Research Article

Evaluation of Antianxiety Activity of Xanthine Oxidase Inhibitors in Albino Mice

***Swetha E. S., Sathisha Aithal, Ayesha Rubina**

S. S. Institute of Medical Sciences & Research Centre, NH-4 Bypass Road, Davangere, 577004, Karnataka, India.

ABSTRACT

Background: Anxiety the most common mental health disorder affecting majority of the population. Serotonin is involved to be a major neurotransmitter in causing anxiety. Tryptophan is the only precursor of serotonin and xanthine oxidase is the endogenous activator of tryptophan pyrrolase enzyme which can result in reduced serotonin level. Xanthine oxidase inhibitors putatively inhibit the metabolism of tryptophan therefore leading to increase in serotonin level. Hence the study was planned to evaluate the antianxiety effect of xanthine oxidase inhibitors, allopurinol and febuxostat. Objective: To evaluate the antianxiety activity of Allopurinol 39mg/kg and Febuxostat 15.6mg/kg in comparison with control, Diazepam0.5mg/kg and with Fluoxetine10mg/kg. Methods: **Elevated plus maze**-Pre-treated animals were placed individually for 5mins in the maze. The number of entries into the open and closed arm, time spent in each arm and the no. of entries in both the arms was noted**. Social interaction test**-Pre-treated mice were isolated for 1hour before the test. In the test arena, the mice were observed for cumulative time spent in social interaction for a period of 10mins. All the results are expressed as Mean±SEM. Data are analyzed by ANOVA using Graph Pad Instat (GPIS) package, version 3.05. $P < 0.05$ was considered as significant. Results: **Elevated plus maze-** Administration of Allopurinol and Febuxostat significantly increased the time spent in open arms, $p<0.001$&$p<0.018$ respectively in comparison with control. **Social interaction test**- Administration of Allopurinol and Febuxostat significantly increased the time spent in social interaction, $p<0.001$ as compared to control. Conclusion: Both Allopurinol and Febuxostat possessed significant antiaxiety effects.

Keywords: Anxiety, elevated plus maze, social interaction test, serotonin, tryptophan

Received 18 July 2014 Received in revised form 30 July 2014 Accepted 05 August 2014

***Address for correspondence:**
Swetha E. S.,
Department of Pharmacology, S. S. Institute of Medical Sciences & Research Centre, NH-4 Road, Davangere, 577004, Karnataka, India.
E-mail: shwetha.es@gmail.com

INTRODUCTION

Anxiety disorders are the most common mental health conditions affecting 18% of the world's population and have become an important area of research in psychopharmacology [1]. Anxiety is a state of fear characterized by motor tension, sympathetic over activity, apprehension and vigilance. Several neurotransmitters like glutamate, GABA, catecholamines, serotonin etc. are known to mediate different components of anxiety [2]. Brain serotonin ($5HT_1$) is important in modulating various physiological activities like mood, impulse control, sleep, vigilance, eating, libido etc. and hence an important modulator of anxiety. Recent guidelines recommend that except in acute states of anxiety, Selective serotonin reuptake inhibitors (SSRIs) are used in almost all types of anxiety disorders. Diazepam is preferred for acute anxiety states [3]. Tryptophan is an aromatic precursor of serotonin and it is metabolized by the enzyme tryptophan pyrrolase. Xanthine oxidase is the enzyme which endogenously activates tryptophan pyrrolase thus resulting in increased metabolism of

tryptophan [4]. Thus its level can be increased by inhibiting xanthine oxidase. The role of tryptophan in anxiety has been established by various studies [5,6]. In the present study xanthine oxidase inhibitors, allopurinol and febuxostat are used. Both these drugs are presenty used in the treatment of hyperuricemia and gout [7]. They interfere in the metabolism of tryptophan thereby indirectly could increase the level of tryptophan and hence serotonin in the body. Therefore allopurinol and febuxostat can be hypothesized to have antianxiety property. Hence the present study was planned to evaluate their antianxiety effect in comparison with the control and standard drugs diazepam and fluoxetine.

MATERIALS AND METHODS [8]:

Ethical approval was taken from the institutional ethics committee of SSIMSRC, Davangere. The mice inbred in in the central animal house of S.S. Institute of Medical Sciences and Research Centre is used. A total of 30(n=30) animals will be divided into 5 groups of 6 each. The previous day of the experiment, the animals were weighed and were randomly housed in cages as 6 animals per cage. The temperature was maintained at 21±3^{0}C, with 12-hour light-dark cycle. The animals had free access to food and water, *ad libitum.* Swiss albino mice of either sex weighing 20-25 g were used and divided into five groups of 6 each. Group I received 1 ml distilled water, Group II received Diazepam 0.5mg/kg, GROUP III allopurinol 39mg/kg, group IV febuxostat 15.6mg/kg and group V fluoxetine 10mg/kg. Two models were used Social interaction test and elevated plus maze test.

Social interaction test: Pre-treated mice were isolated for 1 hour before the test. In the test arena, an open topped box (22 × 15 × 12 cm), the mice were observed for cumulative time spent in social interaction for a period of 5mins. The social interaction included genital investigation, sniffing a partner, following, grooming, kicking, biting, wrestling, climbing over and under, neck licking and boxing. Elevated plus maze: The plus maze apparatus consisted of two open arms, measuring 16 × 5 cm, and two closed arms, measuring 16 × 5 × 12 cm, connected to a central platform (5 × 5 cm). The maze was elevated to a height of 25 cm above the floor. Pre-treated animals were placed individually for 5mins in the maze with its head facing the open arm. The number of entries into the open and closed arm, time spent in each arm and the preference to the open arms was noted. **The percent time spent on the open arms was determined as follows:**

% = 100 × Number of seconds spent on open arms/300 total seconds (5 min observation time).

Statistical analysis: All the results were expressed as Mean ± SEM. Data were analyzed by ANOVA in Graph Pad Instat (GPIS) package, version 3.05. $P < 0.05$ was considered as significant.

RESULTS

Social interaction test:

Diazepam (0.5 mg/kg) and fluoxetine 10mg/kg significantly ($P < 0.001$) increased the time spent in social interaction among mice as compared to its effect in the distilled water-treated group. The test groups allopurinol 39mg/kg and febuxostat 15.6mg/kg ($P < 0.001$), significantly increased the time spent in social interaction as compared to distilled water-treated group (**Figure 1**). The results were comparable to standard diazepam as well as fluoxetine treated groups.

Elevated plus maze test:

Administration of diazepam (0.5 mg/kg) and fluoxetine 10mg/kg significantly increased the amount of time spent in the open arms ($P < 0.001$) and the preference to open arms ($p<0.001$) compared to distilled water-treated group (**Table 1**). The test groups allopurinol 39mg/kg and febuxostat 15.6mg/kg ($P< 0.001$), significantly increased the time spent in open arms ($p<0.001$) and also the preference to enter the open arm ($p<0.001$) as compared to distilled water-treated group (**Table 1**). The results were comparable to standard diazepam as well as fluoxetine treated groups.

DISCUSSION

In the present study, in social interaction test as well as in the elevated plus maze both the test drugs i.e., allopurinol 39mg/kg and febuxostat 15.4mg/kg showed significant antianxiety effect. Both the drugs showed significant antianxiety effects in

both the models consistenly. The results were comparable to the standard drugs diazepam, a benzodiazepine which acts on GABA receptor to antagonize it and fluoxetine, a SSRI, which increases the serotonin levels in the synaptic cleft [9]. The significant antianxiety effect shown by the xanthine oxidase inhibitors can be attributed to the increased level of tryptophan by the inhibition of xanthine oxidase.

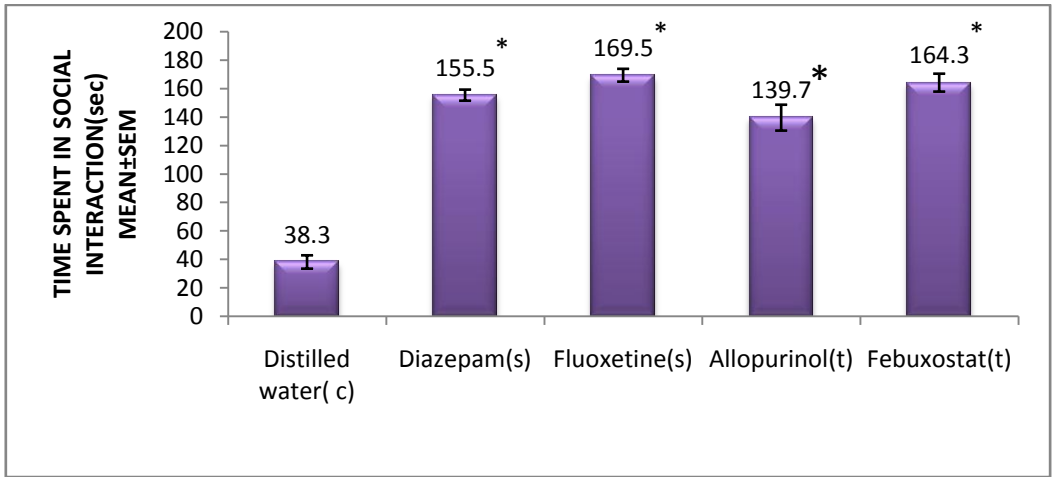

Figure 1: Effect of different drugs in social interaction test in 5 minutes duration
*** - Highly significant, c-control, s-standard, t-test

Table 1: Effect of different treatment groups in elevated plus maze test

Groups	No. of open arm entries	No. of closed am entries	time spent in open arm	time spent in closed arm	% prefeance to open arm
Distilled water(c)	14.3±1.6	11.83±1.13	141.83±13.3	158.16±13.3	6%
Diazepam(s)	8.83±1.13	7.5±1.37	189.5±4.03 *	110.5±4.03	83.3% *
Fluoxetine(s)	10.83±0.83	6.83±0.94	200.5±5.58 *	99.5±5.58	100% *
Allopurinol	11.33±2.2	9±1.82	188.6±2.45 *	111.3±2.45	66.6% *
Febuxostat	12.5±0.88	7.83±1.4	175±2.68 *	125±2.68	83.3% *

*- Highly significant, c-control, s-standard

Many studies have demonstrated that Tryptophan has been used in the treatment of depression in the past [10,11]. Researchers have found that the break-down of L-tryptophan into kynurenine production instead of serotonin production by the enzyme tryptophan pyrrolase is linked to anxiety [12]. There are studies which showed that L-tryptophan ingestion caused a reduction in social anxiety disorder and at the same time depletion induced significant increase of anxiety in treated Seasonal Affective disorder (SAD) patients [5,13,14]. Tryptophan is the only aromatic acid precursor of serotonin, a chief neurotransmitter in the brain which has a key role responsible for various brain physiologies such as alterations in appetite, energy, sleep, mood, libido, and cognitive functioning seen in affective disorders [15]. It is produced in the brain by a short two step process. In the first step, L- Tryptophan is first converted into 5-Hydroxytryptophan by the enzyme tryptophan hydrolase. 5-Hydroxy-tryptophan is then decarboxylated by another enzyme, aromatic enzyme decarboxylase in the second step [16]. For about 80% of our body's total serotonin content, the source is the gut, i.e., the enterochromaffin cells - where it regulates intestinal movements. The rest is synthesized in the serotonergic neurons in the central nervous system as serotonin cannot cross the blood-brain barrier [17].

Therefore serotonin that is used inside the brain must be produced within it. Serotonin facilitates defensive responses to potential threat like inhibitory avoidance related to anxiety. Elena et al. focused on the involvement of serotonergic receptor in the dysfunctional forms in anxiety both by preclinical as well as clinical data [18]. The role of serotonin in anxiety is supported by its modulatory effects on the locus coeruleus and its dense projections to the amygdale [19]. Allopurinol is in the market from many decades now & is tolerated well by most of the patients with few drug interactions unlike TCAs and SSRIs. It is known to cause increase in the volume of distribution of tryptophan [20]. Fever, myalgia, malaise hepatomegaly are some of the rare side effecs caused by allopurinol. Febuxostat is more potent and has better adverse effect profile and it is well tolerated as compared to allopurinol. The most frequently occurring side effects include nausea, diarrhea, headache and alteration in the liver function. It is the non purine xanthine oxidase inhibitor. The activity of these xanthine oxidase inhibitors were studied on depression models and a significant antidepressant activity was seen [21]. The putative role of xanthine oxidase on levels of tryptophan and serotonin has to be studied. If their role in various brain physiologies is validated, it would enhance their therapeutic utility.

CONCLUSION

Both Allopurinol and Febuxostat have got significant antianxiety effect as demonstrated in both social interaction test and elevated plus maze test when compared to control. The antianxiety activity was comparable to the standard drugs diazepam and fluoxetine. This has to be confirmed by other models of anxiety as well as by human trials and if proved, may provide an important alternative in the treatment of anxiety. Also the effects of xanthine oxidase on tryptophan and serotonin levels have to be assessed and validated by other methodologies.

RFERENCES

1. The new National Institute of Mental Health. 1967. Available at: http://www.nimh.nih.gov/health/publications/depression/depression-booklet.pdf. Accessed May 13, 2014.
2. Shin LM, Liberzon I. The neurocircuitry of fear, stress, and anxiety disorders. Neuropsychopharmacology. 2010;35(1):169–91. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3055419&tool=pmcentrez&rendertype=abstract. Accessed May 28, 2014.
3. Generalised Anxiety DISORDER in adults GENERALISED ANXIETY DISORDER IN ADULTS: London: The British Psychological Society and The Royal College of Psychiatrists; 2011.
4. Becking G, Johnson W. The inhibition of tryptophan pyrrolase by allopurinol, an inhibitor of xanthine oxidase. Can J Biochem. 1967;45:1667–72.
5. Argyropoulos S, Hood S, Adrover M, et al. Tryptophan depletion reverses the therapeutic effect of selective serotonin reuptake inhibitors in social anxiety disorder. Biol Psychiatry. 2001;56(1503-9).
6. Kanai M, Funakoshi H, Takahashi H, Hayakawa, Tomoko Mizuno S, Matsumoto, Kunio Nakamura T. Tryptophan 2,3-dioxygenase is a key modulator of physiological neurogenesis and anxiety-related behavior in mice. Mol. Brain. 2009;2(8).
7. Furst D, Ulrich R, Altamirano C. Non-steroidal anti-inflammatory drugs, disease modifying antirheumatic drugs, non opoid analgesics & drugs used in gout. In: Katzung B, Masters S, TrevorAJ, eds. Basics of clinical pharmacology. 12th ed. New Delhi: McGraw-Hill; 2011.
8. Mahendra P, Bisht S. Anti-anxiety activity of Coriandrum sativum assessed using different experimental anxiety models. Indian J Pharmacol. 2011;43(5):574–577.
9. Understanding Anxiety Disorders and Effective Treatment. Am. Psychol. Assoc. 2010. Available at: http://www.apapracticecentral.org/outreach/anxiety-disorders.pdf.
10. Thomson J, Rankin H, Ashcroft G, Yates C, McQueen J, Cummings S. The treatment of depression in general practice: a comparison of L-tryptophan, amitryptiline and a combination of L-tryptophan and amitryptiline with placebo. Psycho Med. 1982;12:741–51.
11. Coppen A, Shaw D, Herzberg B, Maggs R. Tryptophan in the treatment of depression. Lancet. 1967;2:1178–80.
12. Funakoshi H, Kanai M, Nakamura T. Modulation of Tryptophan Metabolism, Promotion of Neurogenesis and Alteration of

Anxiety-Related Behavior in Tryptophan 2,3-Dioxygenase-Deficient Mice. Int. J. Tryptophan Res. 2011;4:7–18. Available at: http://www.la-press.com/modulation-of-tryptophan-metabolism-promotion-of-neurogenesis-and-alte-article-a2586. Accessed July 16, 2014.

13. Young S, Smith S, Pihl R. et al (1985) Tryptophan depletion causes a rapid lowering of mood in normal males. Psychopharmacology (Berl). 1985;87:173–77.
14. Van der Does A. The effects of tryptophan depletion on mood and psychiatric symptoms. J Affect Disord. 2001;64:107–19.
15. Toker L, Amar S, Bersudsky Y, Benajmin J. The biology of tryptophan depletion and mood disorders. Isr J Psychiatry Relat Sci. 2010;47:46–55.
16. Bender DA. Biochemistry of tryptophan in health and disease. Molec. Asp. Med. 1982;6:101–97.
17. Best J, Nijhout HF, Reed M. Serotonin synthesis, release and reuptake in terminals: a mathematical model. Theor. Biol. Med. Model. 2010;7:34. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2942809&tool=pmcentrez&rendertype=abstract.
18. Akimova E, Lanzenberger R, Kasper S. The Serotonin-1A Receptor in Anxiety Disorders. Biol psychiatry. 2009;66(7):627–635. Available at: http://dx.doi.org/10.1016/j.biopsych.2009.03.012.
19. Davis M, Whalen PJ. The amygdala: vigilance and emotion. Mol. Psychiatry. 2001;6(1):13–34. Available at: http://www.ncbi.nlm.nih.gov/pubmed/11244481.
20. Green A, Aronson J, Curzon G, Woods H. Metabolism of an oral tryptophan load: Effect of pretreatment with the putative tryptophan pyrrolase inhibitors nicotinamide and allopurinol. Br. J. Clin. Pharmacol. 1980;10:611–5.
21. Karve AV, Jagtiani SS, Chitnis K . Evaluation of effect of allopurinol and febuxostat in behavioral model of depression in mice. Indian J. Pharmacol. 2013;45(3):244–7. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3696294&tool=pmcentrez&rendertype=abstract. Accessed July 16, 2014.