World Applied Sciences Journal 15 (8): 1104-1109, 2011
ISSN 1818-4952
© IDOSI Publications, 2011

The Effect of Hydro-Alcoholic Extract of Citrus Flower on Pentylenetetrazole and Maximal Electroshock-Induced Seizures in Mice

[1]Mahmoud Hosseini, [2]Pardis Pkan, [3]Hassan Rakhshandeh, [3]Azita Aghaie, [4]Hamid Reza Sadeghnia and [1]Mahboobeh Ghasemzadeh Rahbardar

[1]Neuroscience Research Center and Department of Physiology, School of Medicine, Mashhad, University of Medical Sciences, Mashhad, Iran
[2]University of Binalood, Mashhad, Iran
[3]Pharmacological Research Center of Medicinal Plants and Department of Pharmacology, School of Medicine, Mashhad, University of Medical Sciences, Mashhad, Iran
[4]Neuroscience Research Center and Department of Pharmacology, School of Medicine, Mashhad, University of Medical Sciences, Mashhad, Iran

Abstract: The flowers of *Citrus aurantium L.* are considered to have depressive action upon the central nervous system (CNS). In the present study the effect of hydro- alcoholic extract of *Citrus* flowers on PTZ and maximal electroshock- induced seizures model was investigated in mice. The animals were divided into 5 groups (n=10), as follows: (1) Saline, (2) Ext 100, (3) Ext 500, (4) Ext 1000 and (5) Diazepam. The animals of groups 2, 3 and 4 were injected by 100, 500 and 1000 mg/kg of extract respectively, 30 minutes before each PTZ injection. The animals of groups 1 and 5 received saline (10 ml/kg) and diazepam (3 mg/kg) respectively, instead of extract. PTZ (90 mg/kg, i.p.) was injected and the animals were observed during 60 min. Behavioral responses of the animals such as latency to first minimal clonic seizure (MCS) and latency to the first generalized tonic-clonic seizures (GTCS) were recorded. In electroshock model, alternating current electroshock stimuli (0.2 sec, 50 Hz, 150 mA) were delivered via saline-soaked, cotton-covered ear-clip electrodes. The duration of tonic convulsion (a tonic extension of the hind limb) was recorded. A significant increase in the MCS latency was seen in Ext 500 and Ext 1000 groups in comparison with Saline group ($p < 0.001$ and $p < 0.01$ respectively). The GTCS latencies in Ext 100, Ext 500 and Ext 1000 groups were significantly higher than Saline group. In electroshock model, there was no significant difference between groups.

Key words: Seizure · Citrus aurantium L. · Flower · PTZ

INTRODUCTION

Sour orange, *Citrus aurantium L.* is a tree, about five meters tall, with scented white flowers which grows throughout the world [1]. Different parts of *Citrus auranium L.* such as peel, flowers and leaves are popularly used for their beneficial effects [2]. The fruits of this tree are too sour to use popularly as a nourishment material, but it has been reported that the ripe fruit is eaten by Iranian people [3-4]. It has also been reported that the fresh fruit is sometimes eaten with salt and chili paste in Mexico [1]. Immature fruits are sometimes used as a condiment. The peel of *Citrus aurantium L* is often used in marmalade and dried peel is used in bouquet garni and for flavoring a Belgian beer called Orange Muscat [1]. The flowers are used in tea, whereas the essential oil from the flowers, called neroli, is used in perfumes, liqueurs and orange-flower water, which is used to flavor sweets [1, 5].

In Asian herbal medicine, the dried, entire unripe fruit is used to treat digestive problems [1]. Dried peel of the unripe or ripe fruit is also used in Western herbal medicine to stimulate appetite and gastric. It is a common ingredient in ‘‘Swedish bitters’’ and other gastrointestinal remedies[1, 6]. The results obtained from clinical studies confirms that fruits of *Citrus aurantium* are containing beta agonists and therefore, it's consumption is resulted in weight loss and thermogenesis [1, 7-8]. It has also been suggested that chronic administration of the extracts of

Corresponding Author: Mahmoud Hosseini, Department of Physiology, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran. Tel: +98-511-8828564-5.

fruit may have toxic effects and may act as an oxidative stressesor [9].

In traditional medicine of some nations, it is suggested that the fruit of *Citrus aurantium L.* have depressive action on central nervous system and probably is effective in treatment of anxiety, insomnia and convulsion [10]. In animal experimental models, sedative and anti- anxiety effects of the fruit of Citrus aurantium has been reported [10-11]. The beneficial effects of the flowers in heart ailment and nervous disorders such as hysteria and neural weakness have been reported [2]. In traditional medicine, the flowers of this herb are considered to have depressive action upon the central nervous system and they are occasionally used in folk medicine as a mild sedative [1]. In the present study the effect of hydro- alcoholic extract of citrus flowers on PTZ induced seizures and maximal electroshock model in mice was investigated.

MATERIAL AND METHODS

Chemicals and Plant Extract: PTZ was purchased from Sigma Chemical (St. Louis, Mo) and dissolved in normal saline. *Citrus aurantium L.* flowers were collected from Tabas (middle part of Iran) and identified by botanists. To prepare hydro-alcoholic extract, 50 g of the chopped, dried flowers was extracted with 300 ml ethanol-water (70/30 v/v) using a soxhlet apparatus. The extracts reduced to dryness with a rotary vacuum evaporator [12].

Animals: Eight-week female mice, weighing 25-30g each, were used throughout the study. All of them were housed in the same room under a constant temperature (22-24°C) and on a 12 h light/dark cycle. Food and water were available ad libitum properly. Animal handling and all related procedures were carried out in accordance with Mashhad University of Medical Science, Ethical Committee Acts.

Ptz-Induced Seizures: In order to observe ictal behavior, PTZ (90 mg/kg, i.p.) [13-15] 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[13].

Electroshock-Induced Seizures: An alternating current electroshock stimuli were delivered via saline-soaked, cotton-covered ear-clip electrodes. The stimulus duration was 0.2 sec at a frequency of 50 Hz (sine-wave alternating current) and 150 mA. The duration of tonic convulsion (a tonic extension of the hindlimb) and the percentage of the mortality protection were recorded[16].

Procedure: The animals were divided into 5 groups (n=10), as follows: (1) Saline, (2) Ext 100, (3) Ext 500, (4) Ext 1000 and (5) Diazepam. The animals of groups 2, 3 and 4 were injected by 100,500 and 1000 mg/kg of extract respectively, 30 minutes before each PTZ injection. The animals of groups 1 and 5 received saline(10 ml/kg) and diazepam (3mg/kg) [17] instead of extract.

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

RESULTS

All animals in different treatment groups except Diazepam group were showed MCS and GTCS following PTZ administration. In Diazepam group the minimal clonic seizure (MCS) and first generalized tonic-clonic seizures (GTCS) in all animals were started after 10 min or didn't any seizure occurred. Therefore 60 s latency was recorded for all animals. A significant increase in the MCS latency was seen in Ext 500 and Ext 1000 groups in comparison with Saline group ($p < 0.001$ and $p < 0.01$ respectively; Fig1). There was no significant difference between Ext 100, Ext 500 and Ext1000 groups in MCS latencies (Fig 1). The GTCS latencies in Ext 100, Ext 500 and Ext 1000 groups were 119.54 ± 11.55 s and 109.79± 10.38 s and 105.33± 11.75 respectively and were significantly higher than Saline group(53.91± 3.28 s) ($p < 0.001$, $p < 0.01$ and $p < 0.01$ respectively; Fig. 2). There was no significant difference between Ext 100, Ext 500 and Ext 1000 groups in GTCS latencies. There were no significant differences in mortality rate following PTZ administration between different treatment groups except about Diazepam group which hadn't any mortality. The survival of the animals treated by diazepam as well as 500 and 1000 mg/kg extract was also significantly higher than saline group ($p < 0.05$ to $p < 0.001$) (Table 1).

Table 1: Effect of the Extract on survival of the animals after GTCS induced by PTZ

Treatment	# of surviving animals	% mortality protection
Vehicle (normal saline, i.p.)	0	0
Diazepam 3mg/kg, i.p	10***	100
Ext 100mg/kg, i.p	4	40
Ext 500mg/kg, i.p	5*	50
Ext 1000mg/kg, i.p	7**	70

***p < 0.001 and **p < 0. 01 *p < 0. 05 as compared to Saline group. Fisher's exact probability test was used for statistical evaluation. The animals of Ext 100, Ext 500 and Ext 1000 groups received 100, 500 and 1000 mg/kg hydro-alcoholic extract of citrus flower before injection of PTZ(90 mg/kg).The animals of Saline group were injected by Saline instead of extract.

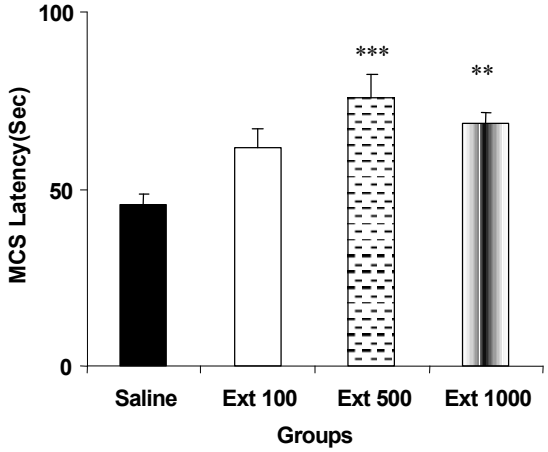

Fig. 1: Latencies to minimal clonic seizures (MCS) onsets in Saline, Ext 100, Ext 500 and Ext 1000 groups. Data are presented as mean ± SEM (n = 10 in each group). ***p < 0.001 and **p < 0. 01 as compared to Saline group. Analysis of variance (ANOVA), followed by Tukey test, were used for statistical evaluation.

The animals of Ext 100, Ext 500 and Ext 1000 groups received 100, 500 and 1000 mg/kg hydro-alcoholic extract of citrus flower before injection of PTZ(90 mg/kg).The animals of Saline group were injected by Saline instead of extract.

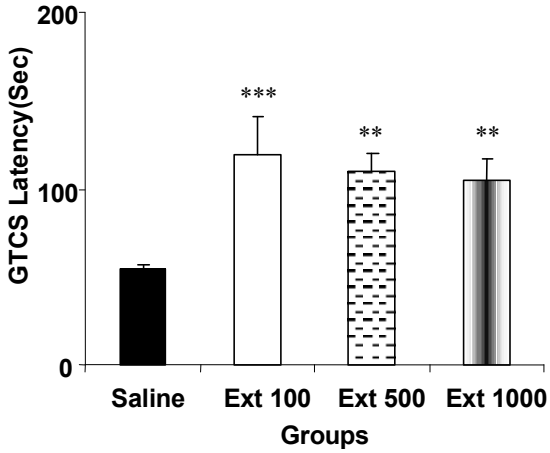

Fig. 2: Latencies to generalized tonic-clonic seizures (GTCS) onsets in Saline, Ext 100, Ext 500 and Ext 1000 groups. Data are presented as mean ± SEM (n = 10 ineach group). ***p < 0.001 and **p < 0.01 as compared to Saline group. Analysis of variance (ANOVA), followed by Tukey test, were used for statistical evaluation.

The animals of Ext 100, Ext 500 and Ext 1000 groups received 100, 500 and 1000 mg/kg hydro-alcoholic extract of citrus flower before injection of PTZ (90 mg/kg).The animals of Saline group were injected by Saline instead of extract.

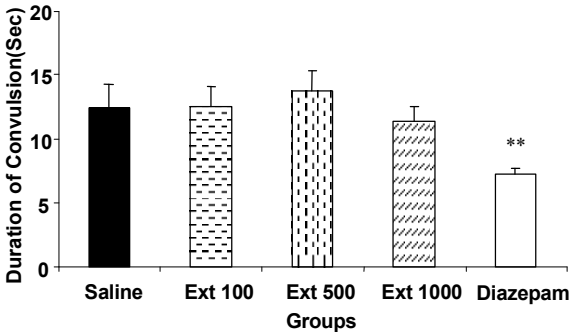

Fig. 3: Duration of tonic convulsion in Saline, Ext 100, Ext 500, Ext 1000 and Diazepam groups. Data are presented as mean ± SEM (n = 10 ineach group). $^{***}p < 0.001$ and $^{**}p < 0.01$ as compared to Saline group. Analysis of variance (ANOVA), followed by Tukey test, were used for statistical evaluation.

The animals of Ext 100, Ext 500 and Ext 1000 groups received 100, 500 and 1000 mg/kg hydro-alcoholic extract of citrus flower before stimulus delivery.The animals of Saline group were injected by Saline instead of extract. The animals of Diazepam group were injected by Diazepam.

In electroshock model the animals of all groups showed a tonic extension of the hindlim; there was no significant difference between groups. Duration of tonic convulsion in Diazepam group was 7.3± 0.39 sec and significantly lower than of Saline group (12.55 ±1.59 s) ($p < 0.01$) (Fig 3). There was no significant difference between extract treated groups in comparison with Saline group. There was no significant difference between groups in mortality rate.

DISCUSSION

As a traditional drug in Islamic medicine and as a folk remedy, the flowers of *Citrus aurantium L* has been used to cure heart ailment and nervous disorders such as hysteria and neural weakness [2]. Depressive action of the flowers upon the CNS is believed in traditional medicine and it has also been confirmed in experimental studies [1, 10, 18]. To the best of our knowledge, there is any study regarding the anti - consultant effects of *Citrus* flowers however, there are some reports about other parts of the plant. Hypnotic, anexiolytic and anti-convulsunt effect of the leaf of *Citrus aurantium L* has been reported by Carvalho-Freitas and co worker [10]. It has also been suggested that the essential oil from *Citrus aurantium* peel have anxiolytic effect in rodents without any motor impairment, even after repeated administration [11]. The results of an study regarding the use of medicinal plants by the patients which was done in the outpatient clinics of five health-care centers in Puerto Rico showed that *Citrus aurantium L.* was most frequently used plant especially for its sedative effect [19]. The results of present study showed that the hydro- alcoholic extract of citrus flowers had anti-convulsant effects in PTZ and maximal electroshock induced seizures model.

The anti-convulsion effect of the extract seen in the present study may be due to each of the ingredients or the synergistic effect of its constituents. The analysis of citrus flowers shows that the main constituents are flavonoids such as naringin, hesperidin and neohesperidin [20]. Flavonoids are complex chemical compounds, which have also been found in other medicinal plants with CNS depressant effects [21]. For example, *Passiflora coerulea*, a medicinal plant which uses as a sedative in South of America is reach of the flavenoid chrysin [21]. Chrysin has been suggested to act as a ligand for the benzodiazepine receptors[21]. Another flavonoid apigenin, which has been isolated from the medicinal plant, *Matricaria chamomilla* showed anticonvulsant effect probably by binding to the central benzodiazepine receptors [22].

In Mexican traditional medicine the flowers of several species of Citrus genera are used for their sedative effects [18]. It has been shown that many flavonoids may act as ligands for the ?- aminobutyric acid type A (GABAA) receptors in the central nervous system and therefore may have benzodiazepine-like effects. This is supported by their behavioral effects in animal models of anxiety, sedation and convulsion [23-25].

It has also been shown that neohesperidin and naringin which are present in *citrus* flowers increased the thiopental-induced sleeping time in mice [23, 26]. They also reduced the spontaneous locomotor activity and the exploratory behaviors [23, 26]. Anexiolytic effects naringin has also been reported [23] and therefore, it has been suggested that these flavenoids are respnible

for general inhibitory effects of citrus flowers in the CNS [23, 26]. Anti- convulsive effects of hesperidin and the synergistic interaction between hesperidin and diazepam has also been reported [23, 26-27]. Therefore, this flavenoid may also take part in anti - convulsant effects of the extract which was seen in the present study.

REFERENCE

1. Fugh-Berman, A. and A. Myers, 2004. Citrus aurantium, an ingredient of dietary supplements marketed for weight loss: current status of clinical and basic research. Exp. Biol. Med. (Maywood), 229(8): 698-704.
2. Zargari, A., 1995. Medicinal plants. Tehrarn University Publications, pp: 484-489.
3. Ling, A.P.K., J.Y. Chia, S. Hussein and A.R. Harun, 2008. Physiological responses of Citrus sinensis to gamma irradiation. World Applied Sciences J., 5(1): 12-19.
4. Hosseinimehr, S.J., H. Tavakoli, G. Pourheidari, A. Sobhani and A. Shafiee, 2003. Radioprotective effects of citrus extract against gamma-irradiation in mouse bone marrow cells. J. Radiat. Res., (Tokyo), 44(3): 237-241.
5. Kiple, K.F. and K.C. Ornelas, 2001. The Cambridge world history of food. Food Service Technol., 1(1): 61-62.
6. Bisset, N.G. and F.C. Czygan, 2001. Herbal Drugs and Phytopharmaceuticals: A Handbook for Practice on a Scientific Basis;[with Reference to German Commission E Monographs]. Medpharm Scientific Publ., pp: 91-95.
7. Preuss, H.G., D. DiFerdinando, M. Bagchi and D. Bagchi, 2002. Citrus aurantium as a thermogenic, weight-reduction replacement for ephedra: an overview. J. Med., 33(1-4): 247-264.
8. Pellati, F., S. Benvenuti, M. Melegari and F. Firenzuoli, 2002. Determination of adrenergic agonists from extracts and herbal products of Citrus aurantium L. var. amara by LC. J. Pharm Biomed Anal., 29(6): 1113-1119.
9. Arbo, M.D., G.C. Schmitt, M.F. Limberger, M.F. Charao, A.M. Moro, G.L. Ribeiro, E. Dallegrave, S.C. Garcia, M.B. Leal and R.P. Limberger, 2009. Subchronic toxicity of Citrus aurantium L. (Rutaceae) extract and p-synephrine in mice. Regul. Toxicol. Pharmacol., 54(2): 114-117.
10. Carvalho-Freitas, M.I. and M. Costa, 2002. Anxiolytic and sedative effects of extracts and essential oil from Citrus aurantium L. Biol. Pharm Bull., 25(12): 1629-1633.
11. Pultrini Ade, M., L.A. Galindo and M. Costa, 2006. Effects of the essential oil from Citrus aurantium L. in experimental anxiety models in mice. Life Sci., 78(15): 1720-1725.
12. Rakhshandah, H. and M. Hosseini, 2006. Potentiation of pentobarbital hypnosis by Rosa damascena in mice. Indian J. Exp. Biol., 44(11): 910-912.
13. Hosseini, M., H.R. Sadeghnia, S. Salehabadi, H. Alavi and A. Gorji, 2009. The effect of L-arginine and L-NAME on pentylenetetrazole induced seizures in ovariectomized rats, an in vivo study. Seizure, 18(10): 695-698.
14. Ebrahimzadeh Bideskan, A.R., M. Hosseini, T. Mohammadpour, R. Karami, M. Khodamoradi, H. Nemati Karimooy and H. Alavi, 2011. Effects of soy extract on pentylenetetrazol-induced seizures in ovariectomized rats. Zhong Xi. Yi. Jie. He. Xue. Bao., 9(6): 611-618.
15. Ebrahimi Hariry, R., 2011. Anticonvulsant Profile of Valeriana officinalis on Pentylenetetrazole-Induced Seizure Threshold in Mice. World Applied Sciences J., 12(8): 1291-1298.
16. Hosseinzadeh, H., G.R. Karimi and M. Rakhshanizadeh, 2005. Anticonvulsant effect of Hypericum perforatum: role of nitric oxide. J Ethnopharmacol., 98(1-2): 207-208.
17. Hosseinzadeh, H. and M. Nassiri Asl, 2003. Anticonvulsant, sedative and muscle relaxant effects of carbenoxolone in mice. BMC Pharmacol., 3: 3.
18. Guzman-Gutierrez, S.L. and A. Navarrete, 2009. Pharmacological exploration of the sedative mechanism of hesperidin identified as the active principle of Citrus sinensis flowers. Planta Med., 75(4): 295-301.
19. Hernandez, L., R.A. Munoz, G. Miro, M. Martinez, J. Silva-Parra and P.I. Chavez, 1984. Use of medicinal plants by ambulatory patients in Puerto Rico. Am J. Hosp. Pharm, 41(10): 2060-2064.
20. Lu, Y., C. Zhang, P. Bucheli and D. Wei, 2006. Citrus flavonoids in fruit and traditional Chinese medicinal food ingredients in China. Plant Foods Hum Nutr., 61(2): 57-65.
21. Medina, J.H., A.C. Paladini, C. Wolfman, M. Levi de Stein, D. Calvo, L.E. Diaz and C. Pena, 1990. Chrysin (5,7-di-OH-flavone), a naturally-occurring ligand for benzodiazepine receptors, with anticonvulsant properties. Biochem Pharmacol., 40(10): 2227-2231.

22. Avallone, R., P. Zanoli, G. Puia, M. Kleinschnitz, P. Schreier and M. Baraldi, 2000. Pharmacological profile of apigenin, a flavonoid isolated from Matricaria chamomilla. Biochem Pharmacol., 59(11): 1387-1394.
23. Fernandez, S.P., M. Nguyen, T.T. Yow, C. Chu, G.A. Johnston, J.R. Hanrahan and M. Chebib, 2009. The flavonoid glycosides, myricitrin, gossypin and naringin exert anxiolytic action in mice. Neurochem Res., 34(10): 1867-1875.
24. Johnston, G.A., 2005. GABA(A) receptor channel pharmacology. Curr. Pharm. Des., 11(15): 1867-1885.
25. Marder, M. and A.C. Paladini, 2002. GABA(A)-receptor ligands of flavonoid structure. Curr. Top. Med. Chem., 2(8): 853-867.
26. Fernandez, S.P., C. Wasowski, L.M. Loscalzo, R.E. Granger, G.A. Johnston, A.C. Paladini and M. Marder, 2006. Central nervous system depressant action of flavonoid glycosides. Eur. J. Pharmacol., 539(3): 168-176.
27. Dimpfel, W., 2006. Different anticonvulsive effects of hesperidin and its aglycone hesperetin on electrical activity in the rat hippocampus in-vitro. J Pharm Pharmacol., 58(3): 375-379.