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Research Article

ANXIOLYTIC ACTIVITY OF BARLERIA PRIONITIS LIN STRESS INDUCED ALBINO RATS

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ABSTRACT

Stress is an inherent component of life that can profoundly influence physical and psychological health. It is defined as the physiological and psychological response of an organism to external or internal stressors that challenge homeostasis (McEwen, 2007). Anxiety usually refers to the experience of fear, apprehensiveness, nervousness, panic, restlessness, tension, and agitation. The percentage of anxiety among people increasing day by day, hence need to focus on it. Anxiety disorders are the most common class of mental disorder in adult. Specific phobias and social anxiety disorder are very common among this disorder. Some Neurotransmitters including serotonin, dopamine, noradrenaline and GABA are responsible for generation anxiety. Serotonin modulators and benzodiazepines are mostly used to improve anxiety. The present paper discusses anti-anxiety potential of Barleria prionitis L leaves. Barleria prionitis L leaves contains flavonoids, steroids, tannins, saponins and alkaloids. Flavonoids were the major constituent for the anxiolytic activity. The effect of methanolic extract of Barleria prionitis L leaves (200mg/kg and 400mg/kg, orally, daily, 21 days) on anxiolytic activity was assessed by using Modified elevated plus maze apparatus and Light-dark box apparatus. Anxiety activity in rats was induced by social isolation. The results shows that Open arm entries were increased and closed arm entries were decreased in modified elevated plus maze apparatus (MEPMA). Light box entries were increased and dark box entries were decreased in light and dark box model (LDB). Thus we can conclude that methanolic extract of Barleria prionitis L leaves exhibits significant anxiolytic activity at low dose (200mg/kg) and high dose (400mg/kg). Thus Barleria prionitis L leaves is a promising herbal option in the pharmaceutical world.

Keywords: Barleria prionitis L, Anxiety, Elevated plus maze apparatus, light and dark box apparatus, Anxiolytic activity

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1. INTRODUCTION

Stress is a natural part of life and can have a significant impact on one's physical and mental well-being. It is described as an organism's physiological and psychological reaction to internal or external stressors that disrupt homeostasis (McEwen, 2007). When we discuss anxiety, we usually refer to emotions such as restlessness, tension, agitation, panic, fear, and apprehension. Anxiety needs to be addressed because it is growing more common among people. Anxiety disorders are the most common type of mental illness in adults. This disorder, which encompasses social anxiety disorder and some phobias, is rather common (J.P. Jhabarmal et al., 2013). Among the symptoms include sweating, trembling, and mild headaches. Changes in heart rate, muscle tone, skin conductance, and elevated blood pressure (BP) are all possible. Serotonin. Among the symptoms include sweating, trembling, and mild headaches. Changes in heart rate, muscle tone, skin conductance, and elevated blood pressure (BP) are all possible. There is a little safety margin between the anxiolytic action of benzodiazepines and serotonin, dopamine, noradrenaline, gamma amino butyric acid (GABA), corticotropin releasing factor (CRF), melanocyte stimulating hormone (MSH), neuropeptides, and

neurosteroids. Anxiety is caused by these neurotransmitters (J. P. Jhabarma et al., 2013).

Physical symptoms of anxiety include headaches, palpitations, increased perspiration, fatigue, and lightheadedness. Anxiety is linked to almost all emotional disorders and physical illnesses. There is evidence that the prefrontal cortex plays a crucial role in putting an end to dread by controlling the amygdala-mediated expression of fear or dread. Although the biological mechanisms underlying negative and positive control of anxiety or dread are not fully understood, many genes have been shown to influence the condition (Mohale D. S et al., 2012).

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Particular phobia was the most common mental illness, according to the Epidemiological Catchment Area (ECA) study, one of the biggest epidemiological studies ever carried out in the United States. Obsessive-compulsive disorder ranked fourth. The same prevalence statistics are found in the National Comorbidity Survey, National Comorbidity Survey-Revised (NCS-R), and other epidemiological studies. Epidemiological studies carried out in other countries revealed comparable incidence rates. Anxiety disorders were the most common mental diseases in the European Union (EU), with a median 12-month prevalence of 12%, according to a review of 27 epidemiological research carried out there between 1990 and 2004 (M. Miyazaki et al., 2017).

Plants are the primary source of medicine and are essential to global health. It has long been recognized that medicinal plants or herbs may provide therapeutic benefits. Medicinal plants are widely used and have a significant part in the global health system. The primary justification for using the plant is that it produces less adverse effects and is more compatible, adaptable, and culturally acceptable with the human body.Atropine, quinidine, physostigmine, reserpine, tubocurarine, artimesinin, morphine, colchicine, quinine, digoxin, aspirin, pilocarpine, taxol, ephedrine, vinblastine, and vincristine are a few significant medications derived from plants.(Olodeji O. et al., 2016)

Barleriaprionitis L. (Family: Acanthaceae), commonly known as Vajradanti, is a medicinal shrub widely distributed in India and other tropical regions. Various parts of the plant are traditionally used for treating inflammation, toothache, fever, cough, and other ailments. Phytochemical studies have identified flavonoids, iridoids, phenolics, terpenoids, and alkaloids, which may contribute to its reported antioxidant, anti-inflammatory, antimicrobial, hepatoprotective, and neuroprotective activities (Banerjee et al., 2012; Talukdar et al., 2015).

Phytochemical investigations have reported the presence of iridoid glycosides, flavonoids, phenolic compounds, terpenoids, alkaloids, tannins and saponins, with compounds such as barlerin, acetylbarlerin, verbascoside, scutellarein, lupeol and β-sitosterol being reported from different plant parts. These constituents may contribute to the plant's diverse pharmacological properties (Talukdar et al., 2015; Bhardwaj et al., 2026).
Experimental studies have demonstrated several biological activities of B. prionitis, including antioxidant, antimicrobial, anti-inflammatory, antidiabetic, hepatoprotective, antinociceptive and neuroprotective effects. Recent reviews further emphasize its potential as a source of bioactive compounds for pharmacological and drug-development research (Banerjee et al., 2012; Chaudhari et al., 2025; Bhardwaj et al., 2026).

2. MATERIAL AND METHOD

Materials:

Animals:- 8 weeks of healthy female Sprague-dawley rats (weighing 150-250 gm) were used for this study. Animals were housed in polyethylene cages with wire mesh top and husk breeding was maintained under controlled condition of light (12h-light, 12h-dark), temperature (25±2ºC), and humidity(60±5%) and fed with a standard pellet diet and water ad libitum, were used for the entire animal study. The experiments were performed during the day time (8.00-16.00 hrs). The rats were housed and treated according to the rules and regulations of CPCSEA and IAEC. The protocol for all the animal study was approved by Institutional Animal Ethics Committee (IAEC).

Method:-

Barleriaprionitis L. plant were collected from local area of Maharashtra (India). The plant material was identified and authenticated By Dr. Punjabrao Deshmukh Krishi Vidyapeeth Akola Vasantrao Naik Collage Of Agriculture Biotechnology, Yavatmal. (Reference No. VNCABT/Ytl/ Hort/ 1087 /2022).

Leaves were dried in a shade and then powdered to get a course powder. This powder was stored in air tight container and used for extraction.

For the extraction of Barleriaprionitis L. leaves methanol was used as a solvents. The extraction needs a glass bottle. The dried powder Barleriaprionitis L. leaves were placed into a glass bottle for extraction. Powdered leaves were macerated during the maceration process; they were occasionally stirred at regular intervals of time. It was filtered. The extract was concentrated and dried at a controlled temperature of 40 °C on a water bath, and the percentage yield was found to be 8.7%. A dried extract of the leaves was used for further investigation. (Mst Mahfuza Khatoon et.al, 2014).

The extract was then screened for the presence of different phytocostituents viz. alkaloid, tanins, saponins, Flavonoids, Phenolic Flavonide etc. (S. Sivakrishnan et.al, 2014).

Experimental Design:-
For this study animals were divided into five groups,
Group I (Vehicle control group)- Rats received only saline solution.
Group II (Negative control group)- Rats were subjected for social isolation for 21 days.
Group III (Low dose group)- Rats were subjected for social isolation and treated with 200mg/kg methanolic extract of Barleriaprionitis L. orally for 21 days.
Group IV (High dose group)- Rats were subjected for social isolation and treated with 400mg/kg methanolic extract of Barleriaprionitis L. orally for 21 days.
Group V (Standard group)- Rats were subjected for social isolation and treated with 2mg/kg Diazepam for 21 days.

Induction of anxious state:-

All groups were subjected for 21 days for social isolation except normal control group which was placed in normal condition in animal house. For the induction of anxiety rats were socially isolated in dark room for 21 days.

Drugs and dosing:-

Diazepam (2mg/kg) was used as standard drug. Diazepam was diluted to 1.5 mg/10ml with distilled water. Two different concentrations (200 and 400 mg/kg) of the Barleriaprionitis L. leaves extract were prepared by dissolving the extract in distilled water. All solutions were prepared freshly on test days and administered orally according to body weight of rats. Low dose group extract were calculated 200mg/kg and high dose group extract were calculated 400mg/kg of rats. Then dosingwere given to rats in the concentrations like 0.1ml, 0.2ml, 0.4ml….etc.
Anxious behavioral state of animals after 21 days were checked by using elevated plus maze apparatus and light and dark box model.

Elevated plus maze apparatus:-

Elevated Plus Maze Apparatus (EPMA) was widely used for the assessment of anxiolytic activity.30 min before the experiment, mice were individually placed in the center of the apparatus facing one of the open arms.The number of entries and time spent on the open and enclosed arms was observed during a period of 5 min. An arm entry was counted when all four paws were in the arm. The percentage of open arm entries and the time spent in open arm were measured using the following formula:

% of open arms time spent = [open arms time/(open arms time + closed arms time)] ×100% of open arms entries = [open arms entries/(open arms entries + closed arms entries)]×100 (Vijendar Kumar et.al, 2013).

Light and Dark apparatus:-

Light and dark apparatus was commony used model for the assessment of anxiolytic activity. This apparatus was consist of two compartment, one-third for the dark compartment while two-third for the light compartment. The light compartment was brightly illuminated with a light source of 400 lux which is placed 35cm above the box. 30 minutes after treatment with the vehicle, methanol extract or diazepam, each mouse was individually placed in the corner of the light compartment, facing away from the entry to the dark compartment. The mice were monitored for a period of 5 min and the following parameters were observed and quantified:

(a) latency of the first crossing from one compartment to the other, (b) time spent into light and dark compartment, (c) the number of transition between the light and dark compartment (Michel Bourin et.al, 2003).

3. RESULTS

Qualitative phytochemical analysis of BP shows presence of Alkoaloids, Flavonoids, Carbohydrates, steroids, tannins, proteins and absence of glycosides in all plant extracts.

Elevated Plus Maze Apparatus Results:-

Table 1 : Effect of methanolic extract of Barleriaprionitis L. leaves in anxious rats using Elevated plus maze test.

Sr No.	Groups	Number entries in closed arm (%) (0day)	Number entries in closed arm (%) (28 day)	Number entries in open arm (%) (0day)	Number entries in open arm (%) (28 day)
1.	Normal control	67.52 ± 1.12	66.16 ± 1.58	32.66 ± 1.21	33.16 ± 1.72
2.	Negative control	66.84 ± 1.58	92.66 ± 1.03@	32.84 ± 1.35	10.66 ± 1.03@
3.	Low dose group	66.85 ± 1.35	72.66 ± 2.25**	32.24 ± 1.35	26.66 ± 2.25**
4.	High dose group	67.12 ± 1.43	64.32 ± 0.89**	31.92 ± 1.29	27.32 ± 0.89**
5.	Diazepam Std	68.33 ± 1.11	64.5 ± 2.88**	32.33 ± 1.11	34.5 ± 2.88**

Values are expressed in Mean±SEM (n=6)
@P<0.0001 Significant increase in closed arm and decrease in open arm entrieswas observed compared to normal control group.
**P<0.0001.Significant decreased in closed arm entries and increased in open arm entries was observed compared to negative control group.
#P>0.05 when compared with negative control.
Table 1 shows that there was a significant (P<0.001) increased in the closed arm entries of negative control as compared to normal control and the low dose, high dose treated group there was significant (P<0.001) decreased

closed arm entries.

Table 1 shows that there was a significant (P<0.001) decreased in the open arm entries of negative control as compared to normal control and the low dose, high dose treated group there was significant (P<0.0001) increased open arm entries.

Table 2: Effect of methanolic extract of Barleria prionitis L. leaves in anxious rats on time spent in closed arm and open arm of.

Sr No.	Groups	Time spent in closed arm (sec) (0day)	Time spent in closed arm (sec) (28 day)	Time spent in open arm (sec) (0day)	Time spent in open arm (sec) (28 day)
1.	Normal control	52.25±0.98	50.25±1.20	41. 33 ± 1.88	41. 68 ± 1.84
2.	Negative control	51.55±1.26	72.56 ± 1.03@	42.14 ± 1.54	28.56 ± 1.03@
3.	Low dose group	51.54±0.99	61.66 ± 2.55**	41.24 ± 1.65	35.66 ± 2.55**
4.	High dose group	50.25±1.26	58.89 ± 1.49**	41.65 ± 1.24	37.89 ±1.49**
5.	Diazepam std	52.25±0.98	52.65 ± 1.88**	40.68 ± 5.11	40.65 ± 2.88**

Values are expressed in Mean±SEM (n=6)

@P<0.0001 Significant increase in time spent in closed arm and decreased in time spent in open arm was observed compared to normal control group.

*P<0.05.Significant increased in time spent in open arm was observed compared to negative control group.

**P<0.001.Significant increased in time spent in open arm was observed compared to negative control group.

#P>0.05 when compared with negative control.

Table 2 shows that there was a significant (P<0.001) increased in the time spent in closed arm of negative control as compared to normal control and in the low dose, high dose treated group there was significant (P<0.001) decreased time spent in closed arm entries.

There was a significant (P<0.001) decreased in the time spent in open arm of negative control as compared to normal control and the low dose, high dose treated group there was significant (P<0.05,P<0.001) increased time spent in open arm entries.

Light And Dark Box Apparatus Results:-

Table 3 : Effect of methanolic extract of Barleriaprionitis L. leaves on Light and dark box model in dark box and light box in anxious rats.

Sr No.	Groups	Number entries in darkbox (%) (0day)	Number entries in darkbox (%) (28 day)	Number entries in lightbox (%) (0day)	Number entries in lightbox (%) (28 day)
1.	Normal control	74. 26 ± 1.26	75.63 ± 1.54	24.56 ± 1.23	24.16 ± 1.26
2.	Negative control	74.54 ± 1.64	87.54 ± 1.26@	24.98 ± 1.25	12.96 ± 1.15@
3.	Low dose group	75.55 ± 1.55	82.56 ± 1.28**	26.28 ± 1.78	18.25 ± 1.52**
4.	High dose group	75.84 ± 1.54	81.26 ± 1.16**	23.85 ± 1.29	19.36 ± 0.89**
5.	Diazepam std	75.56 ± 1.31	75.26 ± 1.26**	42.37±3.95	46.35±4.12***

Values are expressed in Mean±SEM (n=6)

[a] P<0.0001 Significant increase in dark box and decreased in light box entries was observed compared to normal control group.

**P<0.0001.Significant decreased in dark box and increased in light box entries was observed compared to negative control group.

Table 3 shows that there was a significant (P<0.001) increased in the dark box entries of negative control as compared to normal control, the low dose, and high dose treated group there was significant (P<0.001) decreased dark box entries. There was a significant (P<0.001) decreased in the light box entries of negative control as compared to normal control the low dose, and high dose treated group there was significant (P<0.001) increased light box entries.

4. DISCUSSION

Anxiety is defined as an exaggerated feeling of apprehension, uncertainty, and fear. It is an unpleasant state of tension with an anticipation of imminent danger. It may be regarded as a particular form of behavioral inhibition that occurs in response to environmental events that are novel. Anxiety affects one-eighth of the total population worldwide and has become a very important area of research interest in psychopharmacology during this decade (R. S. Adnaik et.al, 2009).

GABAA receptors are involved in anxiety and their direct activation would have an anxiolytic effect. It is well documented that pentylenetetrazole-induced convulsions are produced due to diminution of GABA level in brain (R.S Adnaik et.al, 2009).

Anxiety can be produced by different methods such as using chemicals, social model and stress evoked, sensory models, transitory model, restraint stress etc. One of the commonly used model is restraint stress, which is a modified form of immobilization stress, restraint stress has been widely used as a model of chronic psychoemotional stress to induce depressive and anxiety like behaviours, learning and memory deficits, and hippocampal neuronal damage in mice. Restraint stress method is very economic, easy availability, easy induction of depression and anxiety

and it also shows good results. Hence we choose restraint stress for generation of anxiety in this work (Hanwoong Woo et.al, 2018).

During this procedure inescapable physical and mental stress is induced by placing the animals in a plastic bottle in order to block their movements. This is a validated experimental stressor involving both physical and psychological effects at the same time (Pitman et al., 1988; Jaggi et al., 2011).

Now a days herbal medicine are used to treat intense and constant sicknesses. Herbal remedies have been used for huge number of years . In fact, herbal medicine is the establishment of modern medicine. This medicine also has very less sideeffects.(Olodeji O. et.al,2016).

It has been reported that many flavonoids and neuroactive steroids were found to be ligands for the GABA receptors in the central nervous system; that can act as benzodiazepine-like molecules (Mst Mahfuza Khatoon et.al, 2014).

Literature shows that Grewia genus contains terpenoid, Alkaloid, flavones etc. (Wali Ullah et al 2012)

Flavonoids were the major constituents for the anxiolytic activity (Mst Mahfuza Khatoon et.al, 2014).

Our study confirms the presence of glycosides, saponins, tannins, steroids and flavonoids etc. in methanolic extract of Barleriaprionitis L. leaves.

Flavonoids present in the extracts may be responsible for its CNS depressant activity.(Mst. Mahfuza Khatoon et al, 2014).

There are many models for the screening of anxiety like modified elevated plus maze apparatus, light and dark apparatus, elevated T maze, elevated zero maze, open field test, and white black box. In this study for the assesment of anxiolytic activity we have used modified elevated plus maze apparatus (MEPA) and light and dark model (LDB) due to their economic, easily availability, popularity, accuracy, specificity and shows good results.

In modified elevated plus maze apparatus, closed arm entries of control vehicle, low dose, high dose and standard group after 28 days were decreased as compared to 0 days entries. Only negative control group had more no.of entries in closed arm as compared to 0 day entries. In closed arm, time spent by rats in control vehicle, low dose, high dose and standard group after 28 days were decreased as compared to 0 dayreadings time spent by rats. Only negative control group had more time spent in closed arm as compared to 0 dayreadings time spent by rats.

In open arm, entries of control vehicle, low dose, high dose and standard group after 21 days were increased as compared to 0 days entries. Only negative control group had less no.of entries in open arm as compared to 0 day entries. In open arm, time spent by rats in control vehicle, low dose, high dose and standard group after 28 days were increased as compared to 0 days readings time spent by rats. Only negative control group had less time spent in open arm as compared to 0 dayreadings time spent by rats.

In light and dark model, no.of entries by rats in dark box were decreased by treated groups of rats compared with negative control group. No.of entries by the rats in light box were increased by Treated Groups of rats Compared with negative control.

From this result we can say that methanolic extract of Barleriaprionitis L. leaves exists anxiolytic activity due to the presence of flavonoids as a major constituent.

5. CONCLUSION

The present findings indicates that the methanolic extract of Barleriaprionitis L. leaves exhibits significant anxiolytic activity at low dose (200mg/kg) and high dose (400mg/kg).

Thus Barleriaprionitis L.leaves is a promising herbal option in the pharmaceutical world.

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