Research Article

INTERNATIONAL JOURNAL OF CURRENT MEDICAL AND PHARMACEUTICAL RESEARCH

*ISSN: 2395-6429, Impact Factor: 4.656*
*Available Online at www.journalcmpr.com*
*Volume 5; Issue 01(A); January 2019; Page No. 4009-4013*
*DOI: http://dx.doi.org/10.24327/23956429.ijcmpr201901610*


# COMPARISON OF ANXIOLYTIC ACTIVITY OF ONDANSETRON AND URSOLIC ACID IN CHRONIC ANXIETY USING ELEVATED PLUS MAZE TEST

**Mrunal Pramod Dhole[1]., Seema Bhalerao[1]., Aakash Suresh Kewlani*[1]., Puja Jha[2]., Pratik Rane [1] and Samiksha Shelar[3]**

[1]Department of Pharmacology, Dr. D. Y. Patil Medical College, Hospital and Research Centre, Dr. D. Y. Patil Vidyapeeth, Pune
[2]Drug Safety Physician, APCER Life Sciences, New Delhi
[3]Department of Pharmacology, Swami Ramanand Tirth Rural Government Medical College, Ambajogai, Maharashtra University of Health Sciences, Nashik

**ARTICLE INFO**

***Article History:***
Received 13th October, 2018
Received in revised form 11th November, 2018
Accepted 8th December, 2018
Published online 28th January, 2019

***Key words:***

Chronic anxiety, Anxiolytic activity, Elevated plus maze test, Diazepam, Ondansetron, Ursolic acid

**ABSTRACT**

**Background:** Prolonged use of benzodiazepines to treat chronic anxiety disorders has adverse effects such as impaired cognitive function and motor control, tolerance, dependence and abuse. Ondansetron (5-$HT_3$ receptor antagonist) and Ursolic acid (ubiquitous phytochemical) have anxiolytic activity comparable to Diazepam with no such adverse effects. This study evaluated and compared the effect of Ondansetron and Ursolic acid on chronic anxiety in rats using elevated plus maze (EPM) test, which is a validated animal model to investigate anxiolytic agents.
**Methodology:** Wistar albino rats of either sex (150-250 g) were divided into four groups with eight rats in each group. To induce chronic anxiety, all rats underwent forced swim test daily for 21 days after which the study groups were given distilled water (2 ml p.o.), Diazepam (1 mg/kg i.p.), Ondansetron (1 mg/kg i.p.) and Ursolic acid (0.2 mg/kg p.o.) respectively. Effect of test drugs on anxiety was evaluated using EPM test and parameters such as number of entries in open arms and closed arms as well as time spent in open arms were assessed. Results (mean±standard deviation) were analysed using one-way ANOVA test followed by Bonferroni's correction.
**Results:** Statistically significant increase ($p < 0.05$) was seen in time spent and number of entries in open arms in other three groups compared to Control group, in Diazepam group compared to Ondansetron and Ursolic acid groups, and in Ondansetron group compared to Ursolic acid group.
**Conclusion:** This study revealed that Ondansetron had significant anxiolytic activity compared to Ursolic acid in chronic anxiety.



## INTRODUCTION

Anxiety can significantly interfere with carrying out the daily routine tasks and have an adverse impact on the quality of life of the concerned individual warranting a need for pharmacological treatment. One in 14 people around the world at any given time has an anxiety disorder and one in 9 will experience an anxiety disorder in a given year.[1]

Benzodiazepines are effective in treating acute episodes of anxiety as well as chronic treatment of anxiety-related disorders. On chronic use, they adversely affect cognitive performance and memory, motor control, and potentiate the effects of other sedatives including alcohol and may lead to development of tolerance to the anxiolytic effects.[2] Also, withdrawal of benzodiazepines after chronic treatment can cause anxiety and seizures. Thus, treatment with benzodiazepines has the potential to lead to habituation, dependence and abuse. At the present time, it is the abuse liability of the benzodiazepines which has seen their downfall, and has encouraged concerted efforts to find new treatments.[3]

Ondansetron, a selective 5-HT3 receptor antagonist, used as an antiemetic in post-operative nausea and vomiting (PONV) and cancer chemotherapy induced emesis, produces significant anxiolysis at the antiemetic dose itself in rodents.[4] They lack any sedative action. There are no problems on withdrawal of therapy.[3]Purification of extracts of medicinal herbs such as holy basil, lemon balm, mugwort, and wild sage led to isolation of Ursolic acid which is aphytochemical.[5-8]Acute oral administration of Ursolic acid elicited an anxiolytic-like effect in elevated plus maze (EPM) test in mice.[9]

**Corresponding author:* **Aakash Suresh Kewlani**
Department of Pharmacology, Dr. D. Y. Patil Medical College, Hospital and Research Centre, Dr. D. Y. Patil Vidyapeeth, Pune

Ondansetron and Ursolic acid can prove to be an effective alternative to Diazepam for the management of chronic anxiety disorders. To the best of our knowledge, no studies have been carried out to compare the efficacy of Ursolic acid and Ondansetron for their anxiolytic action.

The aim of this study was to compare anxiolytic activity of Ondansetron and Ursolic acid in chronic anxiety using elevated plus mazetest.

## MATERIAL AND METHODS

The study commenced after IAEC (Institutional Animal Ethics Committee) approval and was conducted in accordance with CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals) guidelines. Wistar albino rats (150-250 g) of either sex were procured from the animal house located in Dr. D. Y. Patil Medical College, Hospital and Research Centre, Pune. They were housed in standard polypropylene cages under standard conditions of temperature (25 ± 5°C) and relative humidity (55 ± 10%) and 12/12-hour light/dark cycle. Apart from daily replenishment of food pellets and drinking water, they were left undisturbed.

### *Forced swim test*[10]

Anxiety was induced in rats using a stress test known as forced swim test. At a given time, only one rat was forced to swim for a duration of 5 minutes in a plastic tank (40 cm x 100 cm x 60 cm) containing tap water. To produce chronic anxiety, all rats underwent forced swim test daily for 21 days at a fixed time after which they were administered the study drugs.

### *Study drugs*

Ondansetron [Cadila Healthcare Ltd., India] and Ursolic Acid [TCI Chemicals (India) Pvt. Ltd., India] were used as test drugs. Ondansetron was diluted to obtain a solution of concentration 0.1mg/ml and administered intraperitoneally at the dose of 1mg/kg.[4,11]Ursolic Acid solution was freshly prepared in distilled water with 10% of Tween 80 and given in a dose of 0.2 mg/kg orally.[11,12]Diazepam[Neon Laboratories Ltd., Mumbai] was used as the standard drug. It was diluted to obtain a solution of concentration 0.1mg/ml and administered intraperitoneally at the dose of 1mg/kg.[11,13] Distilled water was given orally as control in equivalent volume.

Each study drug was given one hour before conducting EPM test.

### *Study groups*

Rats were divided into four groups with eight rats in each group.

Group I: Distilled water, 2.0 ml p.o. (Control group)
Group II: Diazepam, 1.0 mg/kg i.p.
Group III: Ondansetron, 1.0 mg/kg i.p.
Group IV: Ursolic acid, 0.2 mg/kg p.o.

### *Elevated plus maze test*

Effect of study drugs on chronic anxiety was evaluated using EPM test.

The elevated plus-maze consists of two open arms (50 cm x 10 cm x 40 cm) and two enclosed arms (50 cm x 10 cm x 40 cm) with an open roof, arranged so that two open arms are opposite to each other. The maze is elevated to a height of 50 cm.[14] The rat was placed in the centre of the maze, facing one of the enclosed arms, allowing it to explore the maze freely for 5 minutes. The floor of the enclosure was cleaned with 70% ethanol between tests.

The following parameters were measured during a period of 5 minutes.

- Time spent in open arms
- Number of entries into open arms
- Number of entries into closed arms

Arm entries were considered valid only if all four paws entered into an arm. An increase in frequency of entries into open arms and duration of time spent in open arms indicate low levels of anxiety.

### *Statistical Analyses*

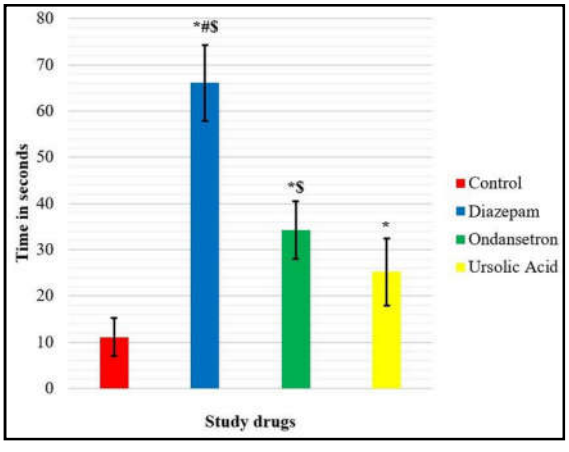
The data was compiled in a Microsoft Excel 2016 spreadsheet. It was analyzed using the statistical packages – WinPepi (Version 11.65) and Primer of Biostatistics (Version 7). Results are expressed in mean and standard deviation (SD). The data passed the Shapiro-Wilk normality test for distribution. One Way Analysis of Variance (ANOVA) was used to determine whether the difference in group means was overall statistically significant. The p-value less than 0.05 was considered to be statistically significant. If the p-value was found to be less than 0.05, Bonferroni correction for multiple comparisons (a post-hoc test) was applied after ANOVA to confirm which specific groups differed.

## OBSERVATIONS AND RESULTS

**Table No.1** Time spent in open arms in EPM

| Group | Mean | SD | p |
|---|---|---|---|
| Control | 11.12 | 4.086 | |
| Diazepam | 66.12 | 8.184 | 0.001 |
| Ondansetron | 34.25 | 6.182 | |
| Ursolic acid | 25.25 | 7.265 | |

Table No 1. shows that difference seen among the study groups for time spent in open arms after applying ANOVA test was significant




**Graph No.1** Time spent in open arms in different groups in EPM

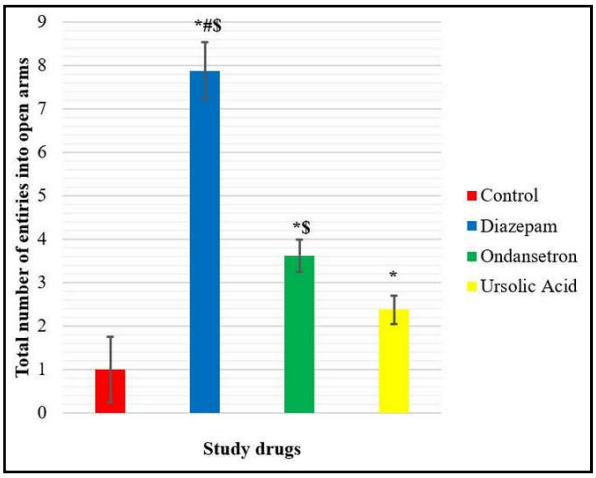
* p < 0.05 as compared to Control group
\# p < 0.05 as compared to Ondansetron group
$ p < 0.05 as compare to Ursolic acid group

Graph No. 1 shows that after applying post-hoc test, statistically significant increase was observed in time spent in open arms in the other three groups when compared with Control group. When these three groups were compared with each other, statistically significant increase in Ondansetron group was seen as compared to Ursolic acid group but both groups were not comparable to Diazepam group.

**Table No. 2** Total number of entries in open arms in EPM

| Group | Mean | SD | p |
|---|---|---|---|
| **Control** | 1 | 0.7559 | |
| **Diazepam** | 7.875 | 0.6665 | |
| **Ondansetron** | 3.625 | 0.375 | 0.001 |
| **Ursolic acid** | 2.375 | 0.3239 | |

Table No.2 shows that difference seen among the study groups for number of entries in open arms after applying ANOVA test was significant.




**Graph No.2** Number of entries in open arms in different groups in EPM

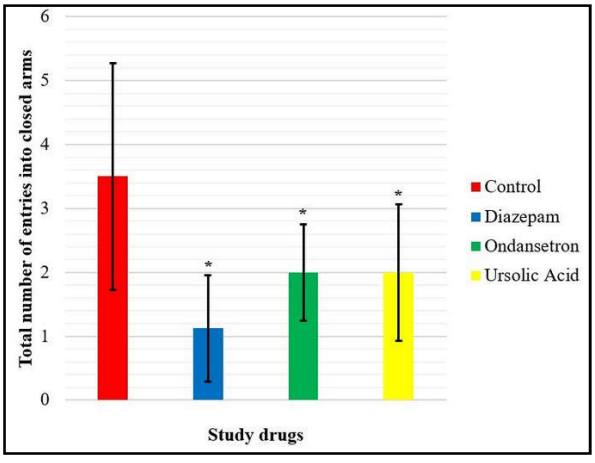
* p < 0.05 as compared to Control group
# p < 0.05 as compared to Ondansetron group
$ p < 0.05 as compare to Ursolic acid group

Graph No. 2 shows that after applying post-hoc test, statistically significant increase was observed in number of entries in open arms in the other three groups when compared with Control group. When these three groups where compared with each other statistically significant increase in Ondansetron group as compared to Ursolic acid group but both groups were not comparable to Diazepam group.

**Table No. 3** Total number of entries in closed arms in EPM

| Group | Mean | SD | P |
|---|---|---|---|
| Control | 3.5 | 1.773 | |
| Diazepam | 1.125 | 0.8345 | 0.004 |
| Ondansetron | 2 | 0.7559 | |
| Ursolic acid | 2 | 1.069 | |

Table No. 3 shows that difference seen among the study groups for number of entries in closed armsafter applying ANOVA test was significant.




**Graph No 3** Number of entries in closed arms in different groups in EPM

* p < 0.05 as compared to Control group

Graph No. 3 shows that after applying post-hoc test, statistically significant decrease was observed in number of entries in closed arms in the other three groups when compared with control group. When these three groups where compared with each other, no statistically significant difference was found among them.

## DISCUSSION

The anxiolytic effects of Diazepam, a benzodiazepine, can be ascribed in part to potentiation of GABAergic pathways that serve to regulate the firing of monoamine-containing neurons known to promote behavioural arousal and to be important mediators of the inhibitory effects of fear and punishment on behaviour. The remarkable safety profile of benzodiazepines likely relates to the fact their effects *in vivo* depend on the presynaptic release of GABA.[15] However, adverse effects of prolonged use of benzodiazepines has led to the search of even better anxiolytic agents.

The present study evaluated and compared the anxiolyticactivity of a phytochemical, Ursolic acid and a 5-$HT_3$ receptor antagonist, Ondansetron with the standard anxiolytic drug, Diazepam in rats using EPM test. It also compared Ondansetron and Ursolic acid for the first time to determine which one of the eitherdrugs effectively attenuate chronic anxiety.

Ondansetron-treated mice showed increased exploratory behaviour in the aversive white compartment in the light/dark exploration test. In both social interaction test in rats and marmoset human threat test, Ondansetron was revealed to have an efficacy equal to Diazepam but a greater potency as an anxiolytic agent.[16] The efficacy and potency of Ondansetron was confirmed in the elevated X-maze test.[17] Thus several pre-clinical studies have consistently shown Ondansetron to have potential anxiolytic activity.

The neurobiological basic research has reported that monoamines; specifically serotonin dysfunction is critically involved in the development of clinical depression and anxiety.

Serotonergic pathways are largely innervated in brain areas such as mid brain (including hippocampus) and frontal cortex where they play a major role in controlling mood and emotional behavior.5-$HT_3$ receptor antagonists block and subsequently prevent the pre-synaptic 5-$HT_3$ auto receptors mediated negative feedback inhibition of serotonin release at the synapse and hence facilitate the serotonin activity at the synaptic cleft. This could be one of the possible cellular pathways by which Ondansetron could have elevated the serotonin levels in the brain as observed in a study by Gupta *et al*. (2014).[18] Increase in serotonergic neurotransmission with several antidepressants has shown to inducehippocampal neurogenesis. Also, stress induced depletion of serotonin has reported to be associated with neuronal cell damage and a significant reduction in neurogenesis.

The active principle, Ursolic acid (2.5%), obtained from the ethanolic extract of *Ocimum sanctum* leaves was responsible for the anti-anxiety effect in a study done by Pemminati *et al*. (2010).[5]Ursolic acid isolated and identified from the methanolic extract of *Melissa officinalis* via bioassay-guided fractionation inhibited GABA transaminase.[19]Ursolic acid isolated by fractionation of *Artemisiaindica* was found to be a positive modulator of $\alpha1\beta2\gamma2$L recombinant $GABA_A$

receptors. This modulation was antagonized by Flumazenil. This validates the hypothesis that the anxiolytic mode of action ofUrsolic acid involves effects on GABAergic systems.[7]

Ursolic acid has been shown to exert an antidepressant-like effect by a serotonergic-mediated mechanism in mice.[20,21] The dose that causes antidepressant-like effect in the forced swimming test is the same that causes anxiolytic activity.[22] The anxiety/depression comorbidity is high. Therefore, Ursolic acid may offer a novel therapeutic strategy for the treatment of comorbidity depression/anxiety.

Forced swimming test or behavioural despair test is a well-established model to test for antidepressant activity.[23] In a study done by Adreatini *et al*. (1999), the mice underwent forced swim test which had an anxiogenic effect.[24] This test was used to induce anxiety in rats in this study.

Elevated plus maze test has been widely used as a tool in the investigation of the psychological and neurochemical bases of anxiety and for screening anxiety-modulating drugs.[22] It is based on the natural aversion of rodents for open spaces and uses conflict between exploration and aversion to elevated open places. Provoked behaviour profiles in the EPM appear to include elements of neophobia, fear of height, exploration, agoraphobia and approach/avoidance conflict and thus the apparatus is often referred to as an unconditioned spontaneous behavioural conflict model. Rats generally taken from their home cages will show a pattern of behaviour characterized by open-arm avoidance with a consistent preference for the closed arms. The rank order preference profile is closed > centre > open, indicative of a penchant for relatively secured sections of the maze.[25]

Lister[26] showed that the behavioural parameters in the EPM provided measures of two independent factors, one reflecting anxiety and one reflecting motor activity. The percentage of open-arm entries and the time spent on the open arms are extremely good measures of anxiety generated by this test and the number of closed-arm entries provided a better measure of motor activity.

This test offers a number of advantages over other paradigms used to assess anxiety that involve food or water deprivation or shock administration. In particular, drug effects on appetite or sensitivity to pain are unlikely to interfere with experimental results.[23]

In this study, when EPM test was done on Day 21 to evaluate the antianxiety effects of study drugs on chronic anxiety, statistically significant increase ($p < 0.05$) in time spent in open arms and total number of entries into open arms was observed in Diazepam, Ondansetron and Ursolic acid groups as compared with the Control group. From these observations, it can be stated that both Ondansetron and Ursolic acid possess significant anxiolytic action.

In a study by Kumar *et al*. (2014), on the seventh day when rats were subjected to EPM test, number of entries and duration of stay in open arms was higher in Diazepam treated group as compared to Ondansetron.[4] However, Rodgers *et al*. showed that sub-chronic treatment with intraperitoneal ondansetron injection (0.1–100 μg/kg) for 21 days in adult male CD1 mice failed to alter the spatiotemporal preference profile (open = closed > centre) seen in the saline control condition in the EPM test, thus producing no evidence of anti-anxiety effect.[27]This result might be attributed to inherent differences among various species.

In the present study, the increase in time spent in open arms and in total number of entries into open arms was significant ($p < 0.05$) in Diazepam group as compared with Ondansetron group. Thus, anxiolytic activity of Ondansetron was not found to be comparable to that of Diazepam.

In a study by Pemminati *et al*. (2011) where Ursolic acid and diazepam were administered for ten days, the antianxiety effects of Ursolic acid in EPM test were comparable to those of Diazepam.[28]

In the present study, increase in time spent in open arms and in total number of entries into open arms was significant ($p < 0.05$) in Diazepam group as compared with Ursolic acid group. Thus, anxiolytic activity of Ursolic acid was not found to be comparable to that of Diazepam.

It showed a statistically significant increase ($p <0.05$) in time spent in open arms and in total number of entries into open arms in Ondansetron group as compared with Ursolic acid group after 21 days. This suggests that Ondansetron has better anxiolytic action compared to that of Ursolic acid in case of chronic anxiety.

Entry into closed arms is suggestive of locomotor activity in rats. Statistically significant decrease ($p <0.05$) was seen in total number of entries into closed arms in Diazepam, Ondansetron and Ursolic acid groups as compared with Control group. However, there was no statistically significant difference seen between Diazepam, Ondansetron and Ursolic acid groups in terms of total number of entries into closed arms.

Thus, the present study revealed that both Ondansetron and Ursolic acid have significant anxiolytic effect on chronic anxiety in rats but it is not comparable to that of Diazepam. However, Ondansetron proved to be a better anxiolytic agent compared to Ursolic acid when evaluated for their effect on chronic anxiety.

Furthermore, Ondansetron is devoid of the adverse effects that are commonly associated with benzodiazepines. However, further studies are required to validate the anxiolytic effect of ondansetron in clinical settings.

Though Ursolic acid was found to be useful as an anxiolytic agent in animal studies, it has not yet been assessed for its antianxiety effect in human studies. Clinical studies are required to evaluate both the safety and efficacy of Ursolic acid as an anxiolytic agent in patients suffering from anxiety disorders.

## CONCLUSION

The present study revealed that both Ondansetron and Ursolic acid have significant anxiolytic effect on chronic anxiety in rats but it is lesser than that of Diazepam. It also showed that Ondansetron has significant anxiolytic activity as compared to Ursolic acid in chronic anxiety.

The mechanism of action and safety profile of Ondansetron has been thoroughly investigated and the same needs to be done for Ursolic acid as well. Ondansetron and Ursolic acid can prove to be an effective add-on therapy for the treatment of chronic anxiety.

**Acknowledgement**

None

## References

1. Baxter AJ, Scott KM, Vos T, Whiteford HA. Global prevalence of anxiety disorders: a systematic review and meta-regression. Psychol Med. 2013 May; 43(5):897-910.
2. O'Donnell JM, Shelton RC. Drug Therapy of Depression and Anxiety Disorders. In: Brunton LL, Chabner BA, Knollmann BC, editors. Goodman and Gilman's The Pharmacological Basis of Therapeutics. 12th ed. New York. The McGraw-Hill Companies; 2011.
3. Costall B, Naylor RJ. Anxiolytic potential of 5-HT3 receptor antagonists. PharmacolToxicol. 1992 Mar;70(3):157-62.
4. Kumar AA, Sravani P, Priya V. Anxiolytic profile of 5-HT3 antagonist ondansetron and its clinical correlation. Int J Pharm Bio Sci. 2014 Apr; 5(2):1-5.
5. Pemminati S, Swati B, Shreyasi C, Chandrasekhar R, GopalaKrishna HN, Pai MRSM. Anxiolytic activity of ethanolic extract of leaves of Ocimum sanctum in rats. Drug invent. today 2010 Feb; 2(2):115-8.
6. Awad R, Muhammad A, Durst T, Trudeau VL, Arnason JT. Bioassay-guided fractionation of lemon balm (Melissa officinalis L.) using an in vitro measure of GABA transaminase activity. Phytother Res. 2009 Aug;23(8):1075-81. doi:10.1002/ptr.2712.
7. Khan I, Karim N, Ahmad W, Abdelhalim A, Chebib M. GABA-A receptor modulation and anticonvulsant, anxiolytic, and antidepressant activities of constituents from Artemisia indicalinn. Evid. Based Complement. Alternat. Med. 2016 Apr;2016(2016):1-12. doi:10.1155/2016/1215393.
8. Kazmi I, Afzal M, Ali B, Damanhouri ZA, Ahmaol A, Anwar F. Anxiolytic potential of ursolic acid derivative-a stearoyl glucoside isolated from Lantana camara L. (verbanaceae). Asian Pac J Trop Med. 2013 Jun;6(6):433-7. doi: 10.1016/S1995-7645(13)60069-3.
9. Colla AR, Rosa JM, Cunha MP, Rodrigues AL. Anxiolytic-like effects of ursolic acid in mice. Eur J Pharmacol. 2015 Jul 5; 758:171-6.
10. Porsolt RD, Anton G, Blavet N, Jalfre M. Behavioural despair in rats: a new model sensitive to antidepressant treatments. Eur J Pharmacol. 1978 Feb 15;47(4):379-91.
11. Oghenesuvwe EE, Ekene, Nwoke E, Lotanna AD. Guidelines on dosage calculation and stock solution preparation in experimental animals' studies. Journal of Natural Sciences Research. 2014; 4(18): 100-6.
12. Pemminati S, Gopalakrishna HN, Venkatesh V, Rai A, Shetty S, Vinod A *et al*. Anxiolytic effect of acute administration of ursolic acid in rats. Res. J. Pharm., Biol. Chem. Sci. 2011 July-Sep;2(3):431-36.
13. Lourdu JA, Shanthi M, Meher AR. Anxiolytic Effect of Ondansetron, a 5-HT3 Antagonist on male albino mice in the Elevated Plus Maze. Res. J. Pharm., Biol. Chem. Sci. 2013 Apr-Jun; 4(2):1665-75.
14. Vogel HG. Drug discovery and evaluation: Pharmalogical assays. 2nd ed. Berlin: Springer-Verlag Berlin Heidelberg; 2002. Chapter E.2.4.4, Elevated plus maze test; p.434.
15. Gupta D, Radhakrishnan M, Kurhe Y. Ondansetron, a 5HT3 receptor antagonist reverses depression and anxiety-like behavior in streptozotocin-induced diabetic mice: possible implication of serotonergic system. Eur J Pharmacol. 2014 Dec 5; 744:59-66.
16. Jones BJ, Costall B, Domeney AM, Kelly ME, Naylor RJ, Oakley NR, Tyers MB. The potential anxiolytic activity of GR38032F, a 5-HT3-receptor antagonist. Br J Pharmacol. 1988 Apr;93(4):985-93.
17. Costall, B, Kelly ME, Tomkins DM, Tyers MB. Profile of action of diazepam and 5-HT, receptor antagonists on the elevated X-maze. J. Psychopharmacol. 1989c; 3, 10P.
18. Mihic SJ, Mayfield J, Harris RA. Hypnotics and Sedatives. In: Brunton LL, Hilal-Dandan R, Knollmann BC, editors. Goodman & Gilman's The Pharmacological Basis of Therapeutics. 13th ed. New York. The McGraw-Hill Companies, Inc.; 2018.
19. Awad R, Levac D, Cybulska P, Merali Z, Trudeau VL, Arnason JT. Effects of traditionally used anxiolytic botanicals on enzymes of the gamma-aminobutyric acid (GABA) system. Can J PhysiolPharmacol. 2007 Sep;85(9):933-42.
20. Machado DG, Neis VB, Balen GO, Colla A, Cunha MP, Dalmarco JB, Pizzolatti MG, Prediger RD, Rodrigues AL. Antidepressant-like effect of ursolic acid isolated from Rosmarinus officinalis L. in mice: evidence for the involvement of the dopaminergic system. PharmacolBiochemBehav. 2012 Dec;103(2):204-11.
21. Colla AR, Oliveira A, Pazini FL, Rosa JM, Manosso LM, Cunha MP, Rodrigues AL. Serotonergic and noradrenergic systems are implicated in the antidepressant-like effect of ursolic acid in mice. PharmacolBiochemBehav. 2014 Sep; 124:108-16.
22. Vogel HG. Drug discovery and evaluation: Pharmacological assays. 2nd ed. Berlin: Springer-Verlag Berlin Heidelberg; 2002. Chapter E.6.2.2, Despair swim test; p.559-61.
23. Andreatini R, Bacellar LF. The relationship between anxiety and depression in animal models: a study using the forced swimming test and elevated plus-maze. Braz J Med Biol Res. 1999 Sep;32(9):1121-6.
24. Belzung C, Griebel G. Measuring normal and pathological anxiety-like behaviour in mice: a review. *Behav Brain Res* 2001:125;141-9.
25. Bourin M. Animal models for screening anxiolytic-like drugs: a perspective. Dialogues Clin Neurosci. 2015 Sep;17(3):295-303.
26. Lister RG. The use of a plus-maze to measure anxiety in the mouse. *Psychopharmacology (Berl).* 1987:92;180-5.
27. Rodgers RJ, Cutler MG, Jackson JE. Behavioural effects in mice of subchronic buspirone, ondansetron and tianeptine. II. The elevated plus-maze. PharmacolBiochemBehav. 1997 Feb;56(2):295-303.
28. Pemminati S, Gopalakrishna HN, Venkatesh V, Rai A, Shetty S, Vinod A, Pai PG, Shenoy AK. Anxiolytic effect of chronic administration of ursolic acid in rats. J Appl Pharm Sci. 2011; 1(3): 68-71.