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# Comparison of anxiolytic activity of ondansetron and ursolic acid with diazepam in acute anxiety

**Mrunal Dhole, Seema Bhalerao, Aakash Kewlani, Rahul Bhalsinge, Pratik Rane and Samiksha Shelar**

## Abstract
**Background:** Anxiety is emotion that people experience whenever there is an impending danger, accompanied with vague sense of apprehension. Diazepam commonly used for treatment of anxiety, have adverse effects like sedation, dependence and abuse etc. Ondansetron, a 5-$HT_3$ receptor antagonist and Ursolic acid, a phytochemical possesses anxiolytic efficacy comparable to Diazepam with low adverse effects profile. This study compares effect of Ondansetron and Ursolic acid with Diazepam on acute anxiety in rats using open field (OF) test and elevated plus maze (EPM) test.
**Methodology:** Wistar albino rats of either sex (150-250g) were divided into four groups with eight rats in each group. To induce anxiety, all rats underwent forced swim test for 5 minutes, after that, study groups were given distilled water (2ml p.o.), Diazepam (1mg/kg i.p.), Ondansetron (1mg/kg i.p.) and Ursolic acid (0.2mg/kg p.o.) respectively. Effect of drugs was evaluated using OF test with parameters like number of lines crossed, central square entries, rearing, grooming, urination, defaecation and EPM test with parameters like number of entries in open arms, closed arms and time spent in open arms. Results (mean±standard deviation) were analysed using one-way ANOVA test followed by Bonferroni's correction for numerical data and Fisher's exact test followed by Likelihood-ratio test for categorical data.
**Results:** In OF test, significant improvement ($p<0.05$) was observed in parameters like lines crossed, central square entries, rearing, grooming and defaecation in all groups compared to control. Parameters like lines crossed, central square entries, rearing and grooming showed significant improvement ($p<0.05$) in Diazepam compared to Ondansetron and Ursolic acid. Significant decrease ($p<0.05$) in urination was seen in Diazepam as compared to Ursolic acid and control. In EPM test, significant improvement ($p<0.05$) was observed in parameters like time spent in open arm and number of entries in open and closed arm in all groups compared to control. Significant improvement ($p<0.05$) was observed in parameters like time spent and number of entries in open arm in Diazepam as compared to Ondansetron and Ursolic acid.
**Conclusion:** This study showed that Ondansetron and Ursolic acid have significant anxiolytic activity but it is less as compared to Diazepam.

**Keywords:** Acute anxiety, open field test, elevated plus maze test diazepam, ondansetron, ursolic acid

## Introduction
Anxiety [1] is a normal emotion under circumstances of threat and is thought to be part of the evolutionary "fight or flight" reaction of survival. It is an adaptive response that prompts a person to take necessary steps to prevent the threat or to lessen its consequences. There are many circumstances in which the presence of anxiety is maladaptive and constitutes a psychiatric disorder. Anxiety disorder is characterized by the concept of core symptoms of excessive fear and worry.
Most commonly group of drugs used to treat anxiety is Benzodiazepines. The therapeutic effects of the Benzodiazepines result from their actions on the CNS. The most prominent of these effects are sedation, hypnosis, decreased anxiety, muscle relaxation, anterograde amnesia, and anticonvulsant activity. Diazepam is a prototypical benzodiazepine. Therapeutic uses of Diazepam include anxiety disorders, alcohol withdrawal, status epilepticus, skeletal muscle relaxation and as a preanesthetic medication. Common side effects of Benzodiazepines are weakness, headache, blurred vision, vertigo, nausea and vomiting, epigastric distress, and diarrhoea; joint pains, chest pains, and incontinence are much rarer. Bizarre uninhibited behaviour may occur in some users, hostility and rage in others. Withdrawal of Benzodiazepines after long-term treatment may cause aggravation of anxiety and convulsions [2].

Ondansetron, a carbazole derivative, is a first-generation drug and prototype in the class of 5-HT3 receptor antagonists. Though, it is commonly used as antiemetic drugs but it is also known to possess antianxiety activity with no sedating activity and also lack withdrawal symptoms as that of Diazepam.[3]Ursolic acid is a secondary plant metabolite, isolated from the stem bark or leaves or fruit peel of various medicinal plants like *Rosmarinus officinalis*, *Origanum majorana*, *Origanum vulgare*, *Salvia officinalis*, *Thymus vulgaris*, *Ocimum Sanctum*, *Lavandula angustifolia* and *Coffea arabica*. [4]. Oral administration of Ursolic acid have shown anxiolytic activity in mice.

In the view of finding a drug which would have a anxiolytic activity comparable to that of Diazepam with no serious adverse effects like it, this study was conducted in which Ondansetron and Ursolic acid were evaluated for their anxiolytic activity in acute anxiety and were compared with Diazepam in rats using OF and EPM test.

## 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 [5, 6]

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 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 [6, 7, 8] 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 [6, 8, 9]. 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.[6,8,10] Distilled water was given orally as control in equivalent volume.

Each study drug was given one hour before conducting OF & 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.

After dividing rats into four groups, drugs were assessed for their anxiolytic activity against acute anxiety by using OF and EPM test.

### Open field (OF) test [11]

The open field apparatus is a box made of thick Perspex material and consists of a multiple unit enclosure. Each enclosure is surrounded by walls of 38 cm and the length and breadth of the base is 48 cm x 48 cm. Open field is divided into symmetric squares of 4x4 with 4 central squares and 12 peripheral squares.

Rats were placed individually into one of the four corners of the open field and allowed to explore the enclosure 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.

- Number of lines crossed with all four paws
- Number of entries in the central square with all four paws
- Number of rearings, i.e., the frequency with which the rat will stand on their hind legs.
- Number of grooming i.e., the frequency with which the rat spent licking or scratching itself while stationary
- Urination i.e. presence of puddles or streaks of urine
- Defaecation i.e. presence of fecal pellets

### Elevated plus maze (EPM) test [12]

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. For analysis of data, one-way ANOVA test followed by Bonferroni's correction was applied for numerical data and Fisher's exact test followed by Likelihood-ratio test was applied for categorical data.

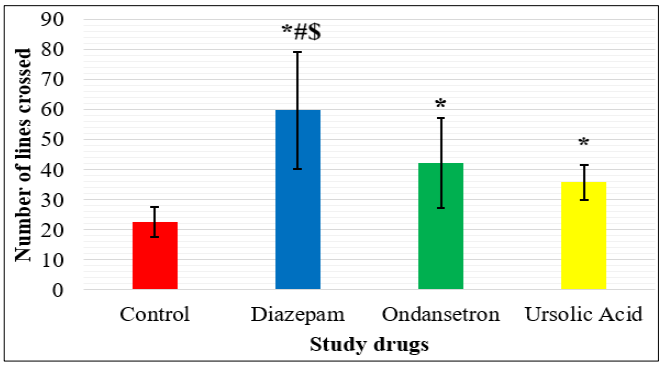
## Results

### A. Open field (OF) test

**Table 1**: Number of Lines crossed

| Group | Mean | SD | p |
|---|---|---|---|
| Control | 22.62 | 4.926 | 0.001 |
| Diazepam | 59.75 | 19.54 | |
| Ondansetron | 42.12 | 15.01 | |
| Ursolic Acid | 35.75 | 5.849 | |

Table No.1 shows that Mean of number of lines crossed was highest in the Diazepam group and lowest in the Control group and the difference seen between the groups was significant




* $p$ <0.05 when compared with control
# $p$ <0.05 when compared with Ondansetron
$ $p$<0.05 when compared with Ursolic acid

**Graph 1**: Effect of different treatment on number of lines crossed in OF test in rats

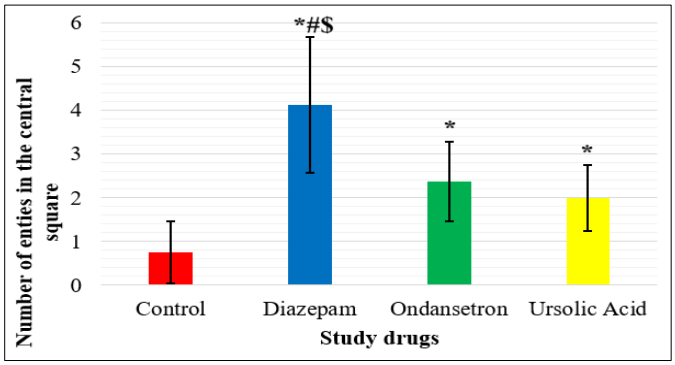
**Graph No. 1 shows**

- The increase in number of lines crossed in the Diazepam, Ondansetron and Ursolic acid groups was significant compared with the Control group.
- The increase in number of lines crossed in the Diazepam group was significant compared with both Ondansetron and Ursolic acid groups.
- No statistically significant difference was seen between the Ondansetron group and the Ursolic acid in terms of number of lines crossed.

**Table 2:** Number of Central square entries

| Group | Mean | SD | P |
|---|---|---|---|
| Control | 0.75 | 0.7071 | 0.001 |
| Diazepam | 4.125 | 1.553 | |
| Ondansetron | 2.375 | 0.9161 | |
| Ursolic Acid | 2 | 0.7559 | |

Table No. 2 shows mean of number of entries in the central square was highest in the Diazepam group and lowest in the Control group and the difference seen between the groups was significant.




* $p$ <0.05 when compared with control
# $p$ <0.05 when compared with Ondansetron
$ $p$<0.05 when compared with Ursolic acid

**Graph 2:** Effect of different treatment on number of central square entries in OF test in rats.

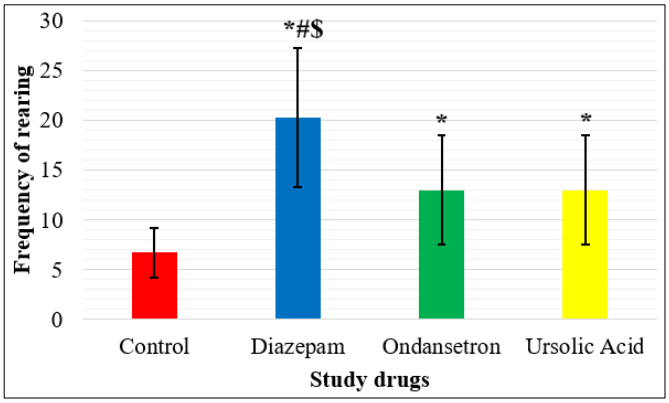
Graph No. 2 shows:

- In comparison with the Control group, the increase in number of entries in the central square was significant in Diazepam, Ondansetron and Ursolic acid groups.
- In the Diazepam group, the increase in number of entries in the central square was significant compared with the Ursolic acid and Ondansetron groups.
- No statistically significant difference was seen between the Ondansetron group and the Ursolic acid in terms of number of entries in the central square.

**Table 3:** Frequency of rearing

| Group | Mean | SD | P |
|---|---|---|---|
| Control | 6.75 | 2.493 | 0.001 |
| Diazepam | 20.25 | 6.985 | |
| Ondansetron | 13 | 5.477 | |
| Ursolic Acid | 13 | 5.477 | |

Table No. 3 shows mean of frequency of rearing was highest in the Diazepam group and lowest in the Control group and the difference seen between the groups was significant.




* $p$ <0.05 when compared with control
# $p$ <0.05 when compared with Ondansetron
$ $p$<0.05 when compared with Ursolic acid

**Graph 3:** Effect of different treatment on frequency of rearing in OF test in rats

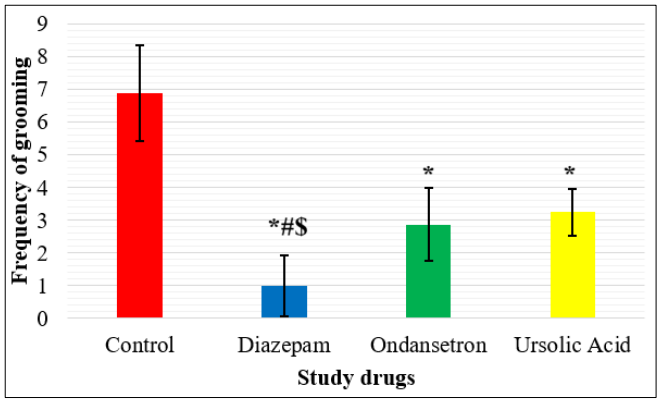
Graph No. 3 Shows:

- The increase in frequency of rearing was significant in Diazepam, Ondansetron and Ursolic acid groups when compared with the Control group.
- The increase in frequency of rearing was significant in the Diazepam group compared with both Ondansetron and Ursolic acid groups.
- No statistically significant difference was seen between the Ondansetron group and the Ursolic acid in terms of frequency of rearing.

**Table 4:** Frequency of Grooming

| Group | Mean | SD | P |
|---|---|---|---|
| Control | 6.875 | 1.458 | 0.001 |
| Diazepam | 1 | 0.9258 | |
| Ondansetron | 2.875 | 1.126 | |
| Ursolic Acid | 3.25 | 0.7071 | |

Table No. 4 shows mean of frequency of grooming was highest in the Control group and lowest in the Diazepam group and the difference seen between the groups was significant.




* p <0.05 when compared with control
# *p* <0.05 when compared with Ondansetron
$ *p*<0.05 when compared with Ursolic acid

**Graph 4:** Effect of different treatment on frequency of grooming in OF test in rats

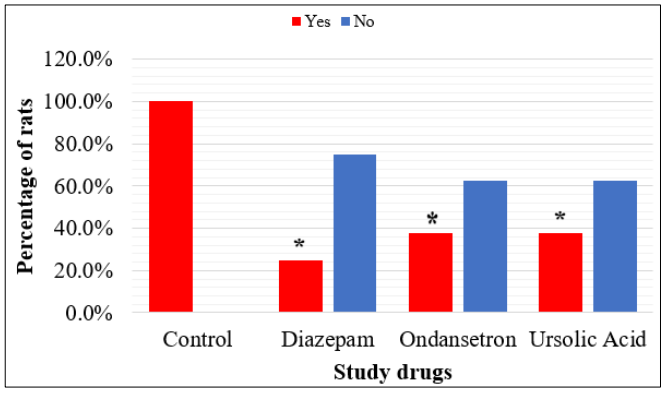
Graph No. 4 shows:

- The decrease in frequency of grooming was significant in Diazepam, Ondansetron and Ursolic acid groups compared with the Control group.
- The decrease in frequency of grooming was significant in the Diazepam group compared with the Ondansetron and Ursolic acid groups.
- No statistically significant difference was seen between the Ondansetron group and the Ursolic acid in terms of frequency of grooming.

**Table 5:** Percentage of rat Defaecated in the field

| | | Yes | No | Total | P |
|---|---|---|---|---|---|
| Group | **Control** | 8 (100%) | 0 (0%) | 8 (100%) | 0.01 |
| | Diazepam | 2 (25%) | 6 (75%) | 8 (100%) | |
| | Ondansetron | 3 (37.5%) | 5 (62.5%) | 8 (100%) | |
| | Ursolic Acid | 3 (37.5%) | 5 (62.5%) | 8 (100%) | |
| | Total | 16 (50%) | 16 (50%) | 32 (100%) | |

Table No. 5 shows percentage of rats who didn't defaecate was highest in the Diazepam group and the difference seen between the groups was significant.




* *p* <0.05 when compared with control

**Graph 5:** Effect of different treatment on defaecation in OF test in rats

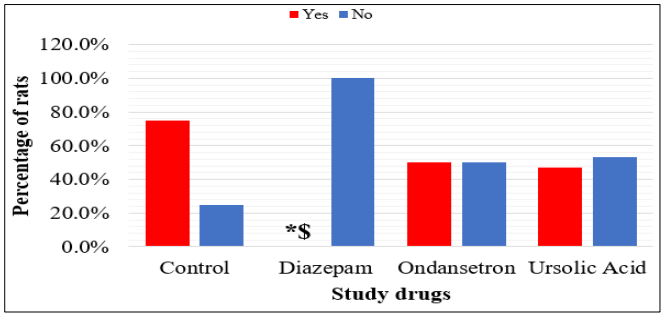
Graph No. 5 shows:

- The percentage of rats who didn't defaecate was significant in Diazepam, Ondansetron and Ursolic acid groups compared with the Control group.
- No statistically significant difference was seen between Diazepam, Ondansetron and Ursolic acid groups in terms of defaecation.

**Table 6:** Percentage of rat urinated in the field

| | | Yes | No | Total | P |
|---|---|---|---|---|---|
| Group | **Control** | 6 (75%) | 2 (25%) | 8 (100%) | 0.015 |
| | Diazepam | 0 (0%) | 8 (100%) | 8 (100%) | |
| | Ondansetron | 4 (50%) | 4 (50%) | 8 (100%) | |
| | Ursolic Acid | 5 (62.5%) | 3 (37.5%) | 8 (100%) | |
| | Total | 15 (46.9%) | 17 (53.1%) | 32 (100%) | |

Table No. 6 shows percentage of rats who didn't urinate was highest in the Diazepam groupand the difference seen between the groups was significant.




* *p* <0.05 when compared with control
$ *p*<0.05 when compared with Ursolic acid

**Graph 6:** Effect of different treatment on urination in OF test in rats

Graph no 6 shows:

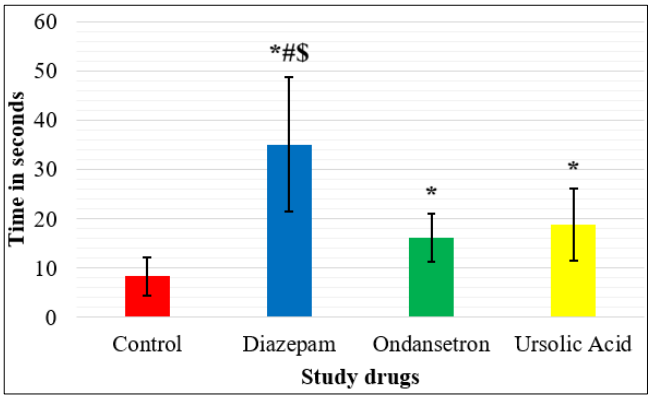
- The percentage of rats who didn't urinate was significant in the Diazepam group compared with the Control group. However, no statistically significant difference was seen in terms of urination when Ondansetron and Ursolic acid groups were compared with the Control group.
- Percentage of rats who didn't urinate was significant in the Diazepam group compared with the Ursolic acid group. However, no statistically significant difference was seen between the Diazepam group and the Ondansetron group in terms of urination.
- No statistically significant difference was seen between the Ondansetron group and the Ursolic acid group in terms of urination.

## B. Elevated plus maze (EPM) test

**Table 7:** Time Spent in Open Arms of EPM

| Group | Mean | SD | P |
|---|---|---|---|
| Control | 8.375 | 3.889 | 0.001 |
| Diazepam | 35.12 | 13.61 | |
| Ondansetron | 16.25 | 4.892 | |
| Ursolic Acid | 18.88 | 7.279 | |

Table No. 7 shows mean of time spent in open arms was highest in the Diazepam group and lowest in the Control group and the difference seen between the groups was significant.




* $p$ <0.05 when compared with control
# $p$ <0.05 when compared with Ondansetron
$ $p$<0.05 when compared with Ursolic acid

**Graph 7:** Effect of different treatment on time spent in open arm of EPM in rats

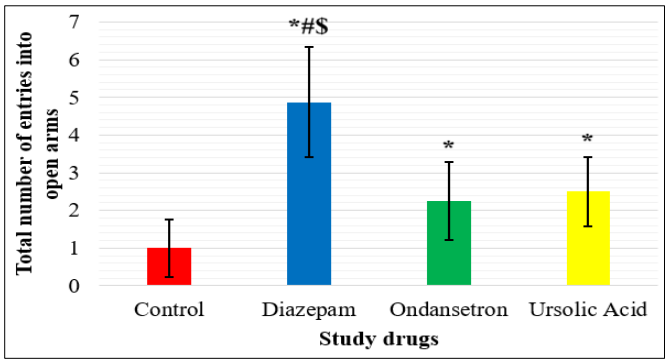
Graph No. 7 shows:

- The increase in time spent in open arms was significant in Diazepam, Ondansetron and Ursolic acid groups when compared with the Control group.
- The increase in time spent in open arms was significant in the Diazepam group compared with both Ondansetron and Ursolic acid groups.
- No statistically significant difference was seen between the Ondansetron group and the Ursolic acid in terms of time spent in open arms.

**Table 8:** Open Arm Entries in EPM

| Group | Mean | SD | P |
|---|---|---|---|
| Control | 1 | 0.7559 | 0.001 |
| Diazepam | 4.875 | 1.458 | |
| Ondansetron | 2.25 | 1.035 | |
| Ursolic Acid | 2.5 | 0.9258 | |

Table No. 8 shows mean of total number of entries into open arms was highest in the Diazepam group and lowest in the Control group and the difference seen between the groups was significant.




* $p$ <0.05 when compared with control
# $p$ <0.05 when compared with Ondansetron
$ $p$<0.05 when compared with Ursolic acid

**Graph 8:** Effect of different treatment on number of entries in open arm of EPM in rats

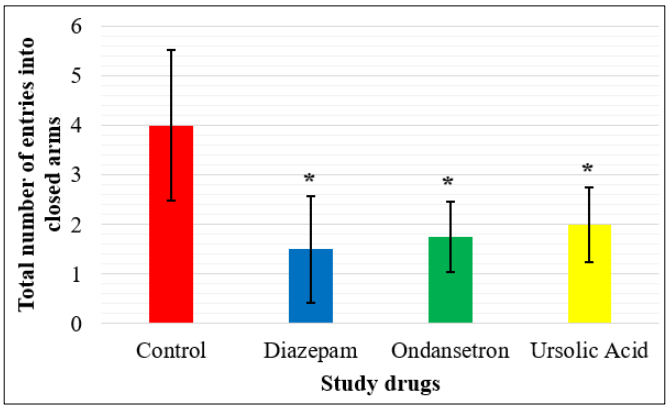
Graph No. 8 shows:

- The increase in total number of entries into open arms was significant in the Diazepam, Ondansetron and Ursolic acid groups compared with the Control group.
- The increase in total number of entries into open arms was significant in the Diazepam group compared with both Ondansetron and Ursolic acid groups.
- No statistically significant difference was seen between the Ondansetron group and the Ursolic acid in terms of total number of entries into open arms.

**Table 9:** Closed Arm Entries in EPM

| Group | Mean | SD | p |
|---|---|---|---|
| Control | 4 | 1.512 | 0.001 |
| Diazepam | 1.5 | 1.069 | |
| Ondansetron | 1.75 | 0.7071 | |
| Ursolic Acid | 2 | 0.7559 | |

Table No.9 shows mean of total number of entries into closed arms was highest in the Control group and lowest in the Diazepam group and the difference seen between the groups was significant.




* $p$ <0.05 when compared with control
# $p$ <0.05 when compared with Ondansetron
$ $p$<0.05 when compared with Ursolic acid

**Graph 9:** Effect of different treatment on number of entries in closed arm of EPM in rats

Graph No. 9 shows:

- The decrease in total number of entries into closed arms was significant in Diazepam, Ondansetron and Ursolic acid groups compared with the Control group.
- No statistically significant difference was seen between Diazepam, Ondansetron and Ursolic acid groups in terms of total number of entries into closed arms.

**Discussion**

Anxiety is a normal emotion that a person experiences whenever there is an impending danger and enables him to take measures to deal with it [13]. Almost 30% of the population may develop an anxiety disorder, due in large part to stressful environments, including exposure to fearful events during normal activities in twenty-first-century society [1].

GABA (γ-aminobutyric acid) is the principal inhibitory neurotransmitter in the brain and normally plays an important regulatory role in reducing the activity of many neurons, including those in the amygdala and those in cortico-striato-thalamo-cortical (CSTC) loops having an inhibitory effect on anxiety. Benzodiazepines, perhaps the best-known and most widely used anxiolytics, act by enhancing GABA actions at the level of the amygdala and at the level of the prefrontal cortex within CSTC loops to relieve anxiety [1].

$GABA_A$ receptors are targets of Benzodiazepines. $GABA_A$

receptor is thought to be postsynaptic in location and to mediate a type of inhibition at the postsynaptic neuron that is phasic, occurring in bursts of inhibition that are triggered by peak concentrations of synaptically released GABA. Theoretically, Benzodiazepines should exert an anxiolytic effect due to enhancement of phasic post synaptic inhibition.[1]

Benzodiazepines increase the affinity of the $GABA_A$ receptor for GABA and thereby enhance GABA-induced Cl– currents. Thus, in terms of channel kinetics, they increase the frequency of opening of the $GABA_A$ receptor $Cl^-$ channel in the presence of GABA. In pharmacodynamic terms, agonists at the benzodiazepine binding site shift the GABA concentration-response curve to the left [2].

Potentiation of GAB Aergic pathway which regulate the firing of monoamine neuron that promotes behavioural arousal might be responsible for anxiolytic behaviour and sedative effect of Benzodiazepines. It is found to be responsible for inhibitory effect of fear and punishment on behaviour [2].

Diazepam being prototype drug in Benzodiazepines, is commonly used in treatment of anxiety but is also associated with serious adverse effects like sedation, muscle cramps, vomiting, sweating, drug dependence and withdrawal syndromes which may cause convulsions [2]. In the search to find drugs having anxiolytic potential comparable to Diazepam with no such adverse effects profile, it was found out that Ondansetron and Ursolic acid have such potential of anxiolytic activity.

A selective 5HT [3] receptor antagonist, Ondansetron used as an antiemetic in the treatment of severe vomiting produces significant anxiolysis even at antiemetic dose itself in rats without any sedative effects. Further, there are no problems on withdrawal of Ondansetron [6, 7].

Exploratory behaviour was found to be increased in aversive white compartment in black:white test in Ondansetron treated mice. In social interaction test in rats, Ondansetron was found to be equally efficacious but highly potent then that of Diazepam as an anxiolytic drug. The efficacy and potency of Ondansetron was confirmed in elevated X-maze test. Potential anxiolytic activity of Ondansetron was consistently proven in various pre-clinical studies [6].

Ursolic acid, a biologically active plant metabolite found in various medicinal plants have shown significant anxiolytic activity in mice after oral administration [14]. Pemminati *et al* (2010) showed Ursolic acid obtained from ethanolic extract of Oscimum sanctum leaves have a potent anti-anxiety effect [15].

Anxiolytic activity of Ursolic acid involve effects on GABAergic system. One of the mechanisms other than $GABA_A$ receptor binding to increase GABA levels in the brain is to inhibit the enzyme GABA transaminase (GABA-T) so that anxiety is controlled. Aqueous extract of *Melissa officinalis* is the best inhibitor of *in vitro* GABA-T activity from rat brain. The triterpenoid Ursolic acid isolated and identified from the methanolic extract of M. officinalis via bioassay-guided fractionation inhibited GABA-T by 13.6% at 10 µg/mL and by 20% at 100 µg/mL in *in vitro* enzyme assay (2007) [6, 16].

Ursolic acid has shown to produce anti-depressant effect in mice by serotonergic mediated mechanism [6, 17, 18]. Thus, it may offer a novel therapeutic strategy for the treatment of co-morbid condition i.e. depression and anxiety [6].

Forced swimming test that was used to induce anxiety in rats in this study or is a well-established model to test for antidepressant activity in rodents [19].

The OF test, originally designed by Hall in 1934, consists of placing the rat in an unknown environment with surrounding walls. It is a reliable method to test the behaviour and locomotor activity of rats. OF test is a rodent model of anxiety, sensitive to the anxiolytic-like effects of BZD and 5-$HT_{1A}$ receptor agonists [11]. Anxiety behaviour in the open field is triggered by two factors: individual testing and agoraphobia.

EPM is the most commonly and widely used test for the screening of anti-anxiety drugs and psychological and neurochemical mechanism of anxiety [12]. This test is based on the conflict of animal behaviour between the aversion and exploration to elevated open spaces. It is also used to study the provoked behavioural elements such as fear of height, neophobia, exploration, agoraphobia as well as avoidance/approach conflict. Therefore, this model is also known as 'unconditioned spontaneous behavioural conflict model. Generally, when rats are taken from their home cages their behavioural pattern changes i.e. rats will avoid open arm spaces and consistently prefers for the closed arm spaces indicates there liking for secured closed space of the maze. This tendency is suppressed by anxiolytics and potentiated by anxiogenic agents [6].

EPM measures two independent behavioral parameters, anxiety and motor activity. This test showed that percentage of open-arm entries and time spent in open arms are extremely good measures of anxiety and number of closed-arm entries is a better measure of motor activity [20]. The major advantage of this test over other paradigms that are used for the assessment of anxiety is that it involves food or water deprivation or shock administration.

In OF test, the increase in number of lines crossed, number of entries in the central square, frequency of rearing and decrease in frequency of grooming and defaecation in the Diazepam, Ondansetron and Ursolic acid groups was statistically significant compared with the Control group. The percentage of rats who didn't urinate was highly significant in the Diazepam group compared with the Control group. These observations indicate that both Ondansetron and Ursolic acid possess significant anxiolytic activity.

The increase in number of lines crossed, number of entries in the central square, frequency of rearing and decrease in frequency of grooming in the Diazepam group was highly significant compared with both Ondansetron and Ursolic acid groups. This shows that the anxiolytic activity of Ondansetron and Ursolic acid is not comparable to that of Diazepam.

Percentage of rats who didn't urinate was significant in the Diazepam group compared with the Ursolic acid group. However, no statistically significant difference was seen between the Diazepam group and the Ondansetron group in terms of urination.

The results of a study by Colla *et al*. (2015) study showed that the oral administration of Ursolic acid (10 mg/kg), similar to Diazepam (2 mg/kg), was able to produce an increase in the total time spent in the centre of open field and a reduction in the number of rearings in the open field test [14].

In this study, no statistically significant difference was seen between the Ondansetron group and the Ursolic acid in terms of number of lines crossed, number of entries in the central square, frequency of rearing, frequency of grooming, defaecation and urination. This suggests that Ondansetron and Ursolic acid are equivalent in terms of alleviating acute anxiety.

In EPM test, there was a statistically significant increase in time spent in open arms and total number of entries into open

arms in Diazepam, Ondansetron and Ursolic acid groups compared with the Control group. The decrease in total number of entries into closed arms was highly significant in Diazepam, Ondansetron and Ursolic acid groups compared with the Control group. The improvement seen in these parameters indicate that both Ondansetron and Ursolic acid possess significant anxiolytic activity.

The increase in time spent in open arms and total number of entries into open arms was highly significant in the Diazepam group compared with both Ondansetron and Ursolic acid groups. This demonstrates that the anxiolytic activity of Ondansetron and Ursolic acid is not comparable to that of Diazepam.

Kumar *et al*. (2014), showed an increase in entries and duration of stay in open arms in Ondansetron treated rats compared to control. Entries and duration of stay in open arms was higher in Diazepam treated group as compared to Ondansetron [7].

In this study, no statistically significant difference was seen between the Ondansetron group and the Ursolic acid group in terms of time spent in open arms, total number of entries into open arms and total number of entries into closed arms. This suggests that Ondansetron and Ursolic acid have equivalent anxiolytic activity in acute anxiety.

## Conclusion

This study concludes that both Ondansetron and Ursolic acid possess potent anxiolytic activity against acute anxiety but it is less as compared to Diazepam.

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## References

1. Stahl SM. Stahl's Essential Psychopharmacology: Neuroscientific Basis and Practical Application. 4th ed, Cambridge University Press, New York, 2013, 388-419.
2. Brunton LL, Hilal-Dandan R, Knollmann BC. Goodman & Gilman's The Pharmacological Basis of Therapeutics. 13th ed, The McGraw-Hill Companies, New York, 2018, 339-354.
3. Ondansetron. Stat Pearls [Weblink: http://www.ncbi.nlm.nih.gov/books/NBK499839/] [Visited on 28 April, 2018]
4. Woźniak Ł, Skąpska S, Marszałek K. Ursolic Acid-A Pentacyclic Triterpenoi with a Wide Spectrum of Pharmacological Activities. Molecules. 2015; 20(11):20614-41.
5. Porsolt RD, Anton G, Blavet N, Jalfre M. Behavioural despair in rats: a new model sensitive to antidepressant treatments. Eur J Pharmacol. 1978; 47(4):379-91.
6. Dhole MP, Bhalerao S, Kewlani AS, Jha P, Rane P, Shelar S. Comparison of Anxiolytic activity of Ondansetron and Ursolic acid in chronic anxiey using elevated plus maze test. International Journal of Current Medical and Pharmaceutical Research. 2019; 5(1A):4009-13.
7. Kumar AA, Sravani P, Priya V. Anxiolytic profile of 5-HT3 antagonist ondansetron and its clinical correlation. Int J Pharm Bio Sci. 2014; 5(2):1-5.
8. 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.
9. 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; 2(3):431-36.
10. 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; 4(2):1665-75.
11. Prut L, Belzung C. The open field as a paradigm to measure the effects of drugs on anxiety-like behaviors: a review. Eur J Pharmacol. 2003; 463(1-3):3-33.
12. Vogel HG. Drug discovery and evaluation: Pharmalogical assays. 2nd ed, Berlin: Springer-Verlag Berlin Heidelberg, 2002, 434-561.
13. Sadock BJ, Sadock VA, Ruiz P. Kaplan and Sadock's Synopsis of Psychiatry: Behavioral Sciences/Clinical Psychiatry. 11th ed, Wolters Kluwer (India) Pvt. Ltd., New Delhi, 2015, 387-413.
14. Colla AR, Rosa JM, Cunha MP, Rodrigues AL. Anxiolytic-like effects of ursolic acid in mice. Eur J Pharmacol. 2015; 758:171-6.
15. 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; 2(2):115-8.
16. 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 Physiol Pharmacol. 2007; 85(9):933-42.
17. Machado DG, Neis VB, Balen GO, Colla A, Cunha MP, Dalmarco JB *et al*. Antidepressant-like effect of ursolic acid isolated from *Rosmarinus officinalis* L. in mice: evidence for the involvement of the dopaminergic system. Pharmacol Biochem Behav. 2012; 103(2):204-11.
18. Colla AR, Oliveira A, Pazini FL, Rosa JM, Manosso LM, Cunha MP *et al*. Serotonergic and noradrenergic systems are implicated in the antidepressant-like effect of ursolic acid in mice. Pharmacol Biochem Behav. 2014; 124:108-16.
19. 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; 32(9):1121-6.
20. Lister RG. The use of a plus-maze to measure anxiety in the mouse. Psychopharmacology (Berl). 1987; 92:180-5.