Innovare Academic Sciences

International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 6, Issue 4, 2014

Original Article

STUDY OF ANTICONVULSANT EFFECT OF ETHYL ACETATE FRACTION OF *MATRICARIA RECUTITA* EXTRACT IN MICE

MAHA NOORI HAMAD[2], AMAL AJAWEED SULAIMAN[1]*, INTESAR TARIK NUMAN[1] AND SAIF AL DEEN ABDULWAHAB ABDUL RAZAK[3]

[1] Department of Pharmacology and Toxicology, College of Pharmacy, University of Baghdad, Iraq , [2] Department of Pharmacognosy and Medicinal Plants, College of Pharmacy, University of Baghdad, Iraq, [3] Ministry of health, medical city, Baghdad, Iraq.

Email: amal3_ajaweed@yahoo.com

Received: 04 Feb 2014 Revised and Accepted: 25 Feb 2014

ABSTRACT

Objective: The current study was aimed to identify the active phytochemicals of ethyl acetate fraction of *Matricaria recutita* and evaluate the protection offered by this fraction against strychnine induced seizure in mice.

Methods: Ethyl acetate fraction of the plant extract was initially analyzed for the presence of flavonoids by high pressure liquid chromatography (HPLC) analysis. The anticonvulsant effect of plant extract (25mg / Kg) was investigated in comparison with standard drug (diazepam 0.5 mg/Kg) using animal model of strychnine induce seizure. Both treatments were given intraperitoneally 60 minutes before the animals challenged with strychnine proconvulsant. Latency to the onset and duration of seizure were determined relative to untreated control group.

Result: HPLC analysis indicated the presence of (apigenin-7-O-glycoside, as a major constituent together with apigenin, rutin, and quercetin, while kaemferol was not detected) in ethyl acetate fraction of the extract. Both tested fraction and standard drug showed significant increase in the onset time to seizure and the survival time with obvious decrease in the severity of the attack compared to control.

Conclusion: The obtained data revealed the potential protective effect of ethyl acetate fraction of M. recutita against epileptic seizure induced by strychnine in mice. However, further studies are needed to investigate the dose response relationship of this fraction.

Keywords: Matricaria recutita, Anticonvulsant, Strychnine

INTRODUCTION

Epilepsy is a group of heterogeneous neurological disorders that characterized by spontaneous and recurrent seizures due to sudden excessive discharge of impulses by a group of cerebral neurons in the brain. It's affect 1% of the population worldwide, being the second most common neurological disorder after stroke, that associated with a variety of causative factors as; trauma, oxygen deprivation, tumors, infection and metabolic derangements producing long lasting plastic changes in the brain affecting neurotransmitters release and transport, the properties of receptors and channels, regulation of gene expression, synaptic reorganization and astrocyte activity [1,2]. However, no specific factors are found in about half of patients suffering from epilepsy[3,4]. Actually, Modern drug therapy, even multi-drug therapy of epilepsy is not effective in some patients and associated with unwanted effects, dose-related and chronic toxicity, teratogenic effects and around 30% of the patients continue to have seizures with current AED (Anti epileptic drug) therapy, therefore extensive research directed towards remedies from plants especially those claimed to have beneficial effect against serious disorders such as epilepsy [5,6]. *Matricaria recutita* is one of the most popular and widely used medicinal plants, of the family *Asteraceae.* Extracts prepared from M. recutita have been reported for their diverse ranges of pharmacological actions including; antispasmodic, anti-platelet, antipruritic, anti-inflammatory, immune-modulatory, anti-diabetic, anti-proliferative, antimicrobial, antiviral, antiulcerogenic and antioxidant activities[7-13]. In addition, some research groups have also investigated plant extracts for central nervous system (CNS) effects and they were documented the CNS depressant and sedative effects of aqueous Chamomile extracts [14]. As, this herb found to contain a lot of bioactive constituents such as phenolic acids, flavonoids (apigenin, luteolin, quercetin), terpenoid, α-bisabolol, and its oxide chamazulene. [13,15]. Viola *et al*. tested a purified fraction of an aqueous Chamomile extract containing apigenin and suggested anxiolytic, sedative, myorelaxant and anticonvulsive potential for this flavonoid [16]. Later studies exhibited that apigenin, major compound found in Chamomile extracts, affects benzodiazepine receptors differently than classical benzodiazepine receptor ligands [16-19]. While, clinical study was reported that chamomile have modest anxiolytic activity in patients with mild to moderate generalized anxiety disorder (GAD). However, Future studies are needed to replicate these observations[20]. On the other hand, documents related to antiepileptic effects of ethyl acetate fraction were not found in a comprehensive literature survey. Therefore, we aimed to investigate this effect in mice performing epileptic seizure, Since, there is no previous study has been reported on model of strychnine induced convulsion in mice, keeping this in view, the present study was designed to evaluate the anticonvulsant activity of the ethyl acetate organic extracts of *Matricaria recutita* in model of strychnine induced seizure on mice.

MARERIALS AND METHODS

Chemicals

All chemicals used in this study were of high quality. strychnine was purchased from BDH (Germany). Diazepam was purchased from Roche Company (Switzerland). Propylene glycol was purchased from Merck(Germany). Standard flavonoids from E. Merck AG. Darmstadt.

Extraction of the plant

Dry powdered flowers (10 gm) of *M. recutita* was extracted by soxhlet using organic solvent of increasing polarity starting from petroleum ether, chloroform, ethyl acetate and methanol (500 ml of each fraction) for 6 hrs., each fraction was filtered and evaporated to dryness under vacuum using rotary evaporator.

Phytochemical screening of ethylacetate fraction

The plant extract fraction was phytochemically screened for the qualitative detection of alkaloids, and flavonoids using standard

techniques as following; To conduct the test for alkaloids, few drops of ethyl acetate fraction was treated with drops of Mayer's reagents, while the presence of flavonoids in ethyl acetate fraction was confirmed by treating few drops of the extract with 5% ethanolic KOH

HPLC analysis of ethyl acetate fraction

Ethyl acetate fraction was analyzed by HPLC (Waters) using C_{18} column with a mobile phase composed of acetonitrile: water as isocratic mixture (30:70 v/v) and a flow rate of 1 ml/min, wave length 335nm, run time 10 min. Comparison was done with standards flavonoids, rutin, quercetin, and kaemferol, while that of apigenin 7-O-glycoside and apigenin were compared with the reported one (21).

plant materials

M.recutita was cultivated in the botanical garden of medicinal plant, college of pharmacy, university of Baghdad, it was collected in April, dried in a shed area, and was kept at the department of pharmacognosy.

Animals

Eighteen male albino mice on 35-40 old-day, weighting 18-22g were used in this study, they were obtained from the animal house of the college of pharmacy /University of Baghdad. The animals were housed under standard laboratory conditions and maintained at 23°C on a 12 h light-dark cycle. They had free access to food and water. Animals were allocated into three groups each of six animals and they were treated as follows: control group; animals received (0.3 ml)of vehicle (propylene glycol), test group; animals treated with ethyl acetate extract of Matricaria (25mg/Kg), standard group; animals treated with benzodiazepine (5mg/Kg). All treatments were given by I.P route. One hour later all animals challenged by strychnine (0.5mg/Kg) and observed for a period of one hour post strychnine administration.

The mean onset time of seizure, latency (sec.) (the time between the injection and the onset of first jerk or clonus), duration of clonus (sec.), and death latency (sec.) were estimated. The experimental protocols were approved by local Institutional Review Board (IRB)/Animal Ethical Committee.

STATISTICAL ANALYSIS

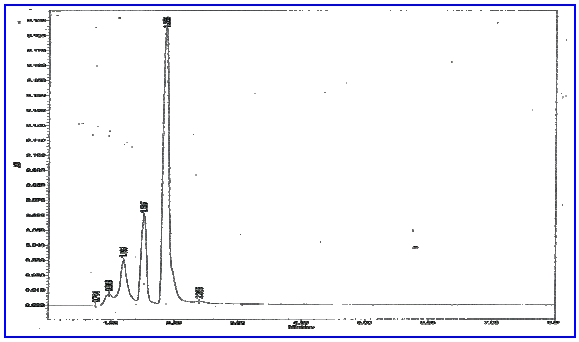
The results were expressed as mean± SD; then the differences in onset time to seizure, duration of seizure and latency to death among groups were evaluated using unpaired student's t-test and one-way ANOVA. P value less than 0.05 was the critical criterion for statistical significance.

RESULTS

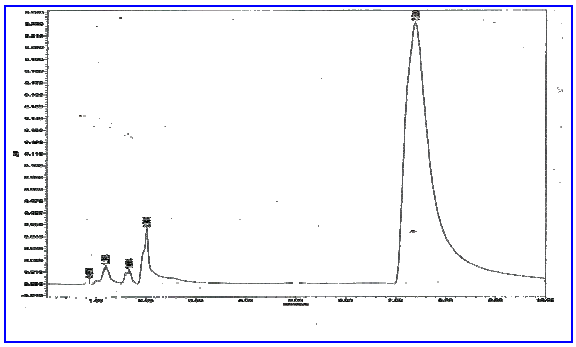
The phytochemical investigation of ethylacetate fraction revealed the presence of flavonoids, as a yellow color was obtained by addition of 5% ethanolic KOH and disappearance of the color was observed by addition of acid. Moreover, negative result was obtained by using Mayer's reagent which indicated the absence of alkaloids in the tested fraction.

The HPLC analysis for flavonoids in ethyl acetate fraction revealed the presence of apigenin-7-O-glycoside, as a major constituent of the fraction together with apigenin, rutin, and quercetin, while kaemferol was not detected in comparison with retention time of standard flavonoids (Rutin=1.859, quercetin= 8.6 and kaemferol= 7.38) minutes as shown in figures (1 and 2).

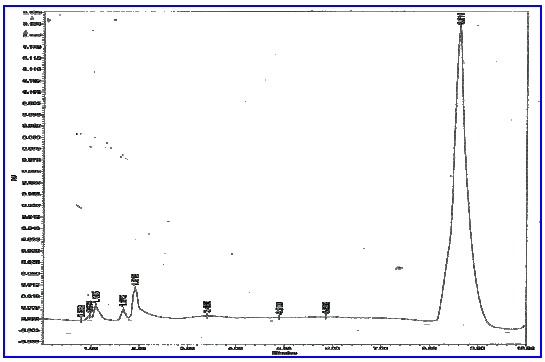
In addition the results of the present study showed that pretreatment with ethyl acetate extract of *M. recutita* at the dose 25m/Kg significantly increased ($p<0.01$) the seizure latency up to 228 seconds and reduced the severity of tonic convulsion as well as seizure duration of strychnine (0.5 mg/Kg i.p) induced convulsions which was started after 23.8 seconds post strychnine treatment. while non-significant difference was obtained in comparison to diazepam pretreated group (figure 3). Moreover, both matricaria extract and diazepam significantly delay the time elapsed to death compared with control ($P<0.001$) (figure 4).



(a)



(b)



(c)

Fig. 1 (a, b and c): HPLC chromatograms showing the retention time of standard flavonoids (rutin, quercetin and kaemferol)

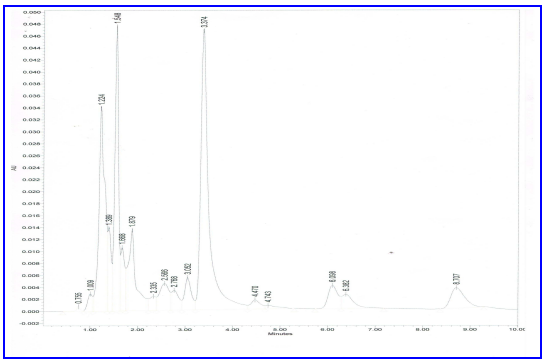


Fig. 2: HPLC chromatogram showing the retention time of flavonoids present in ethyl acetate fraction of *Matricaria recutita* extraxt

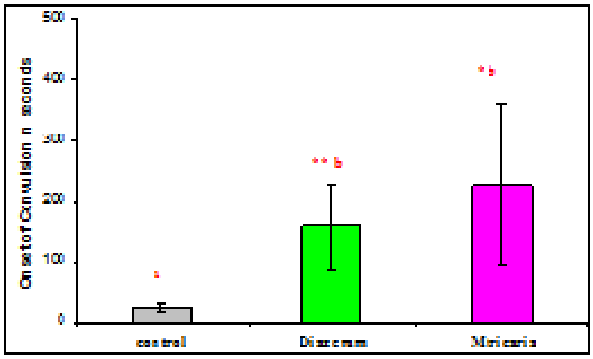


Fig. 3: Bar chart showing the effect of *M. recutita* extract and diazepam on the onset time(seconds) to epileptic attack in comparison with control

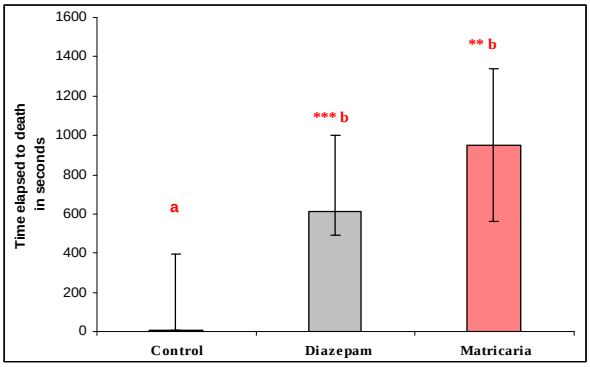


Fig. 4: Bar chart showing the effect of M. recutita extract and diazepam on the time elapsed to death(seconds) in comparison with control

DISSCUSSION

The data of the present study showed that pretreatment of animals with *Matricaria* extract significantly increase both the latency and onset of seizure induced by strychnine as well as dampened the severity of tonic convulsion compared to untreated control animals. These results suggested the protective effect of the ethyl acetate fraction against known epileptic agents, and this action could be attributed to the presence of bioactive substances within the tested fraction mainly flavonoids including apigenin-7-O- glycoside with apigenin, rutin, and quercetin which previously reported as main component of matricaria essential oil that possess an analgesic activity and benzodiazepine like action suggesting the sedative potential of Matricaria recutita [21, 22]. Benzodiazepines are known to be an inhibitory agent for anxiety, pain, and potentiate GABA-induced chloride current [23].

As previously reported that apigenin competitively binds to the benzodiazepine binding site of the GABA type A receptor, producing clear anxiolytic activity in mice when administered intraperitoneally, without showing evidence of sedation or muscle relaxant effects at doses similar to those used for classical benzodiazepines[24,25]. Meanwhile, others revealed that flavonoid as quercetin has neuroprotective effect against electrical kindling in rats [26]. Based on these observations it is possible that the anticonvulsant effect of *Matricaria recutita* in the present study is related to these active ingredients, which suggested to have benzodiazepine-like activity, that may inhibit binding of strychnine with glycine receptor or may enhance glycine or GABA binding to their receptors, in other word the observed effect in this study was probably associated with the major components detected in tested fraction of the extract. These components may act synergistically or other minor constituents may also contribute to the observed activity, since phytochemical screening of the plant showed that extracts containing flavonoids, sterols, glycosides and saponins possess a considerable anticonvulsant activity [27,28]. However, investigations on CNS-related pharmacological activities of these constituents are still under progress**.** In Conclusion; Ethyl acetate fraction of *M.recutita* extract containing bioactive flavonoids considered a good candidate natural remedy for treatment of some epileptic convulsion, and more comprehensive experiments with this fraction using different doses are worthwhile to elucidate the anticonvulsant action of this plant and to revise the finest use of more fractionated extract.

AKNOWLDGMENT

The authors thank University of Baghdad/College of pharmacy for supporting the project.

REFERENCES

1. Scheuer ML, Pedley TA. The evaluation and treatment of seizures. New Engl J Med. 1990;323:1468–74.
2. Kamboj P, Singh I, Mahadevan N, Chaudhary G. Anticonvulsants from nature. Pharmacog Rev. 2009;3(5):108–17.
3. Sierra-Paredas G. Recent advances in the neurochemistry of epilepsy. Eur Neurol Rev. 2008;3(1):96–8.
4. S. Engelbrghs, R. D'hooge, P. P. DE Deyn. Pathophysiology of epilepsy. Acta neurol. belg. 2000; 100: 201-213.
5. B. SamrCn, C. M. vanDuijn, S Koch, V. K. Hiilesmaa, H. Klepel, A. H. Bardy,G. Beck Mannagetta, *et al*. Maternal Use of Antiepileptic Drugs and the Risk of Major Congenital Malformations: A Joint European Prospective Study of Human Teratogenesis Associated with Maternal Epilepsy. Epilepsia 1997; 38(9):981-990.
6. Sunil Kumar, Gaurav Sharma, Abhishikha Sharma, Mathew Gorge, Lincy Joseph. Anticonvulsant effect of chloroform extract of phyllostachys bambusoides. International journal of pharmacy and pharmaceutical science 2011; vol3(5):25-27.
7. Uteshev BS, Laskova IL, Afanasev VA. The immunomodulating activity of the heteropolysaccharides from German chamomile (Matricaria chamomilla) during air and immersion cooling. Eksp Klin Farmakol 2004; 62(6):52-5.
8. Cemek M, Kağa S, Simşek N, Büyükokuroğlu ME, Konuk M. Antihyperglycemic and antioxidative potential of Matricaria chamomilla L. in streptozotocin-induced diabetic rats. J Nat Med -Tokyo 2008; 62(3):284-93.
9. Srivastava JK, Gupta S. Antiproliferative and apoptotic effects of chamomile extract in various human cancer cells. J Agric Food Chem. 2007; 55(23):9470-8.
10. Nayak BS, Raju SS, Rao AV. Wound healing activity of Matricaria recutita L. extract. J Wound Care 2007; 16(7):298-302.
11. Nogueira JC, Diniz Mde F, Lima EO. In vitro antimicrobial activity of plants in Acute Otitis Externa. Braz J Otorhinolaryngol 2008; 74(1):118-24.
12. Mahran LG, Kafafi YA, Okpanyi SN. Antiulcerogenic effect of some gastrointestinally acting plant extracts and their combination. Arzneimittelforschung 2001; 51(7):545-53.
13. McKay, D.L., Blumberg, J.B. A review of the bioactivity and potential health benefits of Chamomile tea (Matricaria recutita L.). Phytother. Res. 2006; 20: 519–530.
14. Della Loggia, R., Traversa, U., Scarcia, V., Tubaro, A. Depressive effects of Chamomilla recutita (L.) Raush, tubular flowers, on central nervous system in mice. Pharmacol. Res. Commun.1982;14:153–162.
15. Orav, A., Raal, A., Arak, E. Content and composition of the essential oil of Chamomilla recutita (L.) Rauschert from some European countries. Nat. Prod. Res. 2010; 24: 48–55.
16. Viola, H., Wasowski, C., Levi de Stein, M., Wolfman, C., Silveira, R., Dajas, F., Medina, J.H., Paladini, A.C., Apigenin, a component of Matricaria recutita flowers, isa central benzodiazepine receptors-ligand with anxiolytic effects. Planta Med. 1995; 61: 213–216.
17. .Raza M and Choudhary M I. Anticonvulsant medicinal plants. *In*: Studies in Natural Product Chemistry. Atta-ur-Rahman. Elsevier Science, Netherlands. 1999; 22: 507-533.
18. Franke, R., Schilcher, K.H.. Chamomile: Industrial Profiles. CRC Press/Taylor and Francis. Medicinal and Aromatic Plants series. 2005; pp. 253–263.

19. Sashidhara, K.V., Verma, R.S., Ram, P. Essential oil composition of Matricaria recutita L. from the lower region of the Himalayas. Flavour Fragr. J. 2006;21: 274–276.
20. Amsterdam JD, Li Y, Soeller I, Rockwell K, Mao JJ, Shults J. A randomized, double-blind, placebo-controlled trial of oral Matricaria recutita (chamomile) extract therapy for generalized anxiety disorder. J Clin Psychopharmacol 2009; 29(4):378-82.
21. Srivastava JK, Gupta S, Extraction, characterization, stability & biological activity of flavonoids isolated from Chamomile flowers. Molecular & Cellular Pharmacology 2009; 1(3):138-147.
22. Esam QNAIS Th e analgesic effect of the ethanolic extract of *Matricaria aurea.*Turk J Biol 2011; 35: 347-352.
23. Claudia Campo-Soria, Yongchang Chang, and David S Weiss. Mechanism of action of benzodiazepines on $GABA_A$ receptors. Br J Pharmacol. 2006; 148(7): 984–990.
24. Suresh Kumar,1, Reecha Madaan, Gundeep Bansal, Anupam Jamwal, Anupam Sharma. Plants and Plant Products with Potential Anticonvulsant Activity. Pharmacognosy Communications, 2012; 2 (suppl 1): 3-99.
25. Viola H, Wasowski C, Levi de SM et al. Apigenin, a component of *Matricaria recutita* flowers, is a central benzodiazepine receptors-ligand with anxiolytic effects. Planta Med. 1995; 61: 213- 216.
26. Tourandokht Baluchnejadmojarad1, Mehrdad Roghani, Homayoun Homayounfar1. Inhibitory Effect of High Dose of the Flavonoid Quercetin on Amygdala Electrical Kindling in Rats. Basic and clinical Neuroscience, 2010; 1 (3) : 57-61.
27. Jalal Uddin Bhat, Shabir Ahmad Parray, Mohammad Aslam, Shahid Ansari,Qudsia Nizami, Razia Khanam, Aisha Siddiqui, Mohd Aftab Ahmad. Anti-seizure activity of flower extracts of Nepeta bractaeta in swiss albino mice. EXCLI Journal, 2012;11:531-537.
28. Abhishek Singh, Umesh Kumar Sharma, Umashankar Sharma, Niranjan Sutar, Vimlesh Misra, Garima Yadav. Anticonvulsant activity of Kiglia pinnata bark extract. International journal of pharmacy and pharmaceutical science. 2010;Vol2(4):147-149.