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Comparison of antianxiety action of *Arnica montana* and alprazolam for acute anxiety in wistar rats

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Abstract
Background: Anxiety affects large number of population worldwide. Benzodiazepines are preferred anxiolytic agents and are still frequently used in spite of the side effect profile. 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 with Alprazolam which is used as anxiolytic drug.
Materials and Methods: Forced swim test was used to induce anxiety. Anxiolytic action of study drugs which were given orally, was evaluated using Elevated plus maze test (EPM) in healthy wistar rats of either sex. Number of open arm entries, closed arm entries and time spent in open arms 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+0.08 mg/kg. Statistical analysis was done using ANOVA (p<0.05).
Results: Time spent in open arms 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 closed arm entries was highly significant (p<0.001) in Alprazolam and combination group and was also significant (p<0.05) in AME group.
Conclusions: *Arnica montana* may have considerable therapeutic benefit as an anxiolytic agent 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*, Elevated plus maze test

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 [1] and has become a very important area of research interest in psychopharmacology.
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 [2]. ß-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 [3]. New synthesized compounds as well as drugs derived from traditional herbs may have a possible therapeutic relevance in the treatment of anxiety [4].
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 [5]. 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 [6] include:
- Ashwagandha (*withania somnifera*)
- Taalisha *(Abies pindrow)*
- Windflower (*Anemone pulsatilla*)
- Hiranpadi (*Convolvulus arvensis*)
- Jatamansi (*Nardostachys jatamansi*)
- Brahmi (*Bacopa monnieri*)
- Sarpagandha (*Rauvolfia serpentina*)

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 [7].
Arnica montana at the dose of 100mg/kg is known to possess significant anxiolytic effect [4]. 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.

Materials and methods
The study was commenced after IAEC (Institutional Animal Ethics Committee) approval was granted and is conducted in accordance with CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals) guidelines.

Study Animals
The animals selected for the study were experimentally naive. The rats with following characteristics were selected:
- Species: *Rattus norwegicus*
- 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 Fibre: 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 12/12 hour 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:

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

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

Models that was used to assess the anxiolytic effect is
Elevated Plus Maze Method
The elevated plus maze (EPM) perhaps the most employed animal model of anxiety in current practice was first proposed by Handley & Mithani [8] and further validated by File [9] *et al*. EPM is an unconditioned spontaneous behavioural conflict model. EPM is a useful test to investigate both anxiolytic and anxiogenic agents. Elevated plus-maze apparatus consisting of two open arms (43 cm x 15 cm x 0.5 cm) and two enclosed arms (43 cm x 15 cm x 23 cm) extending from a central platform (5 cm x 5 cm x 0.5 cm) and raised 50 cm above floor level. EPM is in the form of a 'plus' with two open elevated arms facing opposite to each other and separated by a central square and two arms of the same dimensions but enclosed by walls [10]. The maze is raised off the ground so that the open arms combine elements of unfamiliarity, openness and elevation. EPM 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. Rats generally taken from their home cages will show a pattern of behavior 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 [11].

Procedure

- For testing vehicle or drug treated rats were placed in the elevated plus maze individually on the platform joining the four arms and the rat was observed for a period of 3 min.
- The number of open arm entries, closed arm entries and the amount of time spent in open arms was recorded
- Anxiolytic drugs increase the open arm entries and the time spent in open arms and decreases closed arm entries

The anxiolytic drug treated rodents show more number of open arm entries and more time spent in open arms as compared to control, less effective drug or anxiogenic drug treated rats [12].

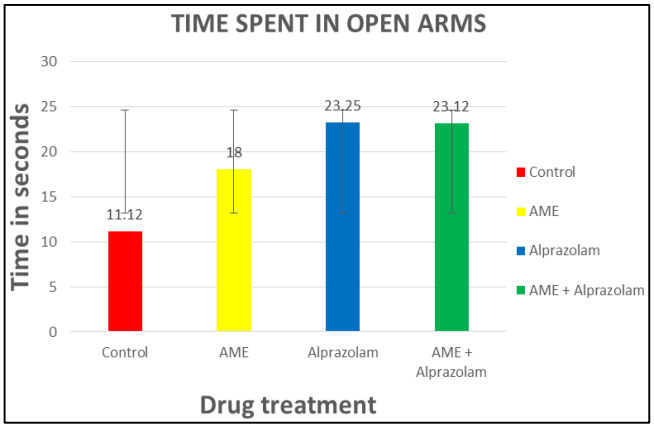
- The data was compiled and analysed using Primer of biostatistics version 5.0 and results were expressed as Mean ± S.D. Statistical significance was analysed using One-way analysis of variance (ANOVA) and P value <0.05 was considered to be statistically significant

Results

1. Time spent in open arms

Alprazolam and combination group showed increase in time spent in open arms and increase was highly significant and AME showed significant increase in time spent in open arm as compared to control

Drug treatment				
Group	**Control**	**AME**	**Alprazolam**	**AME + Alprazolam**
Mean	11.12	18	23.25	23.12
S.D.	4.086	3.546	6.205	4.549

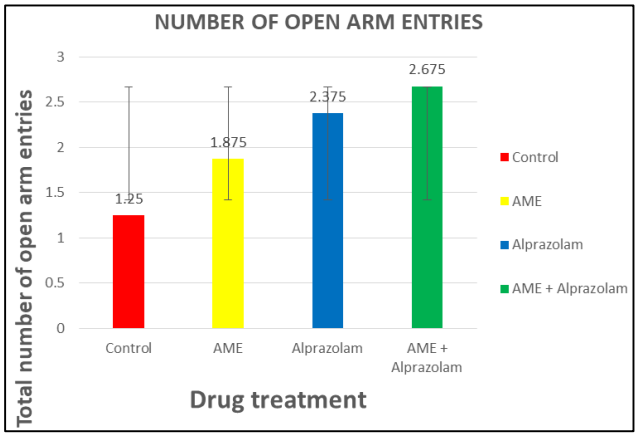


Graph 1

2. Number of open arms entries

Alprazolam and combination group showed increase in number of open arm entries and increase was statistically significant but AME did not show any significant increase in open arm entries as compared to control

Drug Treatment				
Group	**Control**	**AME**	**Alprazolam**	**AME + Alprazolam**
Mean	1.25	1.875	2.375	2.675
S.D.	0.4629	0.9161	0.9161	0.9170

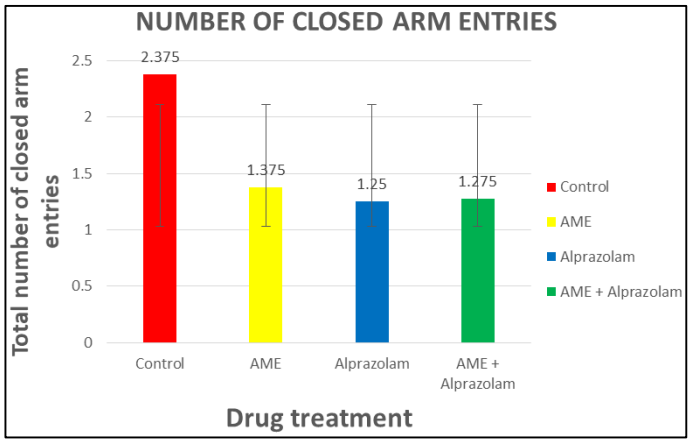


Graph 2

3. Number of closed arms entries

Alprazolam and combination group showed decrease in number of closed arm entries and decrease was highly significant and AME also showed significant decrease in frequency of closed arms entries as compared to control

Drug treatment				
Group	**Control**	**AME**	**Alprazolam**	**AME + Alprazolam**
Mean	2.375	1.375	1.25	1.275
S.D.	1.188	0.4629	0.4629	0.5175



Graph 3

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.

Elevated plus maze (EPM) model is based on the test animal's aversion to open spaces and anxiety is expressed by the animal spending more time in the enclosed arms [10]. Alprazolam and AME plus Alprazolam group showed highly significant results and even AME treated rats showed increase in duration of time spent in open arms which shows its anxiolytic effect.

Number of open arm entries increases in anxiolytic state. Alprazolam and combination group showed increase in number of open arm entries which is positively correlated

with risk assessment behavior but AME treated rats did not show any significant increase in this study.

EPM relies upon rodents' proclivity toward dark and enclosed spaces. Alprazolam and AME plus Alprazolam treated rats showed decrease in number of closed arm entries which was highly significant and result were also significant for AME treated rats in this study.

In conclusion, this study 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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