Asian Journal of Pharmaceutical Education and Research
Vol -8, Issue-3, July-September 2019
ISSN:2278 7496

RESEARCH ARTICLE Impact Factor: 7.014

STUDY OF BIOACTIVE CONSTITUENTS AND ANTIDEPRESSANT POTENTIAL OF HYDROALCOHOLIC EXTRACT OF *URTICA URENS* L. IN MICE

Umme Atifa[1], Hemant Kumar Sharma[1], Prabhakar Budholiya[1], Prabhat Jain[2]

[1]College of Pharmacy, Sri Satya Sai University of Technology and Medical Sciences, Sehore (M. P.)

[2] Scan Research Laboratories, Bhopal (M.P.)

*Corresponding Author's E mail: Ummeaatifa@gmail.com

Received 08 June 2019; Revised 12 June 2019; Accepted 20 June 2019, Available online 10 July 2019.

ABSTRACT

Majority of the antidepressant drugs improve depressive symptoms, but they exert multiple unwanted side effects. The search for more efficacious and well tolerated drugs is in progress. Owning to this, the present study was designed to evaluate the antidepressant activity of hydroalcoholic extract of the aerial parts of *UrticaUrens* L (HAUU) in mice. It was evaluated using the Tail Suspension Test (TST) and Forced Swimming Test (FST) in mice. The HAUU (250 and 500 mg/kg, po) was administered orally in separate groups of Swiss albino mice weighing 20-25 for 14 days in TST and FST tests. Phytochemical analysis revealed the presence of alkaloids, glycosides, phenols, flavonoids, tannins. The HAUU showed a dose dependant reduction in duration of immobility in mice. The dose of 500 mg/kg of HAUU significantly reduced the immobility time of mice in both FST and TST. The efficacy of extract was found to be comparable to fluoxetine (20 mg mg/kg, po). It was found to be toxicologically safe with no deaths of mice when administered orally at the dose of 2000 mg/kg. From the present study, it can be concluded that the HAUU possess potent antidepressant activity as shown by the TST and FST tests and is toxicologically safe.

Keywords: UrticaUrens L, Depression, Tail suspension test, Forced swimming test, Fluoxetine.

INTRODUCTION:

Depression is the leading cause of disability and the 4thleading contributor to the global burden of disease in 2000. Today, depression is already the 2ndcause in the age category 15-44 years for both sexes combined. The lifetime risk of depression varies from 5% to 12% in men and 10% to 25% in women. Suicide is the major consequences in most of the depressive illnesses. About 60% deaths are due to depression and related disorders [1]. It is characterized by emotional and physical manifestations, such as feelings of worthlessness, helplessness, hopelessness, guilt or indecision, change in appetite, change in sleep habits, loss of concentration, loss of energy, loss of interest, loss of pleasure, agitation, mental and motor slowing, and social withdrawal [2].Antidepressant drugs such as tricyclic antidepressant and selective serotonin reuptake inhibitors are used to treat depression showing various side effects and thus the search for a new antidepressant herb without side effects is deemed are

Centellaasiatica, *Rauwolfia serpentine*, *Hypericumperforatum*, and *Withaniasomnifera*[3]. Decades of basic and clinical neuroscience research have greatly improved our understanding of the neurobiology of depression. Based on a solid foundation, basic and clinical neuroscience research is progressing rapidly with many exciting developments on the horizon. Importantly, as the pathophysiology of depression becomes better understood, a number of novel treatment targets are being identified. The antidepressant activity of this plant has not been reported scientifically. Therefore, our study was focused on the evaluation of antidepressant potential of *UrticaUrens* Linn in laboratory animals [4]. *Urticaurens* L. (Urticaceae) leaves commonly known as annual nettle, dwarf nettle, small nettle, dog nettle or burning nettle have a relatively high level of protein (66%), which is of better quality if compared with the proteins of other leafy vegetables [5]. The leaves of nettle are good sources of different significant minerals and vitamins[6,7]. Nettles contain flavonoids, fatty acids, terpenes, protein, vitamins, and minerals. Stinging nettle leaves are rich in vitamin C, B groups vitamins, vitamin K, and some minerals mainly calcium, iron, magnesium, phosphorus, potassium, and sodium [8]. Nettle leaves contain nine carotenoids: Lutein, lutein isomers and b-carotene are the basic carotenoids [9]. Thus, the objective of the present study was to evaluate the antidepressant activity of hydroalcoholic extract of the aerial parts of *UrticaUrens* L in mice using the Tail Suspension Test (TST), ForcedSwimming Test (FST) in mice.

MATERIALS AND METHODS

Plant material

Aerial partsof *Urticaurens*were collected from local area of Bhopal (M.P.) in the month of December, 2018.

Chemical reagents

All the chemicals used in this study were obtained from HiMedia Laboratories Pvt. Ltd. (Mumbai, India), SigmaAldrich Chemical Co. (Milwaukee, WI, USA), SD Fine Chem. Ltd. (Mumbai, India) and SRL Pvt. Ltd. (Mumbai, India).All the chemicals used in this study were of analytical grade.

Extraction of plant material

Arial parts of *Urticaurens* were collected, washed and rinsed properly. They were dried in shade and powdered mechanically. About 100 gm of the plant powder was macerated with 80% methanol and stored for 48 hours for the extraction of phytochemicals. At the end of the 48 hrs extract was filtered using whatmann No. 1 filter paper to remove all un-extractable matter, including cellular materials and other constitutions that are insoluble in the extraction solvent. The entire extract was concentrated to dryness using rotary flash evaporator under reduced pressure and stored in an air tight container free

from any contamination until it was used. Finally the percentage yields were calculated of the dried extracts.

Qualitative phytochemical analysis of plant extract

The *Urticaurens*extract obtained was subjected to the preliminary phytochemical analysis following standard methods by Khandelwal and Kokate[10, 11]. The extract was screened to identify the presence or absence of various active principles like phenolic compounds, carbohydrates, flavonoids, glycosides, saponins, alkaloids, fats or fixed oils, protein and amino acid and tannins.

Animals

In the present investigation the Swiss albino mice (20-25 gm ofeither sex) were group housed (n= 6) under a standard 12 h light/dark cycle and controlled conditions of temperature and humidity (25±2 °C, 55–65%). Mice received standard rodent chow and water *ad libitum*. Mices were acclimatized to laboratory conditions for 7 days before carrying out the experiments. All the experiments were carried in a noise-free room between 08.00 to 15.00 h. Separate group (n=6) of mices was used for each set of experiments. The animal studies were approved by the Institutional Animal Ethics Committee, (IAEC No. COPSSUTMS/ANIL/19-10).constituted for the purpose of control and supervision of experimental animals by Ministry of Environment and Forests, Government of India, New Delhi, India.

Acute oral toxicity

Acute toxicity studies were performed according to OECD (Organization for Economic Co-operation and Development) 425 guideline. Animals were acclimatized to laboratory condition five days prior to the experiment. Body weight of animals was recorded and individual identification was done, fixed dose method: Test procedure with a starting dose of 2000 mg/Kg body weight of hydroalcoholic extract aerial parts of *Urticaurens* L. Starting dose of extract was administered orally to 5 animals and animals were observed for behavioral changes and death. No animals were found dead after 14 days. The study was repeated with same dose and again no death was observed [12].

Evaluation of antidepressant activity

Forced swimming test (FST)

The FST is the most widely used pharmacological *in vivo* model for assessing antidepressant activity. Mice were individually placed in cylinder (45×20 cm) containing 15 cm water (25±2°C), so that it could not touch the bottom of the cylinder with its hind limb or tail, or climb over the edge of the chamber. Mice were divided into groups of 4 and received the hydroalcoholic extract aerial parts of *Urticaurens* L at different doses *viz*. 250 and 500 mg/kg and fluoxetine (20mg/kg) was used as standard drug. One hour post administration each mice were placed individually in a tank. Period of immobility

(i.e. the total time the animal remained floating in water without struggling and making only those movements necessary to keep its head above water) during the 6 min test period was measured [13].

Tail suspension method

Each mouse in the group was suspended individually by the end of tail (50 cm above the floor) with adhesive tape placed approximately 1cm from the tip of the tail. Mice were divided into groups of 4 and received hydroalcoholic extract aerial parts of *Urticaurens* L at different doses *viz*. 250 and 500 mg/kg control and fluoxetine (20mg/kg) was used as standard drug on the test day after 60 min of the administration of last dose. The Duration of immobility was observed for a period of 8 minutes. After the early escape oriented actions, the rat rapidly turns out to be immobile and immobility (when it did not show any movement of body and hanged passively) was recorded during last 5 min of observation period [14].

Statistical Analysis

The data were expressed as mean ±standard error mean (SEM). The significance of differences among the groups was assessed using one way analysis of variance (ANOVA). The test was followed by Dunnett's'-test, p values less than 0.05 were considered as significance.

RESULTS

The crude extracts so obtained after maceration extraction process was concentrated on water bath by evaporation the solvents completely to obtain the actual yield of extract. The yield of extracts obtained from the aerial part of the plants using hydroalcoholic (Water: Methanol 20:80) as solvents are depicted in the Table 1. The results of preliminary phytochemical screening tests revealed the presence of saponins,diterpines, carbohydrates and amino acids in the crude extract (HAUU) Table 2. The extract HAUU was studied for acute toxicity at doses of 2000 mg/kgb.wt., po. The extract was found devoid of mortality of all animals. So, the doses selected for theantidepressant activity were 250 and 500 mg/kg, po.In forced swim test, the immobility time of control, test (250and500mg/kg) and standard was 132±1.80, 102±1.64 *, 78±2.42** and 65±0.65** respectively. The immobility time of test and standard was significant (**$p < 0.01$) and more significant (***$p< 0.001$) respectively. The immobility time decreases with increase in dose of the extract. The immobility time of test was gradually decreases when compared to control.Mice pretreated with hydroalcoholic extract of aerial parts of *Urticaurens* L show significant improvement in the swimming time as compared to standard control. The antidepressant effect of the *Urticaurens* L was prominent at 500mg/kg. In the forced swimming test all the doses administered were able to reduce immobility time and simultaneously enhance swimming Table 3.In the tail suspension test, the mice shown immediate sign of struggles or escape like behaviors when they were suspended in the air followed by temporary increasing periods of immobility. The tail

suspension method revealed in the present study that anti stress activity increases with decrease in immobility time compared to control mice of *Urticaurens* L. In tail suspension test, the immobility time of control, test (250and500 mg/kg) and standard was 218±2.64, 198±3.74*, 174±2.68** and 162±2.48** respectively. The immobility time of test and standard was significant (**p < 0.01) and more significant (***p < 0.001) respectively. The immobility time decreases with increase in dose of the extract. The immobility time of test was gradually decreases when compared to control Table 4.

Table 1: % Yield of hydroalcoholic extract

S. No.	Plant	% Yield (w/w)
1.	**Aerial partsof *Urticaurens***	3.8%

Table 2: Result of phytochemical screening of hydroalcoholic extract

S. no.	Constituents	Hydroalcoholic extracts
1.	**Alkaloids**	
	Dragendroff's test	-ve
	Wagner's test	-ve
	Mayer's test	-ve
	Hager's test	+ve
2.	**Glycosides**	
	Generalglycosides test	-ve
3.	**Flavonoids**	
	Lead acetate test	+ve
	Alkaline test	-ve
5.	**Phenolics**	
	$Fecl_3$ test	-ve
6.	**Amino acids**	
	Ninhydrin test	+ve
7.	**Cabohydrates**	
	Molichs test	+ve
8.	**Diterpines**	
	Copper acetate test	+ve
9.	**Saponins**	+ve

Table 3: Effect of extract of *Urticaurens* Lon animal in forced swim test

Group	Treatment	Immobility time (in Sec.)
Group 1	Control-Water	132±1.80
Group 2	HAUU (250 mg/kg, p.o.)	102±1.64 **
Group 3	HAUU (500 mg/kg, p.o.)	78±2.42**
Group 4	Fluoxetine (20 mg/kg. p.o.)	65±0.65***

Each values represents the mean±SEM; (n=6), *p<0.05, **p<0.01, ***p< 0.001 respectively when compared with control group (one-way ANOVA followed by Dunnett's test).

Table 4: Effect of extract of *Urticaurens* Lon animal in tail suspension test

Group	Treatment	Immobility time (in Sec.)
Group 1	Control-Water	218±2.64
Group 2	HAUU (250 mg/kg, p.o.)	198±3.74 **
Group 3	HAUU (500 mg/kg, p.o.)	174±2.68**
Group 4	Fluoxetine-(20 mg/kg. p.o.)	162±2.48**

Each values represents the mean±SEM; (n=6), *p<0.05, **p<0.01, ***p< 0.001 respectively when compared with control group (one-way ANOVA followed by Dunnett's test).

DISCUSSION

The antidepressant drugs used in the health center today have heterogeneity in the therapeutically response, multiple side effects and high monetary cost. Furthermore, treatment of depression with conversional antidepressant drugs provides a complete diminution in 70% of the individuals treated[15]. Therefore, the study of the antidepressant-like effects of herbs is an increasing attention[16]. Medical therapies with herbs may be effective alternatives in the treatment of depression and the research of their effects has progressed significantly since the past decade [17,18]. In this regard, aerial parts of *UrticaUrens* L have been studied. It was observed that HAUU at doses of 250 and 500 mg/kg b.wtexhibited significant reduction in immobility time in dose dependent manner when compared to control group in TST and FST tests. Similarly, the animals treated with Fluoxetine (20 mg/kg b.wt) as expected showed significant decrease in immobility time. Both the swimming and climbing behaviors in the FST are increased when the animals are treated by a drug which increases serotonin, norepinephrine and dopamine levels in the nerve terminals[19]. An increase in all the three neurotransmitters could be by inhibition of monoamineoxidase (MAO) activity in the brain. A growing body of research indicates that besides depletion of serotonin and catechoamine neurotransmitters, depression could result from various other pathophysiological mechanisms as well. Researchers suggest that depression may inhibit neurogenesis in the hippocampus [20, 21]. This idea is supported by the finding that antidepressants can promote neurogenesis[22].The hydroalcoholic extract of the aerial parts of *UrticaUrens* L possesses potential antidepressant activity in mice as shown by the TST and FST tests and could be considered as toxicologically safe with no deaths of mice when administered orally at the dose of 2000 mg/kg. The HAUU showed a dose dependant reduction in duration of immobility in mice. The efficacy of extract was found to be comparable to fluoxetine (20 mg/kg, po).

CONCLUSION

From the present study, it can be concluded that The hydroalcoholic extract of the aerial parts of *UrticaUrens* Lpossess potent antidepressant activity as shown by the TST and FST tests and is toxicologically safe.

References

1. Meti V, Ruckmani A, Chandrashekhar K, Konda VG, Madhavi E, *et al*. Antidepressant activity of ethanolic extract of *Piper betle*leaves in mice. Current Res Neurosci 2012; 2:11-6.
2. Santosh P, Venugopal R, Nilakash AS, Kunjbihari S, Mangala L. Antidepressant activity of methanolic extract of *Passiflorafoetida*leaves in mice. Int J Pharm PharmSci 2011;3(1):112-5.
3. Sharma VK, Chauhan NS, Lodhi SR, Singhai AK. Antidepressant activity of *Zizyphusxylopyrus*. Int J Phytomed 2009; 1:12-7.
4. Holtzheimer PE 3rd, Nemeroff CB. Future prospects in depression research. Dialogues ClinNeurosci 2006; 8(2):175-89.
5. Hughes RE, Ellery P, Harry T, Jenkins V, Jones E. The dietary potential of the common nettle. J Sci Food Agric. 1980; 31:1279-1286.
6. Adamski R, Bieganska J. Studies of chemical substances present in Urticadioica leaves. Part I, Trace element. HerbaPolonica. 1980; 26:177-180.
7. Kukric ZZ, Topalic-Trivunovic LN, Kukavica BM, Matos SB, Pavicic SS, Boroja MM, Savic AV. Characterization of antioxidant and antimicrobial activities of nettle leaves (Urticadioica L.). Apteff. 2012; 43:259-272.
8. Upton R. Stinging nettles leaf (Urticadioica L.): extraordinary vegetable medicine. J Herb Med. 2013; 3:39-38.
9. Guil-Guerrero JL, Rebolloso-Fuentes MM, Torija-Isasa ME. Fatty acids and carotenoids from stinging nettle (Urticadioica L). J Food Compos Anal. 2003; 16:111-119.
10. Khandelwal KR. Practical pharmacognosy technique and experiments. 23rd Ed. NiraliPrakashan; 2005.
11. Kokate CK, Purohit AP and Gokhale SB. Pharmacognosy; 23 ed., Niraliprakashan:2006; pp.493-497.
12. Guideline Document on Acute oral Toxicity Testing, Series on Testing and Assessment No. 423. Paris: Organization for Economic Co-Operation and Development, OECD Environment, Health and Safety Publications; 1996. Available from: http//www.oecd.org/ehs.

13. Porsolt RD, BertinA and JalfreM.Behavioral despair in mice: A primary screening test for antidepressants. Arch. Int. Pharmacodyn. Ther. 1977; 229: 327-336.
14. Steru L, ChermatR, ThierryB and SimonP. The tail suspension test: A new method for screening antidepressants in mice. Psychopharmacology.1985; 85: 367-370.
15. Fava M and RushAJ. 2006. Current status of augmentation and combination treatments for major depressive disorder: A literature review and a proposal for a novel approach to improve practice. Psychotherapy Psychosomatics, 75: 139-153.
16. Newman DJ, CraggGM and SnaderKM. Natural products as sources of new drugs over the period 1981-2002. J. Nat. Prod.2003; 66: 1022-1037.
17. Hasrat JA, PietersL, De BackerJP, VauquelinG and VlietinckAJ. Screening of medicinal plants from Suriname for 5-HT1A ligands: Bioactive isoquinoline alkaloids from thefruit of *Annona muricata*. Phytomedicine.1997; 4: 133-140.
18. Hasrat JA, BruyneTD, BackerJP, VauquelinG and VlietinckAJ.Isoquinoline derivatives isolated from the fruit of *Annona muricata*as 5 HTergic 5 HT1A receptor agonists in rats: Unexploited Antidepressive (Lead) products. J. Pharm. Pharmacol.1997; 49: 1145-1149.
19. Reneric JP and LuckiI. Antidepressant behavioral effects by dual inhibition of monoamine reuptake in the rat forced swimming test. Psychopharmacology.1998; 136: 190-197.
20. Sapolsky RM. The possibility of neurotoxicity in the hippocampus in major depression: A primer on neuron death. Biol. Psychiat.2000; 48: 755-765.
21. Henn FA and VollmayrB. Neurogenesis and depression: Etiology or epiphenomenon? Biol. Psychiat.2004; 56: 146-150.
22. Rajkowska, G, Miguel-HidalgoJJ, WeiJ, DilleyG and PittmanSD*et al*. Morphometric evidence for neuronal and glial prefrontal cell pathology in major depression. Biol. Psychiatry.1999; 45: 1085-1098.