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Original Article

Effects of Hydroalcoholic Extract of *Matricaria Recutita L.* on Lipid Peroxidation and Nitric Oxide in Pentylenetetrazole-kindled Mice

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Abstract

Matricaria recutita L. (chamomile) is a well-known medicinal plant. As the main constituents of chamomile, flavonoids such as apigenin, act on benzodiazepine/GABA receptors. This study was designed to determine the protective effect of hydroalcoholic Matricaria recutita (MR) extract (hMRxt) on pentylenetetrazole (PTZ)-induced kindling model and lipid peroxidation in mice. Forty-eight male albino mice (25-30 g) were randomly divided into six groups (n=8). Kindling was induced by 13 PTZ injections (35 mg /kg; i.p.) every other day for 26 days. The seizure score was observed and noted until 30 minutes after the PTZ injection. Finally, the mice were decapitated and their brains were dissected out so as to assess some biochemical factors, namely malondialdehyde (MDA) and nitric oxide metabolites (NOx) in the brain tissue. One-way ANOVA was used for analysis in Prism 6.0. The results showed that hMRxt (400 mg/kg) and valproate (VAP) (150 mg/kg) significantly decreased the progression and duration of seizure induced by PTZ ($P < 0.001$). NOx level in the hMRxt (400 mg / kg), VAP (150 mg / kg) and the combination of hMRxt (50 mg / kg) + VAP (50 mg / kg) groups was significantly reduced compared to the PTZ group. Also, hMRxt (50 and 400 mg/kg) significantly increased the MDA level ($P < 0.001$) compared to PTZ and control groups. This study revealed that hMRxt protects mice against the PTZ-induced epilepsy via NO signaling with no effect on lipid peroxidation.

Keywords: Epilepsy, Pentylenetetrazol, Kindling, Malondialdehyde, Nitric oxide, Mice.

1. Introduction

Epilepsy refers to a group of chronic brain disorders characterized by recurrent seizures due to the abnormally excessive electrical discharges of cerebral neurons [1]. Approximately 70 million people around the world suffer from epilepsy [2]. Stafstrom et al. divided generalized seizures

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to different subtypes, each manifested with different signs, namely, absence: no clear response to external stimuli; generalized tonic-clonic: unconsciousness followed by bilateral symmetric convulsions of limbs; myoclonic: sudden, brief attacks with no obvious unconsciousness, and atonic: no muscular tone [3]. According to the International League Against Epilepsy classification, pentylenetetrazole (PTZ) kindling is an accepted model for simulating the primary generalized epilepsy [4].

PTZ kindling is a chronic epilepsy model characterized by a sustained increase in seizure susceptibility. At the molecular and cellular scales, PTZ kindling changes the levels of some substances responsible for the oxidative stress and cause neurodegenerative changes in the hippocampus [5]. Various pathophysiological mechanisms have been proposed for epilepsy, including oxidative stress, neurotransmitter alterations and the imbalance between gamma-aminobutyric acidergic (GABA-ergic) and glutamatergic system [6]. Nitrosative stress and oxidative stress resulting from excessive free radical release is well implicated in the initiation and progression of epilepsy [7]. Initially, oxidative stress was described as an imbalance between the generation and elimination of reactive oxygen species (ROS) and reactive nitrogen species (RNS) [8]. Production of ROS has a key role in the regulation of biological function, damage to cell structures, as well as in the pathogenesis of neurodegenerative diseases of the central nervous system (CNS), including epilepsy [9]. ROS are capable of attacking the polyunsaturated fatty acids (PUFA) within the cell membrane and cause chain reactions of lipid peroxidation (LPO) in the membrane [10]. The final product of lipid peroxidation is malondialdehyde (MDA) [9]. Moreover, increased MDA content in some tissues, such as the brain, may contribute to the increased generation of free radicals [5]. Furthermore, LPO is reported to be significantly higher in patients with seizures than that in normal people [11]. Likewise, ROS accumulation, mitochondrial dysfunction, LPO and brain edema have been observed following the prolonged epileptic seizures [8, 12].

Produced by NO synthase (NOS), nitric oxide (NO) is an important signaling molecule that essentially regulates various functions. NO acts as an intercellular messenger and is also involved in intracellular signaling and modulation of neuronal transmission. Studies report conflicting findings regarding the

involvement of NO in the pathophysiology of epilepsy [13]. Furthermore, *Matricaria recuitta* (MR), also known as chamomile [14], inhibits NO production and inducible NOS (iNOS) gene expression, which may suggest the indication of chamomile as an effective anti-inflammatory agent [15]. Different endogenous systems produce ROS and RNS in normal conditions. Nitrosative/oxidative stress representing an imbalance between the production and elimination of RNS and ROS combined with the reduced production of antioxidants causes oxidative toxic stress [16]. However, the role and function of NO in the induction or containment of seizures has not yet been fully elucidated.

Despite the existence of a number of antiepileptic drugs (AEDs), seizures remain refractory in approximately 40% of patients [17]. Therefore, patients with epilepsy need new treatment approaches. Recently, medicinal herbs have attracted the public attention due to their milder complications [18].

MR belongs to the Asteraceae family with 130 species, and is an annual plant indigenous to Ardebil, Iran [14]. The main constituents include several phenolic compounds, primarily the flavonoids, such as apigenin, quercetin, patuletin, luteolin, and their glycosides [14]. Examining the pharmacological profile of apigenin reveals that it acts on benzodiazepine receptors in the CNS [19]. However, there is no study available on the chronic administration of MR extract (MRxt) in the PTZ induced-kindling model of epilepsy in mice. Furthermore, the mechanism of action of MRxt on epilepsy has not been clearly determined. In the current study, we aimed to examine the effects of hydroalcoholic extract of MR (hMRxt) on LPO and NO in PTZ-kindled mice.

2. Material and Methods

2.1. Animals

Adult male NMRI mice (25–30 g) were used in the present study. Animals were obtained from the animal house of Kashan University of Medical Sciences (KUMS). Mice were housed under standard conditions including 22-25 °C and a 55±5% relative humidity in 12 h light/dark cycle. They were acclimated to laboratory conditions for a week before experimentation. Animals had free access to standard food and water [20]. The experimental protocol was approved by the Ethics Committee of Kashan University of Medical Sciences (IR.KAUMS.REC.1396.38) [21].

2.2. Drugs

PTZ, phosphate buffer saline (PBS), trichloroacetic acid, thiobarbituric acid, sodium nitrite, vanadium chloride, sulfanilamide, N-[1-naphthyl] ethylene-diamine-dihydrochloride, bovine serum albumin (BSA) and Bradford reagent were all purchased from Sigma (USA). Sodium valproate (VAP) purchased from Raha Pharmaceutical Co. (Isfahan) was used as a positive control in the present study. All

drugs were dissolved in the sterile saline and administered intraperitoneally (i.p.). hMRxt was diluted to the desired concentrations by adding sterile isotonic saline and administered (i.p) during the test days. P-cumaric acid apigenin 7-glucoside, luteolin and apigenin were obtained from Carl Roth (Karlsruhe, Germany).

2.3. Plant Material

The flowers of MR were collected from the Fandoghlo area in Ardabil (38.3822° N, 48.5550° E, altitude: 1600 m) during May 2018. The plant was identified by the Institute of Forests and Pastures of Iran; Herbarium number: 19856.

2.4. Extraction and Preparation of Test Samples

The plant was dried and milled, and the dried powder was poured into special containers with 300 g/L of ethanol (80%), and left to rest for 48 hours. After filtering, ethanol was removed from the solution by the rotary device. The extract (hMRxt) was further diluted with saline to obtain different doses [22]. The selected applied doses of hMRxt in our study (50 and 400 mg/kg) were based on the previous dose-response pilot study performed on broad dose ranges (12.5, 25, 50, 100, 200, 400, 800, 1600, and 3200 mg/kg) to determine the LD50 in mice [23]. The mice were also observed for major signs of toxicity (e.g. motor coordination, righting reflex and respiratory changes). Moreover, no abnormal behavior or mortality was reported in the aforementioned study. Our applied doses were far less than the reported LD50 value (3200 mg/kg/b.w.) for MR.

2.5. Standardization of Herbal Formulation by RP-HPLC

Herbal formulation was standardized according to the phenolic and flavonoid compounds by reverse-phase high-performance liquid chromatography (RP-HPLC). The quantitative analysis was performed with external standardization by measurement of the peak areas using the Lab Solutions software (Shimadzu, Japan). Four different concentrations of p-cumaric acid (1-10 µg/ml), apigenin 7-glucoside (5-20 µg/ml), luteolin (1-10 µg/ml) and apigenin (1-10 µg/ml) were used for the calibration curve. The HPLC column was a Spherisorb ODS-2 (5µm) reversed-phase 4.6 mm × 250 mm and the flow rate of the mobile phase (MeOH in H_2O/acetic acid (5–100% MeOH) was carried out at a 1.0 mL/min. The detector wavelength was set between 210 to 380 nm and detection was set at 340 nm. Different parameters including UV spectra, retention times, and comparison with phenolic and flavonoid standards were used for the identification of compounds. Each sample was injected into HPLC system three times.

2.6. Experimental Grouping

Forty-eight mice were divided into 6 groups (n=8). Group 1: control group was given saline (i.p.); Group 2: PTZ group was given PTZ (35 mg/kg/i.p.) for 13 consecutive sessions every 48 hours; Group 3: hMRxt50 + PTZ group was pretreated with hMRxt (50

mg/kg/i.p.), and then administered with PTZ (35 mg/kg/i.p.) for 13 consecutive sessions every 48 hours, Group 4: hMRxt400 + PTZ group was pretreated with hMRxt (400 mg/kg/i.p.), and then administered with PTZ (35 mg/kg/i.p.) for 13 consecutive sessions every 48 hours; Group 5: hMRxt50 + VAP50 + PTZ group was pretreated with hMRxt (50 mg/kg/i.p.) and VAP (50 mg/kg/i.p.), and then administered with PTZ (35 mg/kg/i.p.) for 13 consecutive sessions every 48 hours; Group 6: VAP 150 + PTZ group was pretreated with VAP (150 mg/kg/i.p.), and then administered with PTZ (35 mg/kg/i.p.) for 13 consecutive sessions every 48 hours.

2.7. PTZ Kindlin

All animals, except for the control group, were kindled by the injections of PTZ (35 mg/kg/i.p) on every alternate day for 26 days (13 injections), according to the standardized procedure [24]. In the four treatment groups (different doses of hMRxt and VAP), PTZ was administrated 45 min after treatment with different doses of hMRxt and VAP [25]. After each injection of PTZ, seizure behaviors were monitored for 30 min and seizure score was evaluated using the following modified scale [26]: 0 no response; 1 ear and facial twitching; 2 convulsive waves axially through the body; 3 myoclonic; 4 generalized clonic convulsions turn over into side position; 5 generalized convulsions with tonic extension episode and status epilepticus (the latency and duration are presented in Table 2).

2.8. Brain Tissue Preparation and Biochemical Assays

At the end of the experiment period, all animals were sacrificed and their brains were immediately removed and washed in cold saline twice. The brains were placed into a glass bottle and stored in a deep freeze (-80˚ C). The brain tissues were homogenized with ice-cold 0.1 M PBS (pH 7.4), and the homogenized solution was centrifuged for 5 min at 10000 ×g to remove residue. After centrifugation, the clear upper layer was taken and used for the estimation of biochemical parameters (MDA and NO).

2.9. MDA Evaluation

The MDA level in the supernatant was measured according to the following protocol. Thiobarbituric acid was added to the supernatant, then mixed and incubated at 100˚C for 30 min. After being cooled on ice, the samples were centrifuged at 3500×g for 10 min and the absorbance of the supernatant was read at 532 nm. The results were expressed in nmol MDA/g protein [27].

2.10. NO Metabolites (NOx) Measurement

The levels of NOx (nitrite and nitrate) were considered as an indicator of nitric oxide activity in brain tissue. The brain NOx measurement was based on the Griess reaction. Briefly, 0.1 mL of homogenated solution was deproteinized by adding 0.2 mL of acetonitrile solution and centrifuged for 5 min at 10000 × g. An amount of 0.1 mL of

supernatant, pure water (as blank) or sodium nitrite (as standard) was mixed with 0.1 mL of vanadium chloride. An amount of 0.05 mL sulfanilamide (0.01 %) and 0.05 mL N-[1-naphthyl] ethylene-diamin-dihydrochloride (NED, 0.01 %) were incubated at 37 °C for 30 min in a dark place. The absorbance was read at 540 nm with a spectrophotometer. NO concentration was expressed as nmol/mg protein [28].

2.11. Protein Measurement

The protein content of the brain homogenate was analyzed based on the Bradford method. BSA was used as standard. Brain homogenate (5 µl) was added to 200 µl of Bradford reagent and incubated at 37°C for 10 min. The absorbance was recorded at 595 nm by a microplate spectrophotometer [29].

2.12. Statistical Analyses

All data were analyzed using one-way analysis of variance (ANOVA), followed by Dunnett's post hoc test, and are presented as mean ± SEM. A $P < 0.05$ is considered as statistically significant. Statistical analysis was performed in Prism 6.0.

3. Results and Discussion

3.1. HPLC Analysis

Quantitative HPLC analysis indicated that the flavonoid compounds in the herbal formulation were apigenin 7- glucoside (674.0 µg/ml), apigenin (137.8 µg/ml), p-cumaric acid (124.4 µg/ml) and luteolin (139.2 µg/ml). The analysis revealed that apigenin 7- glycoside and apigenin contents in the extract were higher than other flavonoids. The apigenin 7-glucoside content in the crude extracts was much higher than the free apigenin (Table 1 and Figure 1).

3.2. Effect of hMRxt and VAP on Seizure Score Induced by PTZ

Fig. 2 shows the effect of repeated administration of the sub-convulsant dose of PTZ (35 mg/kg) in PTZ treated group on every alternate day (13 injections) resulted in increasing convulsive activity leading to generalized clonic–tonic seizures. Furthermore, pretreatment with hMRxt in hMRxt400 and (hMRxt50+VAPl50+PTZ) groups significantly suppressed the progression of kindling, as evidenced by a decrease in seizure score, compared to the PTZ group. Moreover, the VAP150 group suppressed the process of kindling, compared to the PTZ group.

3.3. Effects of hMRxt on MDA Level in PTZ-Kindled Mice

Fig. 3 shows the effects of effective and ineffective doses of hMRxt (400 and 50 mg/kg, respectively) per se as the pre-treatments, effective and ineffective doses of VAP as the positive control and the coadministration of ineffective doses of hMRxt and VAP, on brain MDA level (nmol/mg protein) in all groups. The one-way ANOVA showed a significant difference among the groups ($P<0.001$). The post-hoc

Dunnett's test also showed a significant difference, compared to the control (P<0.001), and a significant difference compared to the PTZ group (P< 0.01 and P< 0.001), respectively.

3.4. Effects of hMRxt on NO Level in PTZ-Kindled Mice

Fig. 4 shows the effects of effective and ineffective doses of hMRxt (400 and 50 mg/kg, respectively) per se as the pre-treatments, effective and ineffective doses of VAP as the positive control and the coadministration of ineffective doses of hMRxt and VAP on brain NO level (nmol/mg protein) in all groups. ANOVA showed a significant difference among the groups (P<0.001). The post-hoc Dunnett's test also showed a significant difference, compared to control (P<0.001); and a significant difference compared to the PTZ group (P<0.001), respectively.

In the present study, we evaluated the effects of hMRxt on LPO and NOx in the PTZ-kindled mice. We found that the administration of PTZ (35 mg/kg) on alternative sessions for 26 days (13 injections) significantly increased the mean seizure score and developed a generalized tonic-clonic seizure in mice. However, the hMRxt pretreatment (400 mg/kg) and coadministration of a sub-effective dose of hMRxt (50 mg/kg) and VAP (50 mg/kg) significantly attenuated the PTZ induced-seizure score, presented as the longer latency and shorter duration of 5th phase in Table 2. NOx level alterations in mice also indicate the anti-epileptogenic potential of hMRxt.

As mentioned previously, MR belongs to the Asteraceae family, which consists of 130 species, and is an annual plant indigenous to Ardebil, Iran [14]. The main constituents include several phenolic compounds, primarily flavonoids such as apigenin, quercetin, patuletin, luteolin, and their glycosides. Apigenin competitively binds to the benzodiazepine binding site of $GABA_A$ receptors [30].

Benzodiazepines are the main class of psychoactive agents with efficient therapeutic properties and commonly used in clinical practice. Given the mechanism of benzodiazepines as indirect GABA modulators, they can augment the GABAA channel current [31,32].

Mechanistically, considering the similarity of flavonoids to the benzodiazepines, the antiepileptic action of flavonoids occurs via the modulation of the $GABA_A$ channels [6]. Similarly, apigenin acts through its competitive binding to the benzodiazepine site of the $GABA_A$ receptors [19]. It is presumed that apigenin binds to the aforementioned receptors, results in an increase in Cl^- influx, and causes the antiepileptic effect of the hMRxt.

Numerous ingredients with diverse pharmacological actions are identified in chamomile including terpenoids and flavonoids such as apigenin [18]. In particular, among the flavonoids are apigenin 7-glucoside, apigenin, luteolin, and p-cumaric acid. One of the highly potent

flavonoids found in chamomile with a high affinity to benzodiazepine receptors is apigenin [18, 29, 31].

However, according to other animal studies, apigenin exerts a tranquilizing effect via its inverse agonistic action on benzodiazepine receptors and as a result is considered as a weak epileptic agent [33]. In addition, an inhibitory effect against excitotoxicity has been reported for apigenin [34]. The antagonistic action of apigenin on glutamate conductance through kainate receptors may block the onset and spread of epileptic activity. Therefore, it is suggested that apigenin found in high-dose hMRxt (400 mg/kg group) has the potential of alleviating the seizure activity by modulating the GABA channels. Moreover, it is reported that the inflammatory agents (e.g. PGE2) cause the glutamate release from astrocytes [35; 36]. It is believed that chamomile alleviates the epileptic attacks through its anti-inflammatory effect and its inhibitory effect on PGE2 generation, induced by apigenin 7-glucoside and the decreased glutamate release [37].

Considering brain's high oxygen demand and its higher susceptibly to oxidative insult induced by ROS and RNS, the epileptic activity can increase the ROS/RNS generation [38]. The accumulation of free radicals and the augmentation of epileptic activity occur by activation of glutamine synthase and consequently the accumulation of glutamate [39]. NO as a multipotential messenger and transmitter in the nervous system confers different physiological and pathological effects [40]. The involvement of NO in the pathogenesis of epilepsy is also reported [41, 42]. Accordingly, Itoh et al. have reported an increase in the neuronal NOS activity following PTZ kindling [43]. In our study, the NO level in the hMRxt (400 mg/kg) group, VAP (150 mg/kg) group and the coadministration of sub-effective dose of hMRxt50 plus VAP (50 mg/kg) group were significantly reduced, compared to the PTZ group and led to the attenuation of PTZ-induced epilepsy. Hydrophilic components found in chamomile (flavonoids, including apigenin as the major component) are reported to inhibit iNOS expression in activated macrophages and can lead to the inhibition of NO release and synthesis [37].

The above-mentioned reports for the first time demonstrate the finding that hMRxt may act via the same pathway that VAP acts in a kindling model in mice. However, the details of the molecular mechanisms involved in the process require further investigation.

It is reported that the antiepileptic action of VAP is probably due to its effects on the GABAergic system, through enzymatic reaction and a reduction in excitatory neurotransmission [17]. Therefore, since our results revealed that an ineffective dose of hMRxt in combination with an ineffective dose of VAP could attenuate seizure activity and decrease the NOx, it is assumed that hMRxt may act through the same mechanism as VAP, which can potentiate the chamomile effect to improve epileptic seizures. Since NO plays an important role in mediating inflammatory responses and the

pathophysiology of epilepsy, our study supports the use of chamomile as a potentially effective therapeutic anti-inflammatory and antiepileptic agent. Nonetheless, chamomile is classified as safe in FDA reports, only rare mild allergic reactions are mentionable [18]. A limitation of the present study is that the Griess method for assaying the total NO is an indirect method. Hence, we recommend designing similar studies in future to use some direct and precise methods for NO assaying (e.g., electron paramagnetic resonance spectroscopy) [44].

4. Conclusion

The results of our study showed that the use of hMRxt with increasing MDA levels can have oxidizing effects on lipid products, so hMRxt should be applied as a medicinal herb with caution. It is presumed that hMRxt constituents damage the cell membrane and result in MDA release into surrounding tissues. Overall, hMRxt increases the threshold for PTZ-induced epilepsy probably through the NO pathway.

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References

[1] Acharya UR, Sree SV, Swapna G, Martis RJ, Suri JS. Automated EEG analysis of epilepsy: a review. *Knowl. Based. Syst.* (2013) 45: 147-165

[2] Ngugi AK, Kariuki S, Bottomley C, Kleinschmidt I, Sander J, Newton C. Incidence of epilepsy: a systematic review and meta-analysis. *Neurology* (2011) 77(10): 1005-1012.

[3] Stafstrom CE, Carmant L. Seizures and epilepsy: an overview for neuroscientists. *Cold .Spring Harb .Perspect. Med.* (2015) 5(6): a022426.

[4] Sefil F, Arık AE, Acar MD, Bostanci MÖ, Bagirici F, Kozan R. Interaction between carbenoxolone and valproic acid on pentylenetetrazole kindling model of epilepsy. *Int. J. Clin. Exp. Med.* (2015) 8(7): 10508.

[5] Zhu X, Shen K, Bai Y, Zhang A, Xia Z, Chao J, Yao H. NADPH oxidase activation is required for pentylenetetrazole kindling-induced hippocampal autophagy. *Radic. Biol. Med.* (2016) 94: 230-242.

[6] Choudhary N, Bijjem KR, Kalia AN. Antiepileptic potential of flavonoids fraction from the leaves of Anisomeles malabarica. *J. Ethnopharmacol.* (2011) 135(2): 238-242.

[7] Kiasalari Z, Khalili M, Roghani M, Sadeghian A. Antiepileptic and Antioxidant Effect of Brassica nigra on Pentylenetetrazol-Induced Kindling in Mice. *Iran J. Pharm. Res.* (2012) 11(4): 1209-1217.

[8] Aguiar CC, Almeida AB, Araujo PV, de Abreu RN, Chaves EM, do Vale OC, Macedo DS, Woods DJ, Fonteles MM, Vasconcelos SM. Oxidative stress and epilepsy: literature review. *Oxid. Med. Cell. Longev.* (2012) 2012: 795259.

[9] Malinska D, Kulawiak B, Kudin AP, Kovacs R, Huchzermeyer C, Kann O, Szewczyk A, Kunz WS. Complex III-dependent superoxide production of brain mitochondria contributes to seizure-related ROS formation. *Biochim. Biophys. Acta.* (2010) 1797(6): 1163-1170.

[10] Ardjmand A, Shahaboddin ME, Mazoochi T, Ghavipanjeh G. Ameliorative effects of cerebrolysin against isoproterenol-induced myocardial injury in male rats. *Life Sci.* (2019) 227: 187-192.

[11] Menon B, Ramalingam K, Kumar RV. Oxidative stress in patients with epilepsy is independent of antiepileptic drugs. *Seizure* (2012) 21(10): 780-784.

[12] Shin EJ, Jeong JH, Chung YH, Kim WK, Ko KH, Bach JH, Hong JS, Yoneda Y, Kim HC. Role of oxidative stress in epileptic seizures. *Neurochem. Int.* (2011) 59(2): 122-137.

[13] Banach M, Piskorska B, Czuczwar SJ, Borowicz KK. Nitric oxide, epileptic seizures, and action of antiepileptic drugs. *CNS. Neurol. Disord.Drug Targets* (2011) 10(7): 808-819.

[14] Can ÖD, Özkay ÜD, Kıyan HT, Demirci B. Psychopharmacological profile of Chamomile (Matricaria recutita L.) essential oil in mice. *Phytomedicine* (2012) 19(3-4): 306-310.

[15] Bhaskaran N, Shukla S, Srivastava JK, Gupta S. Chamomile: an anti-inflammatory agent inhibits inducible nitric oxide synthase expression by blocking RelA/p65 activity. *Int. J .Mol .Med.* (2010) 26(6): 935-940.

[16] Kheiripour N, Karimi J, Khodadadi I, Tavilani H, Goodarzi MT, Hashemnia M. Silymarin prevents lipid accumulation in the liver of rats with type 2 diabetes via sirtuin1 and SREBP-1c. *J. Basic. Clin. Physiol. Pharmacol.* (2018) 29 (3): 301-308.

[17] Brodie MJ. Antiepileptic drug therapy the story so far. *Seizure* (2010) 19 (10): 650-655.

[18] Srivastava JK, Shankar E, Gupta S. Chamomile: A herbal medicine of the past with a bright future. *Mol. Med. Rep.* (2010) 85(19-20): 663-669.

[19] Keefe JR, Mao JJ, Soeller I, Li QS, Amsterdam JD. Short-term open-label chamomile (Matricaria chamomilla L.) therapy of moderate to severe generalized anxiety disorder. *Phytomedicine* (2016) 23(14): 1699-1705.

[20] Abbas B, Ahmad J, Hasan A, Mohsen T, Abolfazl A, Hosein A. Effects of the alcoholic extract of white mulberry leaves on behavioral performance of rats.*Biomed .Pharmacol.J.* (2015) 8715-719.

[21] Mobasher M, Sasani P, Aledavood SJ, Aramesh K, Larijani B. Revision of the guideline for ethical use of animals. *J. Med. Ethics Hist. Med.* (2012) 5(1): 70-111.

[22] Shoorei H, Khaki A, Ainehchi N, Taheri MMH, Tahmasebi M, Seyedghiasi G, Ghoreishi Z, Shokoohi M, Khaki AA, Raza SH. Effects of Matricaria chamomilla extract on growth and maturation of isolated mouse ovarian follicles in a three-dimensional culture system. *Chin. Med. J.* (2018) 131(2): 218.

[23] Sebai H, Jabri M-A, Souli A, Rtibi K, Selmi S, Tebourbi O, ElBenna J, Sakly M. Antidiarrheal and antioxidant activities of chamomile (Matricaria recutita L.) decoction extract in rats. *J. Ethnopharmacol.* (2014) 152(2): 327-332.

[24] Rahmati B, Khalili M, Roghani M, Ahghari P. Anti-epileptogenic and antioxidant effect of Lavandula officinalis aerial part extract against pentylenetetrazol-induced kindling in male mice. *J. Ethnopharmacol.* (2013) 148(1): 152-157.

[25] Homayoun H, Khavandgar S, Dehpour AR. The role of α2-adrenoceptors in the modulatory effects of morphine on seizure susceptibility in mice. *Epilepsia* (2002) 43(8): 797-804.

[26] Ilhan A, Gurel A, Armutcu F, Kamisli S, Iraz M. Antiepileptogenic and antioxidant effects of Nigella sativa oil against pentylenetetrazol-induced kindling in mice. *Neuropharmacology* (2005) 49(4): 456-464.

[27] Buege JA, Aust SD. Microsomal lipid peroxidation. *Methods Enzymol.* (1978) 52: 302-310.

[28] Sun J, Zhang X, Broderick M, Fein H. Measurement of nitric oxide production in biological systems by using Griess reaction assay. *Sensors* (2003) 3(8): 276-284.

[29] Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* (1976) 72(1): 248-254.

[30] Wasowski C, Marder M. Flavonoids as GABAA receptor ligands: the whole story? *J. Exp. Pharmacol.* (2012) 4: 9.

[31] Jahani R, Mojab F, Mahboubi A, Nasiri A, Tahamtani A, Faizi M. An In-vivo study on anticonvulsant, anxiolytic, and sedative-hypnotic effects of the polyphenol-rich Thymus kotschyanus extract; evidence for the involvement of GABA-A Receptors. *Iran J. Pharm. Res*. (2019) 18(3):1456-1465.

[32] Jahani R, Khaledyan D, Jahani A, Jamshidi E, Kamalinejad M, Khoramjouy M, Faizi M. Evaluation and comparison of the antidepressant-like activity of Artemisia dracunculus and Stachys lavandulifolia ethanolic extracts: an *in vivo* study. *Res. Pharm. Sci*. (2019) 4(6):544-553.

[33] Salehi B, Venditti A, Sharifi-Rad M, Kręgiel D, Sharifi-Rad J, Durazzo A, Lucarini M, Santini A, Souto EB, Novellino E. The therapeutic potential of apigenin. *Int. J. Mol. Sci.* (2019) 20(6): 1305.

[34] Han JY, Ahn SY, Kim CS, Yoo SK, Kim SK, Kim HC, Hong JT, Oh KW. Protection of apigenin against kainate-induced excitotoxicity by anti-oxidative effects. *Biol.Pharm. Bull.* (2012) 35(9): 1440-1446.

[35] Rana A, Musto AE. The role of inflammation in the development of epilepsy. *J. Neuroinflammation* (2018) 15(1): 144.

[36] Srivastava JK, Pandey M, Gupta S. Chamomile, a novel and selective COX-2 inhibitor with anti-inflammatory activity. *Life Sci.* (2009) 85(19-20): 663-669.

[37] Zargaran A, Borhani-Haghighi A, Faridi P, Daneshamouz S, Kordafshari G, Mohagheghzadeh A. Potential effect and mechanism of action of topical chamomile (Matricaria chammomila L.) oil on migraine headache: A medical hypothesis.*Med. Hypotheses* (2014) 83(5): 566-569.

[38] Grosso C, Valentão P, Ferreres F, B Andrade P. The use of flavonoids in central nervous system disorders. *Curr. Med. Chem.* (2013) 20(37): 4694-4719.

[39] Naziroglu M, Akay MB, Celik O, Yildirim MI, Balci E, Yurekli VA. Capparis ovata modulates brain oxidative toxicity and epileptic seizures in pentylentetrazol-induced epileptic rats. *Neurochem. Res.* (2013) 38(4): 780-788.

[40] Hoffman M. A new role for gases: neurotransmission. *Science* (1991) 252(5014): 1788.

[41] De la Torre J, Pappas B, Prevot V, Emmerling M, Mantione K, Fortin T, Watson M, Stefano G. Hippocampal nitric oxide upregulation precedes memory loss and A β 1-40 accumulation after chronic brain hypoperfusion in rats. *Neurol. Res.* (2003) 25(6): 635-641.

[42] Wojtal K, Gniatkowska-Nowakowska A, Czuczwar SJ. Is nitric oxide involved in the anticonvulsant action of antiepileptic drugs. *Pol. J. Pharmacol.* (2003) 55: 535-542.

[43] Itoh K, Watanabe M, Yoshikawa K, Kanaho Y, Berliner LJ, Fujii H. Magnetic resonance and biochemical studies during pentylenetetrazole-kindling development: the relationship between nitric oxide, neuronal nitric oxide synthase and seizures. *Neuroscience* (2004) 129(3): 757-766.

[44] Kozlov AV, Biagini G, Tomasi A, Zini I. Ex vivo demonstration of nitric oxide in the rat brain: effects of intrastriatal endothelin-1 injection. *Neurosci. Lett.* (1995) 196(1-2): 140-144.

Tables:

Table 1. The content of main flavonoids in *Matricaria recutita L.* extract, determined using the HPLC-UV method. The data are presented as mean±SEM.

Compounds Name	**Regression equation**	**R^2**	**RSD (% w/w)**	**Amount (µg/ml)**
Apigenin 7- glucoside	y=105.18x - 77.99	0.993	0.89	674.0±4.9
Apigenin	y=71.15x - 94.42	0.995	0.95	137.8±1.07
P-cumaric acid	y=30.15x + 8.92	0.999	1.17	124.4±1.25
Luteolin	y=23.69x – 19.00	0.997	1.35	139.2±1.54

Table 2. Effect of sodium valproate and hydroalcoholic *Matricaria recutita* extract on the latency and duration of 5th phase seizure. The data were expressed as mean ± SEM (n=8 in each group).

Experimental groups	**Latency of 5th phase (min)**	**Duration of 5th phase(sec)**
PTZ	2.54±0.09	**23.71±2.58**
VAP150+PTZ	**6.84±1.21	***9.5±1.09**
hMRxt 50+PTZ	3.09±0.65	**19.5±1.38**
hMRxt 400+PTZ	*6.12±1.16	***10±1.15**
hMRxt 50+VAP50+PTZ	3.78±1.04	**16.57±3.73**

Figures:

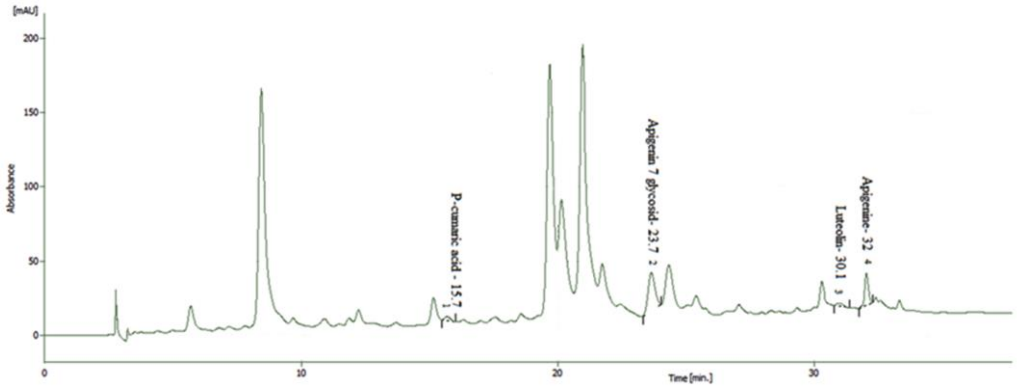

Figure 1. HPLC chromatogram obtained for main flavonoids content in *Matricaria recutita L.* extract. Four different concentrations of p- cumaric acid (1-10 µg/ml), apigenin 7-glucoside (5-20 µg/ml), luteolin (1-10 µg/ml) and apigenin (1-10 µg/ml) were used for the calibration curve. The wavelength of detector was ranged between 210 to 380 nm and detection set at 340 nm. Each sample was injected three times on HPLC system.

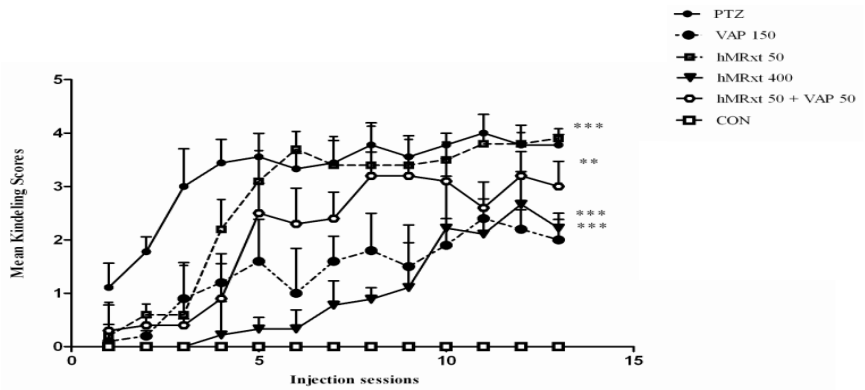

Figure 2. Seizure score in PTZ kindling model. Effect of hMRxt pretreatment on the development of PTZ-induced kindling. Seizure scores are expressed as mean ± SEM (n=8 in each group). $^{**}P<0.01$, $^{***}P<0.001$ compared with PTZ group using one-way ANOVA followed by Dunnett's test as a post-test. PTZ : Pentylenetetrazole; hMRxt : Hydroalcoholic *Matricaria recutita* extract; CON: Control; VAP: Sodium valproate.

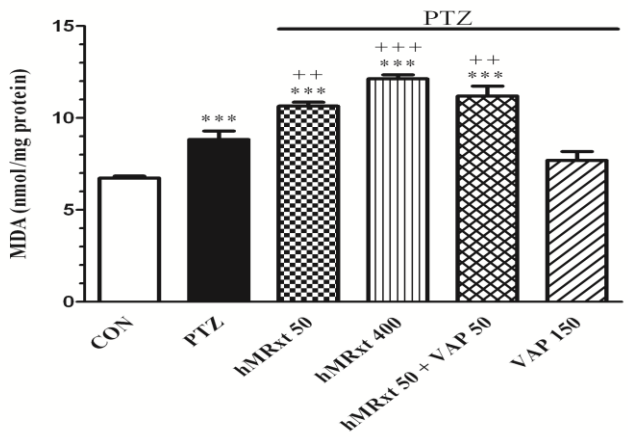

Figure 3. The effects of effective and ineffective doses of hMRxt (400 and 50 mg/kg, respectively) per se, as the pre-treatments, effective and ineffective doses of VAP, as the positive control and the coadministration of ineffective doses of hMRxt and VAP, on brain MDA level (nmol/mg protein) in all groups in the PTZ-kindled mice. Data are expressed as mean ± SEM (n=8 in each group). *** Statistically significant, as compared to the CON group. +++ Statistically significant as compared to the PTZ group. ++ Statistically significant as compared to the PTZ group. PTZ :Pentylenetetrazole; hMRxt: Hydroalcoholic *Matricaria recutita* extract; CON: Control; VAP: Sodium valproate.

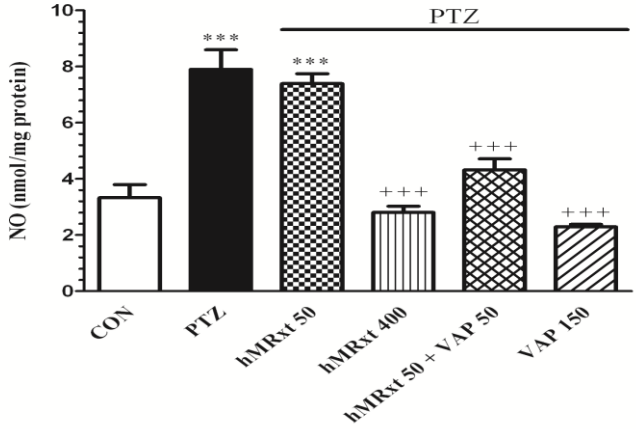

Figure 4. The effects of effective and ineffective doses of hMRxt (400 and 50 mg/kg, respectively) per se, as the pre-treatments, effective and ineffective doses of VAP, as the positive control and the coadministration of ineffective doses of hMRxt and VAP, on brain NO level (nmol/mg protein) in all groups in the PTZ-kindled mice. Data were expressed as mean ± SEM (n=8 in each group). *** Statistically significant compared to the control group. +++ Statistically significant compared to the PTZ group. PTZ :Pentylenetetrazole; hMRxt: Hydroalcoholic *Matricaria recutita* extract; CON: Control; VAP: Sodium valproate.