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Annals of Phytomedicine 1(2): 67-73, 2012

ANNALS OF
PHYTOMEDICINE
An International Journal

Anxiolytic effects of *Nardostachys jatamansi* DC in mice

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Received for publication October 7, 2012; accepted November 7, 2012

Abstract

Anxiety partly caused by stress has emerged to be a common psychiatric manifestation of the modern world and lifestyles. Medications for anxiety *viz*., benzodiazepines, MAOI's have limitations due to unbearable side effects. Herbal anxiolytes promise to alleviate anxiety and other pscychiatric disorders with minimal adverse effects. *Nardostachys jatamansi* DC belongs to plant family Valerianaceae and a prominent herb used in traditional medicine for a wide range of disorders. There is limited scientific literature with regard to its anxiolytic activity. The purpose of the present study was to investigate the anxiolytic effects of water (cold and hot) and 70% ethanolic extract of the rhizome of *Nardostachys jatamansi*.

The effect on anxiety was assessed using Elevated plus maze and Open field test. Pre-treatment of mice with different doses of the extract (125, 250 and 500 mg/ kg body weight) significantly ($p<0.01$) alleviated anxiety by increasing the number of entries on the open arm, time spent on open arms, percent time spent on open arms and decreasing the time spent on closed arms in the elevated plus maze test. Moreover, the 70% ethanolic extract also significantly ($p<0.01$) increased the locomotor activity as observed in the open field test confirming the anxiolytic effects in mice. Diazepam was used as a standard to compare the effects. Correlation analysis showed a linear relationship between antioxidant potency and radical scavenging activity with the polyphenolic content.

Key words: Anxiety, Elevated plus maze test, Open field test, Anxiolytic

Introduction

Anxiety is a central nervous system disorder (Kjernisted and Bleau, 2004) and Weinberger, 2001). Anxiety and fear are normal emotional responses to threatening situations. In human anxiety, disorders-such as panic disorder, obsessive-compulsive disorder, post-traumatic stress disorder, social phobia, specific phobias and generalized anxiety disorder-these responses are exaggerated (Hovatta *et al.*, 2005). Anxiety is the most common mental illness, affecting one eighth of the total population and has become a very important area of research in psychopharmacology in the current decade (Jung *et al.*, 2006). Researchers even in the advanced world are exploring their traditional remedies to find a suitable cure for these 'mind affecting diseases' which have been the outcome of man's zest to win the nature (Dhawan *et al.*, 2001). Medications for anxiety disorders include selective serotonin reuptake inhibitors (SSRIs) which elevate the level of neurotransmitter serotonin *e.g*., fluoxetine, sertraline, paroxetine, citalopram *etc*, benzodiazepines *e.g.*, diazepam, chlordiazepoxide *etc*. which facilitate inhibitory GABA transmission, monoamine oxidase inhibitors (MAOIs) *e.g.*, phenelzine, moclobemide that prevent the breakdown of serotonin and noradrenalin, beta-blockers like propranolol, atenolol which reduce the ability to produce adrenaline etc. The common limitations of these anxiety medications or drug therapy include comorbid psychiatric disorders and increase in dose leading to unbearable side-effects (Cates *et al.*, 1996 and Pilc and Nowak, 2005), such as allergic reactions, drowsiness, coordination problems, fatigue, mental confusion, nausea and addiction liability among others. Plants are known to possess myriad potential to cure ailments from time immemorial with minimal adverse effects. Therefore, research into the use of herbs as curative agents has been intensified over the years. However, the major hurdle in the uninhibited exploitation of herbal medicines into the regular

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practice of prescription is the lack of sufficient scientific data and better understanding of efficacy and safety of the herbal products (Gogtay *et al.*, 2002).

Nardostachys jatamansi DC commonly known as Indian Spikenard,belongs to plant family, *Valerianaceae.* A perennial herb growing to about 10-60 m in height, and commonly found in the alpine Himalayas from Punjab to Sikkim and Bhutan at an altitude of 3,000-5,000 m. Traditionally, the herb was utilised for an array of disorders, including the nervous system. In Ayurveda, the herb is a well known medhya, an intellect promoting herb with various medicinal properties, especially on the nervous system (Joshi and Parle, 2006). The herb has been reported to have antimicrobial activity (Mishra *et al.*, 1995), antianxiety (Jadhav *et al.*, 2009), antidepressant (Dhingra and Goyal, 2008), anticonvulsant (Rao *et al.,* 2005), antiparkinson's (Ahmad *et al.*, 2006), neuroprotective (Salim *et al.* 2003), nootropic (Joshi and Parle, 2006), hepatoprotective (Ali *et al.*, 2000), cardioprotective (Subashini *et al.*, 2006) properties. The dried roots contain sesquiterpenes like Jatamansone (valeranone), spirajatamol, patchouli alcohol, norseychelanone, jatamol A and B, lignans and neolignans like virolin, pinoresinol, jatamansic acid, terpenic coumarins like oroselol and jatamansin. The essential oil is composed of sesquiterpeniods and coumarins. Jatamansone is the principle sesquiterpenoid and renders majority of the biological activity (Hoerster *et al.,* 1977 and Rucker *et al.,* 1978).

The purpose of the present study was to investigate the anxiolytic effects of the rhizome of *Nardostachys jatamansi*, using two behavioural paradigms, employed to specifically assess anxiety in mice-the elevated plus maze and open field test. This was done as the herb has been attributed to have anxiolytic activity but not many scientific reports are available with regard to this.

Materials and Methods

Plant material and preparation of the extract

The plant material (dried roots) was procured from a local supplier at Mysore, Karnataka. The roots were washed thoroughly with distilled water to remove the adhered sand particles and shade dried. The dried roots were powdered and extracted.

The cold water and 70% ethanolic extract was prepared by soaking the powdered roots in water and 70% ethanol and then filtering with Whatmann filter paper No 1.

The hot water extract was prepared by boiling the soaked sample for 15 minutes and then allowed to cool down to filter the contents.

The 70% ethanolic extract was evaporated to dryness under vacuum on a rotary evaporator (Heidolph Rotacool, Germany) into a thick residue. This was subsequently lyophilized in a lyophilizer (Lyolab, India) and the powder was used for analysis. Similarly the filtrate obtained from the hot as well as cold water extract was lyophilized and the powder obtained was utilised for the further analysis.

Chemicals and reagents

Diazepam (Calmpose) used as a standard drug was procured from M/s Ranbaxy laboratories limited, India. 1mg/kg body weight was dissolved and administered. The extract in three different concentrations (125, 250 and 500 mg/kg body weight) was dissolved in distilled water and administered. All the solutions were freshly prepared before administration.

Animals

Swiss albino mice (25-30g) were used to assess the anxiolytic effect of the extract. The experimental protocols were accepted at the 14th Institutional Animal Ethics Committee (IAEC) meeting held on 17th November, 2009 by the IAEC. The animals were housed in groups of six mice per cage with free access to standard laboratory feed and water *ad libitum*. 12 hour light and 12 hour dark cycle was maintained in the animal house. The experiments were carried out between 10:00 h to 14:00 h. Food was withdrawn 3 hours before and during the experiment.

Methods

Behavioural testing

The effect on anxiety was assessed using Elevated plus maze and Open field test. The behavioural testing was recorded using the Any maze software, Stoelting Co., USA.

Elevated plus maze test (EPM)

EPM test is the commonly used behavioural paradigm to assess the anxiolytic behaviour in rodents. Briefly, the apparatus consisted of two open arms (35 cm × 5 cm) and two closed arms (30 cm×5 cm×15 cm) that extended from a common central platform (5 cm × 5 cm). The entire maze was elevated to a height of 60 cm from the floor level (Lister, 1987).

Testing was conducted in a quiet room that was illuminated only by a dim light. Each mouse was placed individually at the centre of elevated plus maze with its head facing towards an open arm and its behaviour was observed for a period of 5 minutes using Any Maze software. During the test period, the number of entries into open arm, closed arm and time spent in each arm and percentage of time spent on the open arms was determined. The percentage of time spent on open arms was calculated as:

$$\% = \frac{100 \times \text{Number of seconds spent on open arms}}{300 \text{ total seconds (5 min observation time)}}$$

After each trial, the maze was wiped with 20% ethanol and dried thoroughly before the next trial could begin. The animals were assigned to five different groups, according to their weight. Group I (control), Group II (diazepam), Group III (125 mg/kg bw), Group IV (250 mg/kg bw), Group V (500mg/kg bw). Six animals per group were used. Drugs were administered 60 minutes before testing, intraperitoneally.

Open field test

Further, to confirm the anxiolytic effects, the mice pretreated with 70% ethanolic extract were exposed to open field test. Open field test is a measure of spontaneous locomotor activity by measuring the total number of line crossings/total ambulatory distance. The apparatus was made of plexiglass; measuring 60 cm. The open field arena usually consists of brightly lit square or round area enclosed by walls with the animal usually being placed in the centre and its behaviour being recorded for a known period of time. The entire apparatus was divided into equal squares of twenty four. The behavioural test lasted for a period of 5 minutes (Kulkarni, 2005). The number of line crossings/the total ambulatory distance was observed and recorded using the Any Maze software. After each trial, the maze was wiped with 20% ethanol and dried thoroughly before the next trial could begin. The animals were assigned to five different groups according to their weight. Group I (control), Group II (diazepam), Group III (125 mg/kg bw), Group IV (250 mg/kg bw), Group V (500mg/kg bw). Six animals per group were used. Drugs were administered 60 minutes before testing, intraperitoneally.

Statistical analysis

All the values were expressed as Mean ± SD from 6 animals and analysed by One-way-ANOVA. The plant extract treated groups and diazepam treated group were compared with control group. p value less than 0.01 was considered as significant.

Results

Elevated plus maze test

ANOVA revealed significant differences between the groups at $p<0.01$. Pretreatment of mice with different doses of the extract (125, 250, & 500 mg/ kg body weight) significantly alleviated anxiety levels. The cold water extract at a dose of 125 mg/kg body weight (Figure 1), hot water extract at a dose of 500 mg/kg body weight (Figure 2) and alcoholic extract at a dose of 250 mg/kg body weight (Figure 3) showed significant increase ($p<0.01$)in the number of entries on the open arm, time spent on open arms, percent time spent on open arms. The standard drug diazepam showed significant anxiolytic behaviour as reported earlier (Soderpalm *et al.*, 1989) by increasing the percentage of time spent as well as the number of entries into the open arm.

Open field test

The alcoholic extract was further assessed for its anxiolytic effects by the open field test. The 70% alcoholic extract at 250 mg/kg body weight (Figure 4) also significantly ($p<0.01$) increased the locomotor activity/the total ambulatory distance. The extract at a dose of 250 mg/kg body weight showed effective results comparable with diazepam.

Correlation analysis

Correlation coefficients (r) calculated between free radical scavenging activity of the extract (assayed using DPPH) with polyphenolic content and antioxidant potency (measured using ferric reducing antioxidant power) with polyphenolic content of the 70% ethanolic extract are shown in Table 1.

Discussion

Herbal plants have been explored for their potential health benefits since time immemorial. Herbal anxiolytes promise to alleviate anxiety and other psychiatric disorders with minimal adverse effects. The 'World Health Organization' has approved that traditional health and folk medicine systems have proved to be more effective in health problems worldwide (Jadhav *et al.*, 2009). In Ayurveda, *Nardostachys jatamansi* is a well known medhya, an intellect promoting herb with various medicinal properties, especially on the nervous system (Joshi and Parle, 2006). The present study attempted to establish the anxiolytic properties of the herb. The most effective concentration of the extract exhibiting significant anxiolytic activity was selected using a variety of concentrations of the extract.

The effect of the cold water, hot water and ethanolic extract of *Nardostachys jatamansi* on anxiety was assessed, using two behavioural paradigms, employed to specifically assess anxiety, Elevated plus maze test and the Open field test. Diazepam as reported increased the number of entries into the open arms as well as the time spent on the open arms and also caused a significant increase in the total ambulatory activity as observed by the open field test. The cold water extract at a concentration of 125 mg/kg body weight showed a significant increase in the number of entries on the open arm, time spent on open arms, percent time spent on open arms and decreasing the percent time spent on closed arms and the number of entries into the closed arms in the elevated plus maze test. Similarly the hot water extract was effective at a dose of 500 mg/kg body weight. The 70% alcoholic extract was effective at a dose of 250 mg/kg body weight in increasing the number of entries on the open arm, time spent on open arms, percent time spent on open in the elevated plus maze test. The elevated plus maze is a novel test for identification of anxiogenic or anxiolytic drug effects in rodents. An aversion to enter open arms is caused by fear or anxiety. The exposure of the rodents to the elevated plus

maze evokes exploratory drive and fear drive and generates approach-avoidance conflict response. Elevation of the maze causes greater fear and more avoidance conflict (Kulkarni, 2005). According to the results obtained, the cold water, hot water and ethanolic extract at a dose of 125, 500 and 250 mg/kg body weight, respectively showed anxiolytic effects in mice. An increase in the time spent in the open arms suggests the anxiolytic effects of the extract at that concentration. This also suggests that the extract at that dose is effective in decreasing the sensitivity of mice to aversive stimuli such as an open arm.

In order to confirm the anxiolytic effects, the mice were, further, exposed to open field test, to assess their locomotor activity. The ethanolic extract was also effective in increasing the locomotor activity/the total ambulatory distance in the open field test at a dose of 250 mg/kg body weight. The results suggest that the extract is working effectively as an anxiolyte and ruling out the fact that the mouse due to anxiety crouched and stayed in one corner of the apparatus. The result confirms the anxiolytic effect of the ethanolic extract in mice. These behavioural changes produced by *Nardostachys jatamansi* extract in the behavioural tests elucidate the anxiolytic effects of the extract, comparable to the positive control *i.e.*, diazepam.

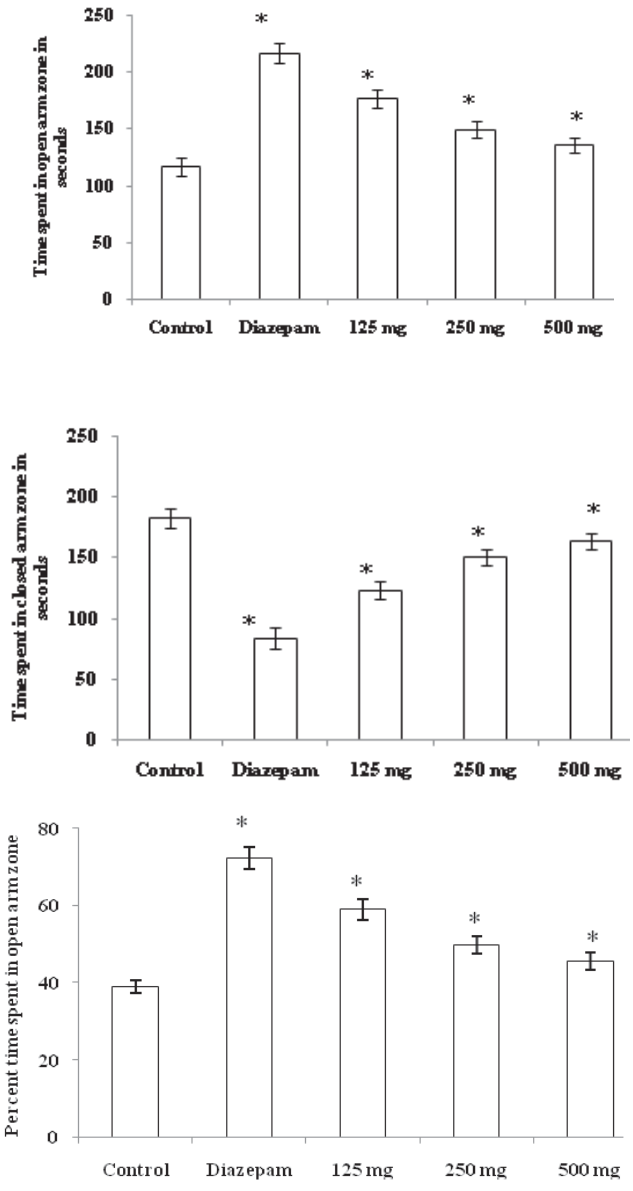
Correlation analysis showed a linear positive relationship between radical scavenging activity and polyphenolic content and antioxidant potency and polyphenolic content suggesting that the herb most likely aids in alleviation of oxidative stress mediated anxiety *via* its antioxidant potency and, further, studies are warranted to study the mode of anxiolytic action of the herb. Literature suggests a positive relationship between total phenolic and antioxidant activity in many plant species (Dasgupta and De, 2004; Dorman and Hiltunen, 2004 and Chen and Yen, 2007).

Current research dealing with anxiety disorders have centered around the gamma amino butyric acid mechanisms, the serotonergic system, noradrenergic mechanisms and neuropeptides (Barchas and Altemus, 1999). So, it becomes essential to know how *Nardostachys jatamansi* exerts its anxiolytic-like effects. GABA is the major inhibitory neurotransmitter, is known to regulate the neuronal excitability and serves as a 'brake' on the neuronal circuitry during stress and is the brain's natural stress reliever (Weeks, 2009). However, Hovatta *et al*. (2005) took a deviation and linked oxidative stress with anxiety by correlating the expression levels of two genes - glyoxalase 1 and glutathione reductase1 both of which take part in the oxidative stress pathway. Several other reports have also suggested for the role of oxidative stress in anxiety (Masood *et al.*, 2008; Bouayed *et al.*, 2007; de Oliveira, 2007and Atmaca *et al.*, 2004 and 2008). All these reports provide new insights into current work focussing on anxiety disorders and stress on the beneficial role antioxidants have in anxiety disorders. Therefore, the results from the present study enunciate the anxiolytic properties of the herb in mice. Further studies are warranted to examine as to how the herb exerts anxiolytic effects. None the less, this herb when incorporated into nutraceuticals promises to provide relief to the anxious.

Correlation analysis	Polyphenolic content
Free radical scavenging activity	r = 0.9845
Antioxidant potency	r = 0.9966

Table 1: Correlation analysis between free radical scavenging activity with polyphenolic content and antioxidant potency with polyphenolic content

Elevated plus maze test



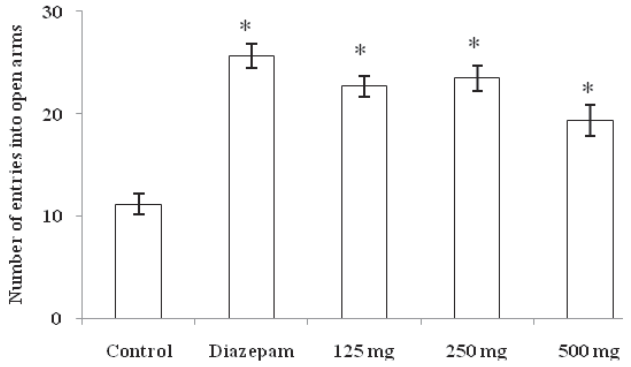
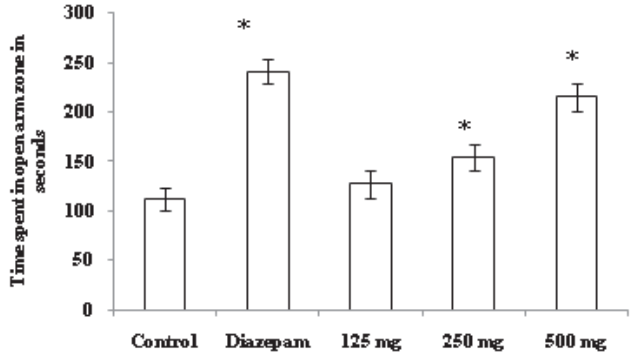
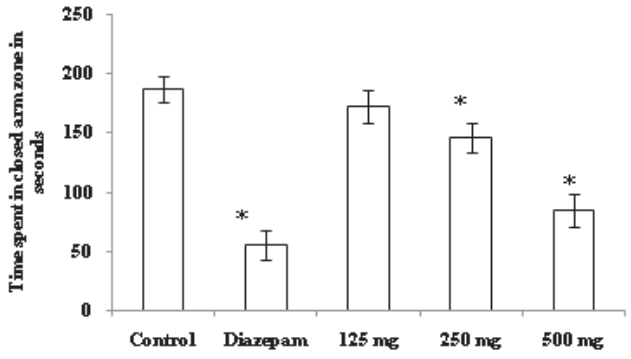
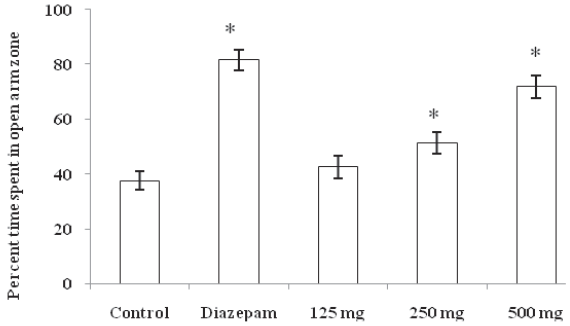


Figure 1: Effects on the behaviour of mice in elevated plus maze following intraperitoneal administration of *N.jatamansi* cold water extract. All values expressed as Mean±SD, *p<0.01.







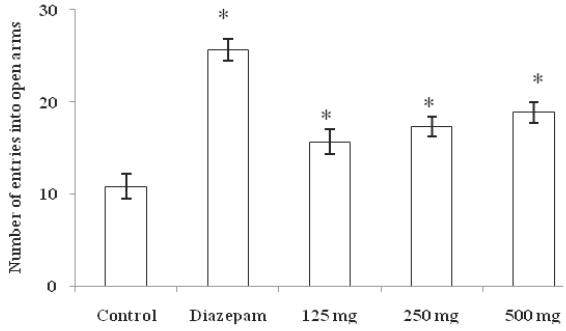
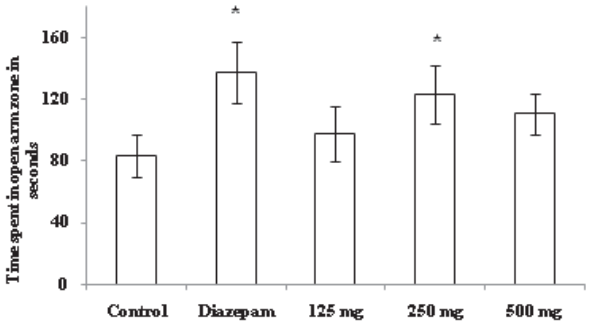
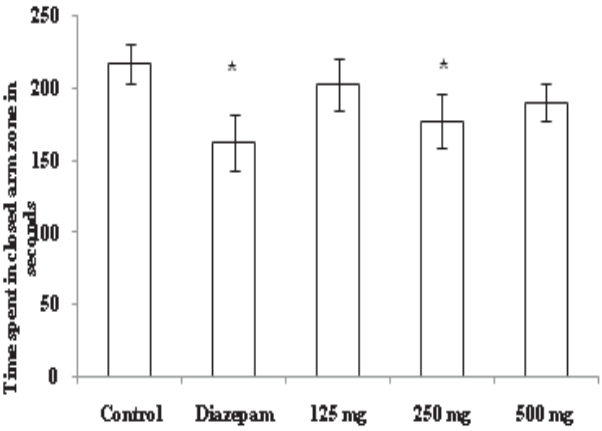
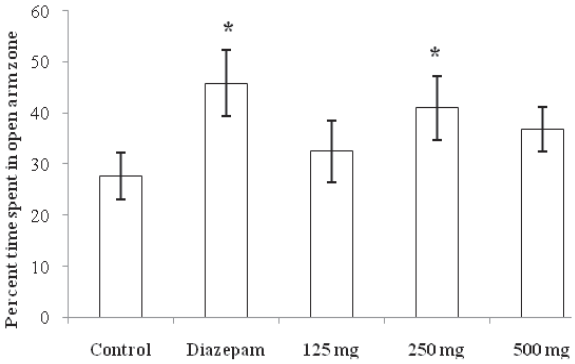


Figure 2: Effects on the behaviour of mice in elevated plus maze following intraperitoneal administration of *N.jatamansi* hot water extract. All values expressed as Mean±SD, *p<0.01.







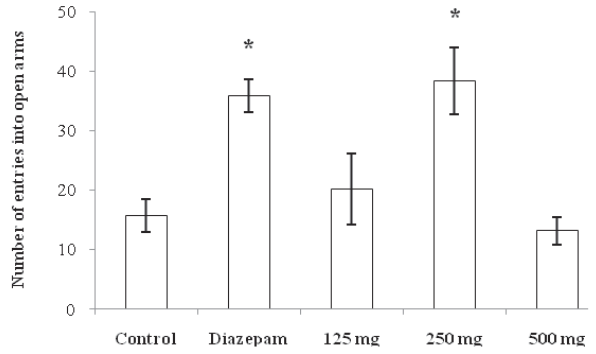


Figure 3: Effects on the behaviour of mice in elevated plus maze following intraperitoneal administration of *N.jatamansi* 70% ethanolic extract. All values expressed as Mean±SD, *p<0.01.

Open field test

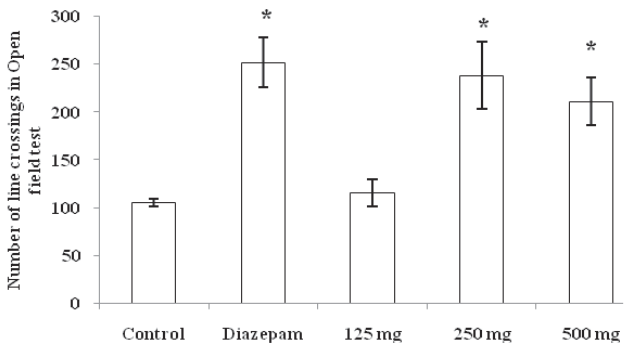


Figure 4: Effects on the behaviour of mice in open field apparatus following intraperitoneal administration of *N.jatamansi* 70% ethanolic extract. All values expressed as Mean±SD, *p<0.01.

Conclusion

The results from the present study enunciate the anxiolytic properties of the herb in mice and suggest that the herb could serve as a new approach in the treatment of anxiety. However, further studies are warranted to examine as to how the herb exerts anxiolytic effects.

Acknowledgement

This work has been carried out for the project # DFR 297 funded by the Defence Research and Development Organisation, India. The authors like to acknowledge the Research Appliances Division of DFRL for fabricating the elevated plus maze and open field test apparatus. The authors also acknowledge the Director, DFRL for his constant support and encouragement.

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