Antidepressant-like effect of extracts from *Urtica dioica* in mice model of depression

Amir Modarresi Chahardehi[1], Darah Ibrahim[1], Farid Abolhassani[2] and Shaida Fariza Sulaiman[3]

[1]Industrial Biotechnology Research Laboratory, [3]Phytochemistry Laboratory, School of Biological sciences, Universiti Sains Malaysia, 11800 Minden, Penang, Malaysia, [2]Department of Anatomy, School of Medical Sciences, University of Tehran, Iran. Corresponding Author: amirmch@yahoo.com

Abstract. *Urtica dioica* (Stinging nettle) leaves have been used as a traditional medicine to treatrheumatoid arthritis, to alleviate rheumatic pain benign prostatic hyperplasia (BPH), diuretic and prevention of kidney stones by local people in Middle East region especially in Iran, Turkey and East Europe. Hence, this investigation evaluates the antidepressant effect of selected crude extracts in the forced swimming test (FST) and tail suspension test (TST), two models predictive of antidepressant activity. The acute treatment of mice with extracts by intra-peritoneal (i.p.) route significantly reduced the immobility time in the FST (50 and 100 mg/kg) and TST (50 and 100 mg/kg), as compared to positive controls (haloperidol and fluoxetine) at 1 and 10 mg/kg, respectively. On the third day of experiment, a significant decrease of mobility was observed for chloroform extract (CE I) and butanol extract (BE II) compared to first day. CE I and BE II led to reduction of immobility time, as the selected extracts with two doses administered were different compared to the control, in the FST method by 65.37% and53.92% for 100 mg/kg, respectively. However, CE I showed the best result compared to our positive controls. Similar results of increased antidepressant effect, that was, of immobility time depending on the concentration administered, were obtained with the TST method. Also our data showed that there was no significant differences between doses (50 and 100 mg/kg). The results suggested that the antidepressant action of the butanol extract and of *U. dioica* its fraction (BE II2) was mediated by an interaction with 5-hydroxytrptamine (5-HT). *U. dioica* showed a potential source for the isolation of important natural products with antidepressant-like properties. However, further studies are still required.
Keywords: *Urtica dioica*, antidepressant activity, forced swimming test (FST), tail suspension test (TST).

Introduction

Depression is considered as an affective disorder characterized by change in mood, lack of interest in the surroundings, psychomotor retardation and melancholia. The prevalence of depression in general population is estimated to be around 5% (Richelson *et al*., 2001). Numerous antidepressant compounds are now available, presumably acting via different mechanisms. Hence, over 20 animal models of depression have been developed. These assays either provide a means of screening for putative antidepressant activity, or allow theories relating to the etiology of depression to be found. Forced swimming test (FST) and tail suspension test (TST) are the models which based on the application of stress to the animal (Porsolt *et al*., 1977; Steru *et al*., 1985).Many plant extracts and different classes of phytochemicals have been shown to have useful activity (Ahangar *et al*., 2011). The search for novel therapeutic natural plants that mitigate depressive illness has been extensively explored over the past decade (Zhang, 2004; Kwon *et al*., 2010).

Urticaceae family was reported as one of the effective medicinal plant (Gunther, 1959). This family known to possess many ethnomedical and biological properties (Modarresi *et al*., 2009). *Urtica dioica* (stinging nettle) is annual and perennial herb which distinguished with stinging hairs. This plant is traditionally used in Northern Iran and Eastern part of Europe (Pourmorad *et al*., 2006) and also have already been known for a long time as medicinal plants in many parts of the world (Charurasia and Wichtl, 1987). This herbs are used to treat stomachache (Gulçin *et al*. 2004; Pourmorad *et al*., 2006). Besides, this herb is used to treat rheumatic pain and for colds and cough (Sezik *et al*., 1997).

CNS-depressant activity has been studied for *Urtica dioica*. It has been shown to produce a reduction in spontaneous activity in rats and mice (Broncano *et al*., 1987). Inhibition of drug-induced convulsions, and a lowering of body temperature in rats. Nettle has been reported to have no effect on the blood pressure of mice, whereas in cats it has produced a marked hypotensive effect and bradycardia (Broncano *et al*., 1987). The stinging hairs of most nettle species contain serotonin, formic acid and histamine (Fu *et al*., 2006). Serotonin or 5-hydroxytrptamine (5-HT) is a monoamine neurotransmitter and is primarily found in the gastrointestinal (GI) tract, platelets, and in the central nervous system (CNS) of animals including humans (Young, 2007). However, there is no report of this plant directed to

antidepressant activity. The antidepressant activity was determined by forced swimming test (FST) and tail suspension test (TST) in order to understand the importance of these extracts.

Materials and Methods

Plant material and extraction

The aerial parts of *U. dioica* including leaves and stems were collected in Iran from Salmanshahr city in Mazandaran province (the geographical coordinates given by GPS: latitude: 36º42'34" N - 51º08'57" E and altitude: 21m) and Tehran city in Tehran province (the geographical coordinates given by GPS: latitude: 35º50'21" N - 51º25'22" E and altitude: 2012m) in August 2007. The Voucher specimens were deposited at the Herbarium of the School of Pharmaceutical Sciences, University of Tehran (Iran) in April 2010 by the code of 6725-TEH. The plant materials were washed, dried and ground to small pieces. The first method (Method I) of extraction included the using of four solvents by following non-polar to polar solvents (by using Soxhlet apparatus). In this method, dried powdered plant was extracted. The solvents used were hexane, chloroform, ethyl acetate and methanol. The second method (Method II) included 5 solvents system (by using partition technique). For Method II, the dried materials were extracted by using Soxhlet extractor with methanol as a solvent for 72 hours at room temperature (30°C). The methanolic extracts were further partitioned by adding distilled water in a separating funnel and then followed using chloroform, diethyl ether, ethyl acetate and butanol as described by Mellidis and Papageorgiou (1993), with a slight modification. The dried extracts were then weighed using microbalance and were kept at 4°C. Abbreviations for crude extract used in this paper namely:

HE I (hexane extract of method I), CE I (chloroform extract of method I), EAE I (ethyl acetate extract of method I), ME I (methanol extract of method I), ME II (methanol extract of method II), CE II (chloroform extract of method II), DEE II (diethyl ether extract of method II), EAE II (ethyl acetate extract of method II) and BE II (butanol extract of method II). According to our previous studies in our lab, antioxidant, antimicrobial, toxicity and anticancer activities, three of these crude extracts (CE I and BE II) were selected for antidepressant activity.

Animals

Mice of either sex purchased from animal house, School of Medical Sciences, University of Tehran (Iran), and weighing 25–35 g were used. Animals were placed at Animal house, Department of Anatomy, School of Medical Sciences, University of Tehran, housed 6 per cage under a normal 12 h/12 h light/dark schedule with the lights on at 07:00 a.m. and had free access to water and food pellets. They were allowed at least 1 week to adapt to the laboratory prior to the administration. All efforts were made to minimize animal suffering and to reduce the number of animal used. All the drugs were administered intraperitoneally (i.p.) 30 min prior to FST.

Forced swimming test (FST)

Mice of either sex were individually forced to swim in an open cylindrical container (diameter 10 cm, height 25 cm), containing 19 cm of water at 25±1 °C. The immobility time, defined as the absence of escape-oriented behaviors, such as swimming, was scored during 6min with the help of stop-watch, as described previously by (Eckeli *et al*., 2000; Zomkowsi *et al*., 2004; Kaster *et al*., 2007). All the mice of either sex were divided in five different groups. The first group assigned as control receiving only vehicle (NaCl 5ml/kg). The other groups received acute dose of extracts (50, 100 mg/kg). The positive group received standard drugs such as Haloperidol (diluted with sterile water) and Fluoxetine (diluted with normal saline) (1 and 10 mg/kg, respectively). The total duration of immobility was recorded during the last 6 min of the 10-min period. Each mouse was judged to be immobile when it ceased struggling and remained floating motionless in the water, making only those movements necessary to keep its head above water. A decrease in the duration of immobility is indicative of an antidepressant like effect. This experiment was injected for two days later with same the doses of selected crude extracts of *U. dioica* (Third day) with a bit modification (Deak *et al*., 2005).

Tail suspension test (TST)

The total duration of immobility induced by tail suspension test was measured according to the method described by Steru *et al*. (1985). Mice both acoustically and visually isolated were suspended 70 cm above the floor by adhesive tape placed approximately 1 cm from the tip of

the tail. The total immobility period was scored manually during 6 minutes test session with the help of stopwatch. Immobility was defined as the absence of any limb or body movements, except for those caused by respiration or when they hung passively and completely motionless. The parameter obtained was the number of seconds spent immobile. Parameter used was the number of seconds spent immobile.

Statistical analysis

Data were expressed as the mean ± standard deviation of mean (S.D.). Comparisons between experimental and control groups were performed by one-way analysis of variance (ANOVA) followed by Tukey`s HSD test when appropriate. $P < 0.001$ and $p < 0.01$ was considered significant.

Results and Discussions

The results presented here showed that the selected crude extract of *U. dioica* given systemically (i.p. route), are effective in producing significant antidepressant-like effects, when assessed in the FST and in the TST. The antidepressant-like effects of these extracts in the FST and TST were comparable to haloperidol and fluoxetine as positive controls.

The data obtained after a single administration of extracts suspension showed that the immobility time of animals decreased dose-dependently. The animals were more active in both employed models, which means that the antidepressant effect were stronger. For all two doses administered there were differences compared to our controls, that are, led to reduction of immobility time, in the FST method for CE I at 50 and 100 mg/kg were 65.37% and 65.55%, for BE II at 50 and 100 mg/kg were 53.92% and 54.52%, and for BE IIf_2 at 50 and 100 mg/kg were 68.48% and 72.87% (Figure 1). Mice pretreated with varying doses (50 and 100 mg/kg) of CE I, BE II and BE IIf_2 to forced swim exhibited no statistically reliable alterations between the first day of forced swim exposure and third day except BE II and BE IIf_2 at 50 mg/kg on the first day (Figure 1, panel left).

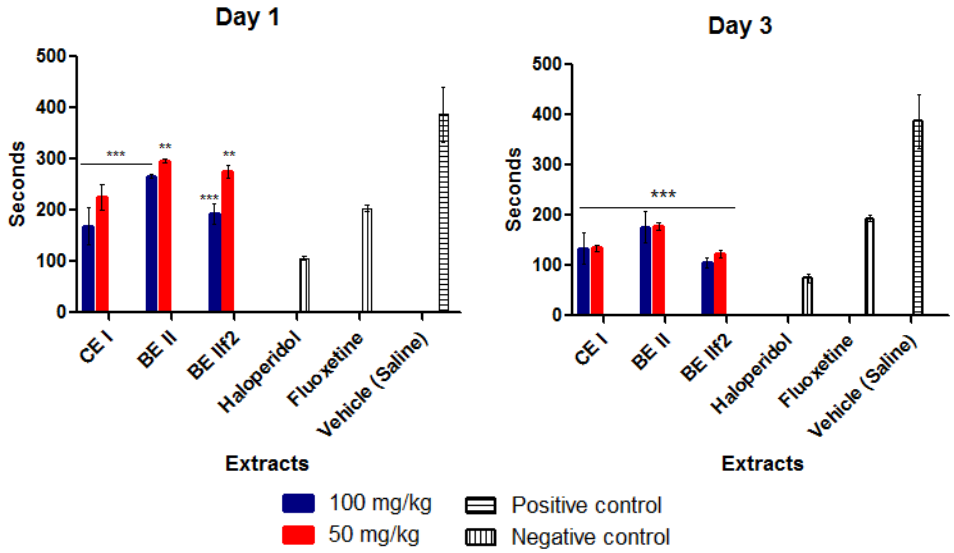

Figure 1. Antidepressant activity of crude extracts of *Urtica dioica* from Forced swimming test (FST). Values were mean ± SD (n= 3 mice). *** Data are significantly different with negative control ($p < 0.001$), ** ($p < 0.01$).

However, statistical significant reduction in mean immobility time during TST was observed with doses of 100 mg/kg for CE I (Figure 2). The reduction time in the TST assay for CE I at 50 mg/kg and 100 mg/kg exhibited 57.41% and 67.82%, for BE II at 50 and 100 mg were 57.10% and 60.98%, and for BE IIf_2 showed 58.63% and 65.99%. Behavioral studies have been shown to play an important part in the evaluation and development of antidepressant drugs (Xu *et al*., 2008). Forced swimming test (FST) and tail suspension test (TST) are among behavioral models that widely and routinely used for screening new antidepressant compound (Cryan *et al*. 2005).

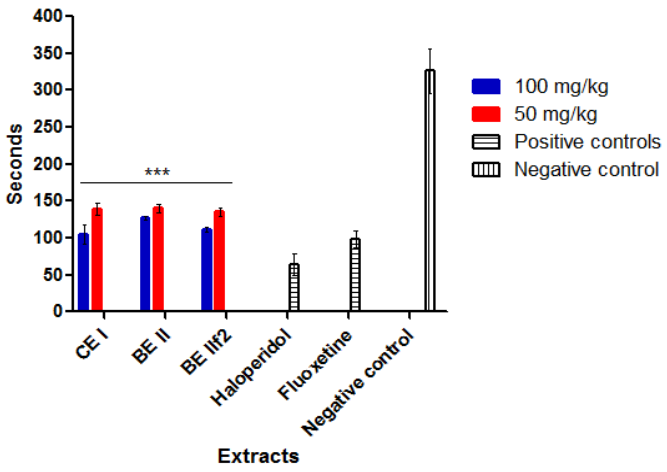

Figure 2. Tail suspension test from some selected crude extracts of *Urtica dioica*. Values were mean ± SD (n= 3 mice). *** Data are significantly different with negative control ($P < 0.001$).

Regarding to high antioxidant, antimicrobial, anticancer activities of these crude extracts, BE II was subjected to get fractions and developed by PC chromatograms using BAW (mobile phase). Four bands obtained in three colours in visible light. BE IIf_1 and BE IIf2 were appeared in light yellow, BE IIf_3 in dense yellow and BE IIf_4 in dark green colour. However, first band (BE IIf_1) exhibited yellow colour, BE IIf_2 in blue (sea green), BE IIf_3 in yellow-green and BE IIf_4 in green colour under UV light. Majority of bands under visible light represented in yellow colour except the last band (fourth band) appeared in dark green colour. These bands (BE IIf_1, BE IIf_2 and BE IIf_3) could be in flavonols group. According to Harborne (1998), flavonols are appeared in bright yellow and bright yellow fluorescent. Although some highly methylated flavonols behave similarly (Harborne, 1998).

Some diterpenoids and triterpenoids isolated from plants of several species of the genus antimicrobial and as an antimicrobial remedy. Also many of them are able to influence the central nervous system investigate (Bonito, 2009). From previous study revealed that one of the main constituent of Salvia divinorum is diterpenoids which may have antidepressant activity (Hanes, 2001). Yu *et al*. (2011) found that course treatment with diterpene alkaloids of *Aconitum baicalense* in mice reduced that time of immobilization in the TST.

According to R_f value of serotonin was 0.34 (34) in TLC chromatogram and same R_f with 35.15 ± 1.27 in BE IIf_2 and also showed blue (sea green) under UV light. BE IIf_2 could be 5-hydroxytryptamine (5-HT) which showed in Figure 3.

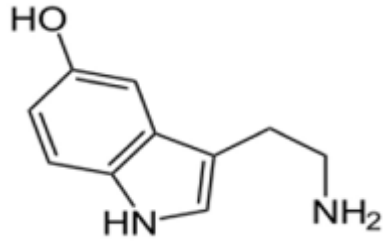

Figure 3. Chemical structure of serotonin or 5-hydroxytrptamine (5-HT)

In this study, a variation of the original Porsolt test was used (1977), which means placing the mice in the water tank in two occasions separated by different days and recording swimming activity automatically.

Rocha *et al*. (2007) reported that *Cecropia glazioui* from Urticaceae family as same as *U. dioica*, had antidepressant-like effect. The effect was enhanced after purification of the active extract. Catechins, procyanidins and flavonoids were the main constituents of the purified active fraction. So far, in vitro studies showed that catechin (4α →8) ent-catechin (Procyanidiin B3 isomer), catechin and epicatechin (4α →8) epicatechin (Procyanidin B2) inhibited 5-HT, NA and DA uptakes in brain synaptosomes but the flavonoids isoorientin and isovitexin did not. Rocha *et al*. found that chatechin and procyanidins are the major active substances *in vitro* and may contribute to the antidepressant-like effect produced by *C. glazioui*

Fluoxetine (Prozac) which it used in this study, in pharmacologic treatments for depression in cancer patients at doses 10-20 mg as starting dose and 20-60 mg as therapeutic range has common side-effect such as varying degrees of gastrointestinal (Pirl and Roth, 1999). However, some consideration should be taking into account that FST and TST is not the only model of depression by which the results obtained using this model should be considered and interpreted with caution due to some differences among experimental animals and clinical studies in human (Farah Idayu *et al*., 2011). It has been reported that the TST is less stressful than FST and has

greater pharmacological sensitivity. Remarkably, TST detects the anti-immobility effects of a wide array of antidepressants. Thus, the activity of *U.dioica* could involve one of the mechanisms of the antidepressant.

Conclusions

In conclusion, the present study indicates that *Urtica dioica* produces a specific antidepressant-like effect in animal models predictive of antidepressant properties, forced swimming test and tail suspension test. Moreover, the effect of the acute or repeated administration of this extract was similar to the action produced by the classical antidepressant fluoxetine and haloperidol.

Acknowledgments

This material is based upon work supported by a grant from Universiti Sains Malaysia (USM). We also thank USM because this research was partially supported by the provided fellowship. Also we thank Dr. Kashani the head of Embryology Lab in School of Medical Sciences, University of Tehran and Ms. Leila Mousavi for her assistant.

References

Ahangar, N., Mirfetros, S. and Ebrahimzadeh, M. A. (2011). Antidepressant activity of polyphenol fraction of *Artemisia absinthium* L. *Pharmacologyonline*, 1: 825 – 832.

Bonito, M. C. (2009). Pharmacological characterization of terpenic secondary metabolites isolated from *Salvia* species. Tesi di dottorato in scienza del farmaco. p 45.

Broncano, J., Rebuelta, M., Vivas, J. M., Diaz, M. P. (1987). Estudio de diferentes preparados de *Urtica dioica* L. sobre SNC. *An Real Acad. Farm*., 53: 284 - 291.

Chaurasia, N. & M. Wichtl. 1987. Flavonol glycosides aus *Urtica dioica. Planta Med.*, 53: 432-434.

Cryan, J.F., Valentino, R. J., Lucki, I., (2005). Assessing substrates underlying the behavioral effects of antidepressants using the modified rat forced swimming test. *Neurosci Biobehav Rev*., 29(4-5): 547 - 569.

Deak, T., Bellamy, C., D`Agostino, G.L., Rosanoff, M., McElderry, N.K. and Bordner, K.A. (2005). Behavioral responses during the forced swim test are not affected by anti-inflammatory agents or acute illness induced by lipopolysaccharide. *Behavioural Brain Research*, 160: 125-134.

Eckeli, A.L., Dach, F., Rodrigues, A.L.S. (2000). Acute treatment with GMP produces antidepressant-like effects in mice. *Neuro Rep*., 11: 1839-1843.

Gunther, R.T. (1959). The Greek herbal of dioscorides. Hafner Publishing, New York, pp 491.

Farah Idayu, N., taufik Hidayat, M., Moklas, M. A. M., Sharida, F., Nurul Raudzah, Shamima, A. R., Apryani, E. (2011). Antidepressant-like effectofmitragynineisolatedfrom Mitragyna speciosa Korth inmicemodelofdepression, *Phytomedicine*, 18(5): 402 – 407.

Fu, H. Y., Chen, S. J., Chen, R. F., Ding, W. H., Kuo-Huang, L. L. and Huang, R. N. (2006). Identification of oxalic acid and tartaric acid as major persistent pain-inducing toxins in the stinging hairs of the nettle, *Urtica thunbergiana*. *Annals of Botany* (London) 98 (1): 57–65. doi:10.1093/aob/mcl089

Hanes, K. R. J. (2001). Antidepressant effects of the herb *Salvia divinorum*: a case report. *J. Clin. Psychopharmacol*., 21: 634 – 635.

Harborne, J. B. (1998). Phytochemical methods: A guide to modern techniques of plant analysis. Third edition, Chapman and Hall, UK, p 292 – 293.

Kaster, M. P., Raupp, I., Binfaré, R. W., Andreatini, R., Rodrigues, S. A. C. (2007). Antidepressant-like effect of lamotrigine in the mouse forced swimming test: evidence for the involvement of noradrenergic system. *Euro. J. Pharmacol.*, 565: 119 - 124.

Kwon, S., Lee, B., Kim, M., Lee, H., Park, H. J. and Hahm, D. H. (2010). Antidepressant-like effect of the methanolic extract from Bupleurum falcatum in the tail suspension test. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, 34: 265 – 270.

Mellidis, A.S. and V.P. Papageorgiou. (1993). Phenolic constituents from *Onosma heterophylla*. *J. Nat. Prod.* 56: 949 - 952.

Modarresi Chahardehi, A., Ibrahim, D. and Sulaiman, S.F. (2009). Antioxidant activity and total phenolic content of some medicinal plants in Urticaceae family. *Journal of Applied Biological Sciences*. 2(3): 01-05.

Pirl, W. F. and Roth, A. J. (1999). Diagnosis and treatment of depression in cancer patients. *In*: Licino, J. and Wang, M. L., Biology of Depression, From novel insights to therapeutic strategies,vol 1, p 378.

Porsolt, R.D., Bertin, A., Jalfre, M. (1977). Behavioral despair in mice: a primary screening test for antidepressants. *Archives Internationales de Pharmacodynamie et de Therapie*, 229: 327–336.

Richelson, E. (2001). Pharmacology of antidepressants. *Mayo. Clin. Proc*., 76: 516 – 527.

Rocha, F.F., Lima-Landman, M.T., Souccar, C., Tanae, M.M., De Lima, T.C. and Lapa, A.J. (2007). Antidepressant-like effect of *Cecropia glazioui* Sneth and its constituents - in vivo and in vitro characterization of the underlying mechanism. *Phytomedicine*, 14(6): 396 – 402.

Sezik, E., F. Yeşilda, M. Tabata, G. Honda, Y. Takaishi, T. Fujita, T. Tanaka & Y. Takeda. 1997. Traditional medicine in Turkey VIII. Folk medicine in East Anatolia Erzurum Ağrı, Kars, Iğdır provinces*. Econ. Bot.* 51: 195-211.

Steru, L., Chermat, R., Thierry, B., Simon, P. (1985). The tail suspension test: a new method for screening antidepressants in mice. *Psychopharmacology*, 85: 367–370.

Xu, Q., Yi, L.T., Pan, Y., Wang, X., Li, Y.C., Li, J.M., Wang, C.P. and Kong, L.D. (2008). Antidepressant-like effectsofmixtureofhonokiolandmagnololfromthebarks of *Magnolia officinalis* in stressedrodents. *Prog. Neuropsychopharmacol. Biol. Psychiatry*, 32: 715 -725.

Young, S.N. (2007). How to increase serotonin in the human brain without drugs. *Rev. Psychiatr. Neurosci.* 32 (6): 394–99.

Yu, V., Nesterova, T.N., Povetieva, N.I., Suslov, A.A. and Pushkarskiy, S.V. (2011). Antidepressant activity of diterpene alkaloids of *Aconitum baicalense* Turcz. *Bull. Exp. Biol. Med*., 151(4): 425–428.

Zhang, Z. (2004). Therapeutic effects of herbal extracts and constituents in animal models of psychiatric disorders. *Life Sci*., 75: 1659–99.

Zomkowsi, A.D.E., Rosa, A.O., Lin, J., Santos, A.R.S., Calixto, J.B., Rodrigues, A.L.S. (2004). Evidence for serotonin receptor subtypes involvement in agmatine antide- pressant like-effect in the mouse forced swimming test. *Brain Res.*, 1023: 256 - 263.