PTZ administration (90 mg/kg). MCS latencies in extract treated groups were not different from PTZ group (Fig. 1). The animals which pre-treated by 200 mg/ kg of AWE had a significant higher latency in comparison with PTZ group (P<0.001, Fig. 2). There were no significant differences in mortality rate following PTZ administration between different treatment groups.

The MDA levels in PTZ group were significantly higher than control animals (P<0.001, Fig. 2). Pretreatment

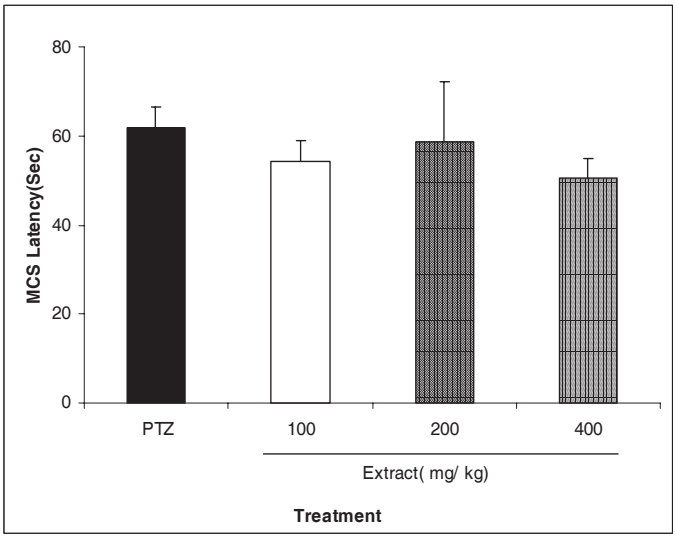

Fig. 1 : Latencies to minimal clonic seizures (MCS) onsets in PTZ and *Achillea wilhelmsii* extract (AWE) treated animals. The animals were treated with saline or AWE (100, 200 or 400 mg/kg) before a single injection (90 mg/kg) of PTZ.

TABLE I: Comparison of the MDA and total thiol concentrations in cortical tissues of Control, PTZ and *Achillea wilhelmsii* extract (AWE) treated animals. The animals were treated with saline or AWE (100, 200 or 400 mg/kg) before a single injection (90 mg/kg) of PTZ. The animals in Control group received saline instead of PTZ.

Groups	*Control (Saline, ml/kg)*	*PTZ (mg/kg)*	*Extract (mg/kg)*		
Dose	2	90	100	200	400
MDA Conc (nM/g tissue)	9.7±0.53	28.59±0.99***	21.62±1.85^{+}	19.73±0.67^{++}	6.23±1.01^{+++}
Total Thiol Conc. (mM)	20.19±1.44	9.08±3.58*	9.6±1.22	21.02±2.14^{+}	28.34±2.26^{++}

*P<0.05, ***P<0.001 as compared to Control group, $^{+}$P<0.05, $^{++}$P<0.01 and $^{+++}$P<0.001 as compared to PTZ group.

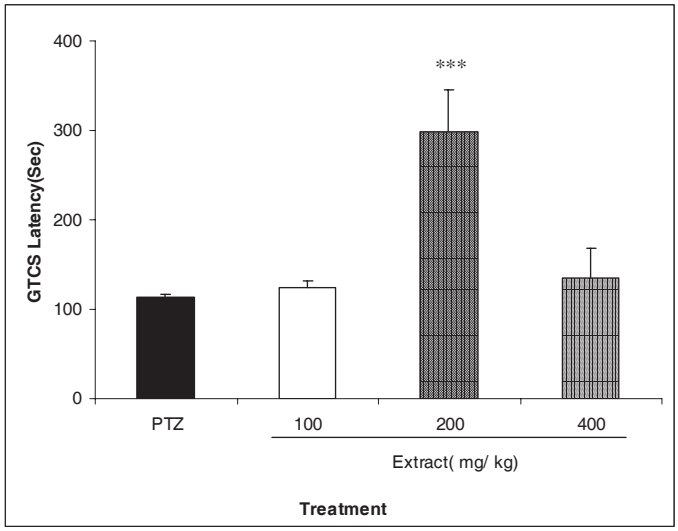

Fig. 2: Latencies to generalized tonic–clonic seizures (GTCS) onsets in PTZ and *Achillea wilhelmsii* extract (AWE) treated animals. The animals were treated with saline or AWE (100, 200 or 400 mg/kg) before a single injection (90 mg/kg) of PTZ. ***P<0.001 as compared to PTZ group.

with all 3 doses of the extract resulted in a significant reduction of the free radical-mediated lipid peroxidation as indicated by a decrease in the MDA levels (P<0.05, P<0.01 and P<0.001 respectively, Table I).

Following PTZ administration, a significant reduction in total SH groups in cortical samples was observed (P<0.05, Table I). Pretreatment with 200 and 400 mg/kg of the extract caused a significant elevation in total thiol concentration, as compared with PTZ group (P<0.05 and P<0.01).

Discussion

Oxidative stress is a basis for many neurological and neurodegenerative disorders. It is also regarded as a possible mechanism in the pathogenesis of epilepsy (1). This disease is accompanied with increased level of reactive oxygen species (ROS), including superoxide anions, hydroxyl radicals and hydrogen peroxide, in the brain (3, 4). It is suggested that brain tissues oxidative damage causes psychiatric and cognitive problems such as depression, anxiety and memory loss (24). Oxidative damage may also be responsible for decreasing of the life span in the epileptic persons (25). The results of present study confirmed that the level of brain tissue concentration of MDA in PTZ treated rats was higher and total SH groups was lower in PTZ treated rats than control ones. PTZ-induced seizure model has been frequently used to examine the drugs or natural compounds for their potential anticonvulsant properties (20, 21). This chemical compound decreases the GABA system function and stimulation while, it increases the activity of glutamate neurotransmission system (26). The contribution of ROS in the neurotoxic effects of PTZ has been suggested (6, 27) therefore, brain tissues oxidative damage which was seen in the present study is conceivable.

On the other hand, the animals pretreated with hydroalcoholic extract of *A. wilhelmsii* extract showed a reduction in the free radical-mediated lipid peroxidation and an elevation in thiol concentrations. Consistent with this finding, some studies reported strong antioxidant activity for *A. wilhelmsii* extract (15). In keeping with these observations in mind, the anticonvulsant activity of several agents with antioxidant effect such as melatonin, vineatrol, trans-resveratrol and alpha lipoic acid has been shown (5). Natural antioxidant compounds are usually

phenolic and polyphenolic including flavonoids compounds (15, 28) and the previous studies reported the considerable anticonvulsant effects of flavonoids (29). Also the presence of these compounds in some *Achillea* species extract has already been reported (30,31). *Achillea* species have also other components including, alkaloids (achilleine), cineol, borneol, α and β pinen, camphor, caryophllene, thujene, rutin, sesquiterpenoids and monoterpenoids (32, 33). Each of these components may have a role in the results of present study. For example the anticonvulsant effects of cineol has been suggested (34). It has also been shown that the plant extracts which contain alkaloids are potent anticonvulsants (35). Both the antioxidant and antioxidant properties of rutin has also been suggested (29, 36). Anticonvulsant activity of sesquiterpenoids of the plants in mouse seizure models has also been reported (37). *A. wilhelmsii* also contains other components including carvacrol, luteolin, apigenin and 1,8-cineole (31, 32, 33). It was previously known that carvacrol has anxiolytic-like effect which is probably mediated by GABA transmission (38). The hydroalcoholic extract of *A.millefolium L* had anxiolytic like effects in elevated plus maze and marble-burying test (39) which may be another evidence for its interaction with GABA system. It the present study, it was found that, *A.wilhelmsii* extract increased only GTCS latency without affecting MCS latency therefore, future studies using other kinds of the extract, other doses or even other animals models are needed to be done.

In contrast to these findings the analgesic effects of *Achillea* species has been attributed to their anti inflammatory effects (40). Taken together the results of the present study suggest that the antioxidant effects of the extract are more considerable than its anticonvulsant effects.

We assessed the effect of the extract by studying its effect on lipid peroxidation, which was measured in terms of MDA concentrations. Studies with human and animal models using the MDA assay generally report an increased lipid peroxidation in brain tissues in seizures and epilepsy (5,6). In our experiments, we observed a significant increase of lipid peroxidation in the brain. Clearly, the administration of the extract intraperitoneally at doses of 100, 200 and 400 mg/kg decreased the MDA levels to a considerable extent in brain tissues of the rats. To the best of our knowledge, this is the first study reporting the protective effect of the *A. wilhelmsii* extract on pentylenetetrazole (seizure model)-induced oxidative brain damage in rat. SH groups are known to be sensitive to oxidative damage and depleted following an oxidative insult (41); therefore, we studied the effect of the extract on total thiol concentrations in brain tissues after seizures. Similar to other studies, thiol (SH) groups were decreased in the brain following seizures injury. Treatment with 200 and 400 mg/ kg doses of the extract increased SH contents following seizures, indicating that *A. wilhelmsii* extract assisted in replenishing the total thiol pool. In our study, the reversal of peroxidative damage in the brain tissues by *A. wilhelmsii* may confirm its antioxidant and antiperoxidative properties as well as its potential role in defense against free radicals however; future studies using other methods are needed to be done to confirm the antioxidant effects of the plant.

In conclusion, our findings, for the first time, demonstrate that hydroalcoholic extract of *A. wilhelmsii* possess antioxidant effect in the brain in PTZ induced seizure model. Isolation the active compound(s) of the extract may yield novel useful agents against deleterious effects of epilepsy on the brain.

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