

## A comparative analysis of anxiolytic activity of *Arnica montana* and alprazolam in rats using open field test

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### ABSTRACT

**Background:** Anxiety affects around 7.3% of the total population worldwide. Benzodiazepines are preferred anxiolytic agents and are still frequently used in spite of the side effect profile including muscle relaxation, memory disturbances, sedation, physical dependence. *Arnica montana*, a traditional herb is known to possess significant anxiolytic effect at the dose of 100mg/kg. In this study, *Arnica montana* has been compared for the first time with alprazolam, a most commonly used anxiolytic drug.

**Methods:** Forced swim test was used to induce anxiety. Anxiolytic action of study drugs which were given orally, was evaluated using Open field test (OFT) in healthy wistar rats of either sex. Behavior of rats, locomotion and number of squares crossed was recorded. Rats were divided into four groups with eight rats in each group. Study groups were Group I Control; Group II Alprazolam 0.08mg/kg; Group III *Arnica montana* extract (AME) 100mg/kg; Group IV AME + Alprazolam group 100mg/kg+0.08mg/kg. Statistical analysis was done using ANOVA followed by Tukey's test ( $p < 0.05$ ).

**Results:** Increase in frequency of rearing was significant ( $p < 0.05$ ) in AME group and highly significant ( $p < 0.001$ ) in Alprazolam and combination group in comparison to control. Decrease in frequency of grooming was highly significant ( $p < 0.001$ ) in Alprazolam and combination group. AME also showed significant ( $p < 0.05$ ) decrease in grooming activity.

**Conclusions:** *Arnica montana* extract showed anxiolytic activity and can be used as an add on drug after further studies and validation in the treatment of anxiety disorders.

**Keywords:** Anxiety, Alprazolam, *Arnica montana*, Grooming, Open field test, Rearing

### INTRODUCTION

Anxiety is defined as a subjective sense of unease, dread or foreboding. It is an emotional state which is seen in response to a significant stress or perceived threat. Anxiety, apprehension, fear and worry are all completely natural human feelings. If these feelings occur in response to seemingly natural stimuli and endure for an extended period, it affects both physical and mental health and leads to clinical anxiety disorders and can indicate a primary psychiatric condition or can be a component of or reaction to a primary medical disease.

Anxiety affects around 7.3% of the total population worldwide and has become a very important area of research interest in psychopharmacology.<sup>1</sup>

Benzodiazepines are preferred anxiolytic agents and are still frequently used in spite of the side effect profile including muscle relaxation, memory disturbances, sedation, physical dependence.<sup>2</sup>  $\beta$ -blockers are used for symptomatic treatment of anxiety like tremors, palpitations, etc. Antidepressants are showing better long-term results for chronic anxiety.

Newer drugs like Azapirones (e.g. Buspirone) are preferred owing to less side effects.

Nevertheless, there is considerable interest in the development of new anxiolytics.<sup>3</sup> New synthesized compounds as well as drugs derived from traditional herbs may have a possible therapeutic relevance in the treatment of anxiety.<sup>4</sup>

In conclusion, strategic approaches aimed at accelerating promising research directions and enhancing quality standards of ongoing investigations into putative psychotropic agents from natural sources are recommended.<sup>5</sup> Thus it is desirable to explore anxiolytic agents derived from herbs.

Anxiety or anxiety neurosis is known since old ages and treatment for it is mentioned in the ancient scriptures. There are herbs which have multiple actions and are used in treatment of various disorders. A peculiarity of herbal treatment especially of ayurvedic preparations is that multiple herbs are used to treat a disorder as they are supposed to act synergistically or decrease side effects of other components. Also, a single herb is used for multiple disease conditions. Herbs with potential anxiolytic activity include:<sup>6</sup>

- Ashwagandha (*withania somnifera*)
- Jatamansi (*Nardostachys jatamansi*)
- Brahmi (*Bacopa monnieri*)
- Sarpagandha (*Rauvolfia serpentina*)
- Taalisha (*Abies pindrow*)
- Windflower (*Anemone pulsatilla*)
- Hiranpadi (*Convolvulus arvensis*).

*Arnica montana* (referred to as leopard's bane, also called as wolf's bane, mountain tobacco and mountain arnica), is a European flowering plant in the sunflower family noted for its large yellow flower head. It has been used in herbal medicines for centuries. It has antiseptic, anti-inflammatory, anti-bacterial, decongestive and antifungal property.<sup>7</sup>

*Arnica montana* at the dose of 100mg/kg is known to possess significant anxiolytic effect.<sup>4</sup> In this study alprazolam is used as standard drug and is having less side effects and *Arnica montana* not yet compared with alprazolam. Hence the present study is undertaken to compare anxiolytic effects of *Arnica montana* with standard antianxiety drug alprazolam in rats.

## METHODS

### Study animals

The animals selected for the study were experimentally naive. The rats with following characteristics were selected:

- Species: *Rattus norvegicus*

- Strain: Wistar albino
- Gender: Both
- Weight range: 150-250g.

The rats for this study were procured from the animal house located in the medical college and hospital.

### Animal feed

Food: Animals were fed with commercially available 'Nutrimix Std-1020' manufactured by Baramati Agro Ltd, acquired from Nutivet Life Sciences, Pune. The nutrition provided by the pellet feed was as follows:

- Energy: 3620 kcal/kg
- Crude protein: 22.15%
- Crude fiber: 62.48%
- Ash: 5.11%
- Sand silica: 1.15%.

Pellets were kept in the space provided for feed in the roof of the cage.

### Water

Drinking tap water supplied by Municipal Corporation was provided to the rats through the feeding bottles with stainless steel nozzle, one per rat cage. Food and water were replenished once daily in the morning.

### Animal housing

Rats were housed in groups of four in standard big polypropylene cages measuring 40 x 27.5 x 13.5 cm which had a wire mesh top with provision for drinking water and space for pellets. Rice paddy husk was used as bedding material in each cage. The rats were housed under standard condition of temperature (25±5°C) and relative humidity (55±10%) and 12/12hour light/dark cycle. Apart from daily replenishment of food and water, rats were left undisturbed.

### Plant material

*Arnica montana* extract (AME) was used in this study as test drug.

### Standard drug

Alprazolam (Intas Pharmaceuticals Ltd., India) was used as standard drug.

### Control

Distilled water was used as control in equivalent volume.

### Study design

Animals were divided into four groups (n=8) as follows:

- Group I: Control (Distilled water)
- Group II: Alprazolam treated group (0.08mg/kg)
- Group III: *Arnica montana* extract (AME)treated group (100mg/kg)
- Group IV: AME + Alprazolam treated group (100mg/kg + 0.08 mg/kg)

The forced swim test was just used as a stressor test to induce anxiety where rats are forced to swim in specially constructed tanks for a particular period (few min to 45 min). Rats were forced to swimming stress for duration of 5-10 minutes in plastic tanks (length 100cm, width 40cm, depth 60cm) containing tap water. Depth of water in the tank was 30cm. One rat was allowed to swim during stress session at one time.

After inducing anxiety, Open field test model was the main model used to assess the anxiolytic effects and behavioural changes were observed.

**Open field test**

This test originally designed by Hall in 1934 on rats consists in placing an animal in an unknown environment with surrounding walls so as to observe a number of behaviour patterns including the tendency to stay on the periphery of the field without entering the centre (called thigmotaxis and often interpreted as anxious behaviour), levels of defecation and urination. It is a reliable method to test the behaviour and locomotor activity of rats.<sup>8</sup> Open field floor is often divided into squares. Animals were tested individually, always being placed in the same position. Anxiety behavior in the open field is triggered by two factors: individual testing and agoraphobia. Higher levels of anxiety should mainly lead to decreases in the number of squares visited in centre. Apparatus consists of a multiple unit enclosure. Each enclosure is surrounded by walls of 15 inches and the length and breadth of the base is 19x19 inches. Open field is divided into symmetric squares of 4x4 with 4 central squares and 12 peripheral squares. High technology and expensive software have been designed to assess the activity of rodents. Rat behaviour and activity were recorded with the help of a video camera for accuracy, reassessment and reproducibility.

**Procedure**

- Rats were placed individually in a single unit of the open field enclosure and the movement of rats is observed and recorded.
- Total number of central squares and number of peripheral squares crossed was recorded.
- Various behavioural responses such as rearing and grooming was recorded.

Also, urination and defecation if done by rats during the test was also recorded. Open-field test may be a rodent model of normal anxiety, sensitive to the anxiolytic-like effects of BZD and 5-HT<sub>1A</sub> receptor agonists.<sup>9</sup> For analysis

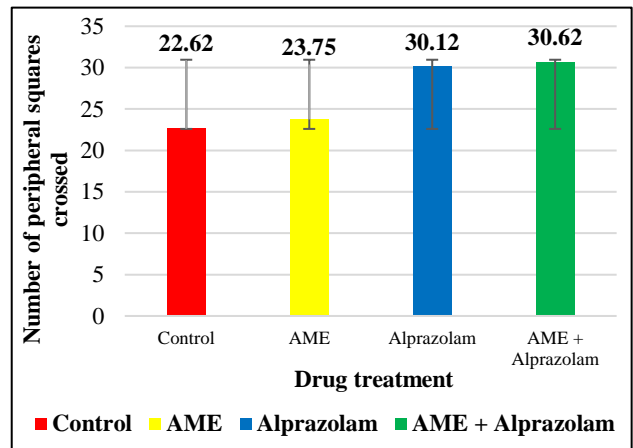
the data was compiled and analyzed using Primer of biostatistics version 5.0 and results were expressed as Mean±S.D. Statistical significance was analyzed using One-way analysis of variance (ANOVA) followed by Tukey’s multiple comparison test and P value <0.05 was considered to be statistically significant.

**RESULTS**

Results were obtained by observing different parameters of open field test which are as follows:

**Peripheral ambulation**

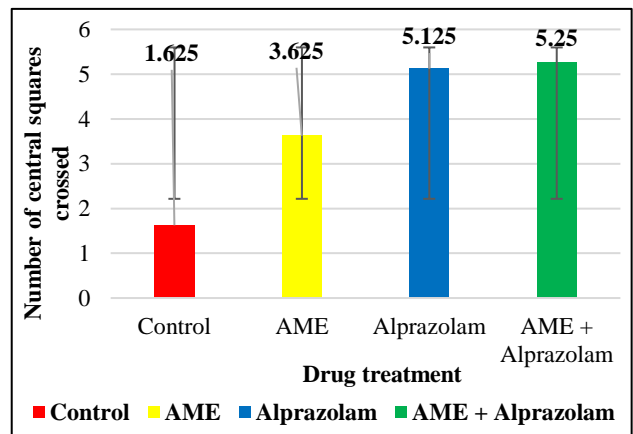
Alprazolam and combination group showed increase in total number of peripheral square crossed in comparison to control and AME and the increase is statistically significant.



**Figure 1: Peripheral ambulation.**

**Central ambulation**

Alprazolam and combination group showed increase in total number of central square crossed and increase is statistically significant and AME did not show any significant result even in comparison to control.



**Figure 2: Central ambulation.**

### Rearing

All three groups showed increase in frequency of rearing and the increase was significant in AME group and the result was highly significant in Alprazolam and combination group in comparison to control.

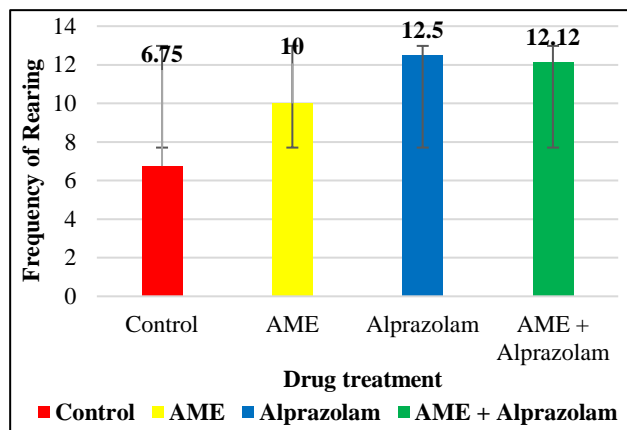


Figure 3: Rearing.

### Grooming

Alprazolam and combination group showed decrease in frequency of grooming and the decrease was highly significant. AME also showed significant decrease in grooming activity.

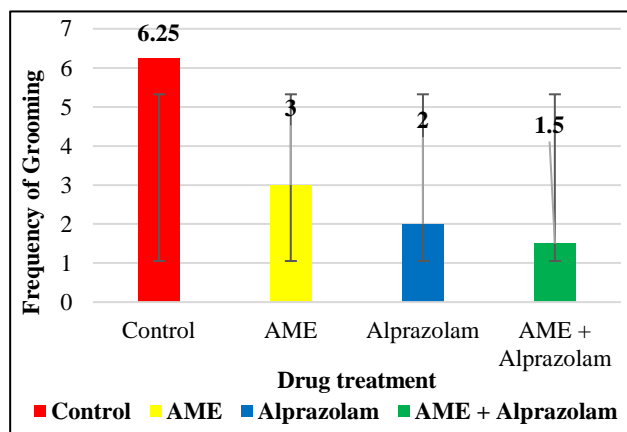


Figure 4: Grooming.

### Defecation

Fisher's exact test was applied to analyze the data and it showed no statistically significant difference for defecation.

## DISCUSSION

Anxiety is associated with decrease in explorative behaviour and an increased preference for closed spaces,

also it is associated with decrease in locomotor activity. Anxiety is also associated with augmented autonomic activity resulting in increased defecation and urination.

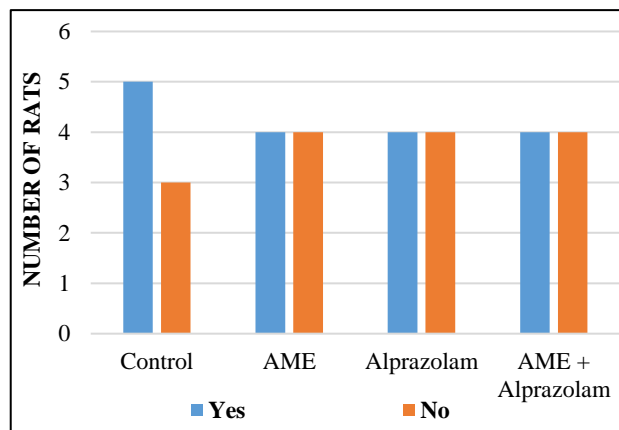


Figure 5: Number of rats that defecated.

Open field test by Walsh and Cummins, provides simultaneous measures of locomotion, exploration and anxiety. Number of peripheral squares crossed, and number of central square crossed are used as measures of locomotor activity but are also used as a measure of exploration and anxiety. Our test drug *Arnica montana* extract (AME) did not show any significant result for these two parameters. alprazolam and AME plus alprazolam (combination group) showed increase in total number of peripheral squares and central squares crossed. A high frequency of these behaviours indicates high exploratory behaviour and low anxiety levels in alprazolam and combination group but not in AME group.

Frequency of rearing are usually used as measures of locomotor activity but also measures of exploration and anxiety. Alprazolam and combination group showed increase in frequency of rearing and the result was highly significant. AME treated rats showed increase in frequency of rearing which was statistically significant in comparison to control which indicates increased locomotion and exploration and a lower level of anxiety in AME group also but not comparable to alprazolam group.

Grooming behaviour is a displacement response and is expected to be displayed in a novel environment by Espejo and grooming increases in anxiety states. Grooming is decreased in AME group and the decrease is significant where as in alprazolam and combination group, decrease is highly significant which shows lower level of anxiety. Thus, AME also reduces level of anxiety but not as effective as alprazolam.

Frequency of defecation and urination was not statistically significant in all groups. Rats in all groups passed urine and fecal pellets in more or less numbers and is similar in comparison to rats in control group. Defecation and urination are often used as measures of anxiety but the validity of defecation as a measure of anxiety has been

questioned by Lister but Bindra and Thompson said that defecation and urination in a novel environment are signs of emotionality which is not to be equated with fearfulness.

Open field test is the best way to observe multiple behavioural responses and is useful for ethological testing in animal models of anxiety and it also is useful for testing locomotor activity and anxiolytic activity.<sup>10</sup>

## CONCLUSION

This suggests that *Arnica montana* may have considerable therapeutic benefit as an anxiolytic agent and as an add on drug for treatment of anxiety disorders after further studies and validation.

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*Ethical approval: The study was approved by the Institutional Animal Ethics Committee*

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