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Research Article

Evaluation of ethanolic extract of *Piper methysticum* leaves on anxiolytic activity of mice

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Abstract

The ethanol extract of *Piper methysticum*, commonly known as kava, has shown promising results in reducing anxiety. Studies suggest it may have anxiolytic effects, potentially offering a natural alternative to conventional anxiety treatments. The ethanolic extract of *Piper methysticum* can increase the duration of action. It can also increase the time spent in open arm, entry in open arm (in elevated plus model) as well as increase the time spent in light field (in Light Dark field) thus we can conclude that it can also possesses Anxiolytic action. The ethanolic extract of *Piper methysticum* possesses an anxiolytic like activity without sedative side effect. The corticosterone level in mice is increased when they got anxiety. So when the plasma corticosterone level of mice is checked in control group its turn out to be 10.28±0.52. It gets decreased with increase in doses. When treated with low dose of Aqueous extract is 7.28±1.44 is less effective than low dose of Ethanolic extract that is 6.22±1.28. Same as that high dose of extract shows the corticosterone level was 6.24±2.28 which was also less effective than high dose of ethanolic extract which was 5.52±0.32. Most effectively stress was decreased when treated with the Standard drug (Alprazolam) which shows plasma corticosterone level was 1.24±0.36. The mice of *Piper methysticum* extract (300 &600 mg/kg/ p.o.) treated group showed significantly ($p<0.05$) increased in body water intake as compared to the control group. Results showed that synthesized extract is very effective for the treatment of anxiety.

Keywords: *Piper methysticum*; anxiolytic activity; Anthraquinones; Light Dark model

1. Introduction

Anxiety is a common mental health condition characterized by feelings of worry, nervousness, or fear that are often disproportionate to the actual situation. Anxiety is different from fear in that fear is defined as the emotional response to a real threat, whereas anxiety is the anticipation of a future threat. It is often accompanied by nervous behavior such as pacing back and forth, somatic complaints, and rumination[1-3]. Persistent anxiety can lead to avoidance of situations that trigger it and may develop into anxiety disorders, such as generalized anxiety disorder or panic disorder, which last for months or even years. Anxiety also plays a significant role in other mental health conditions like obsessive-compulsive disorder and post-traumatic stress disorder[4-6].

The behavioral effects of anxiety may include withdrawal from situations which have provoked anxiety or negative feelings in the past. Other effects may include changes in sleeping patterns, changes in habits, increase or decrease in food intake, and increased motor tension[7,8]. Anxiety can be experienced with long, drawn-out daily symptoms that reduce quality of life, known as chronic (or generalized) anxiety, or it can be experienced in short spurts with sporadic, stressful panic attacks, known as acute anxiety. Symptoms of anxiety can range in number, intensity, and frequency, depending on the person. However, most people do not suffer from chronic anxiety. Anxiety can induce several psychological pains (e.g. depression) or mental disorders, and may lead to self-harm or suicide[9-14].

Piper methysticum (kava plant) is widely cultivated in the south pacific areas. This plant belongs to the Piperaceae family. Kava has a psychoactive activity. In Europe, the usage of kava started around 1900 for the treatment of gonorrhea and nervous disorders and also as a diuretic. In Hawaii, it has been used to treat asthma, skin disorders, urologic problems, and lung disorders. In Germany, kava was used for the treatment of gonorrhea, when penicillin was not discovered [15,16].

Receptor binding assays with kava leaf extracts have shown strong interactions with the GABAA receptor, D2 receptor, opioid receptors (μ and δ), and histamine

receptors (H1 and H2), with weaker interactions at 5-HT6, 5-HT7, and the benzodiazepine site of GABAA. The anxiolytic effects of kava are likely due to GABAA potentiation, while elevated dopamine levels in the brain contribute to its mild psychotropic effects. Although long-term kava use does not impair cognitive function, it has been associated with elevated liver enzymes[17-20].

Kavalactones, the active compounds in kava root, have shown antioxidant properties and potential as chemopreventive or chemotherapeutic agents, particularly in cancer treatment. Studies also reveal dose-dependent anxiolytic effects of kava extract. Kavalactones activate PXR, leading to CYP3A4 gene expression. This study aims to investigate the potential benefits of kavalactones in treating high-fat diet-induced dementia in mice, focusing on their PXR agonistic actions[21-23].

The present work is aimed to evaluate the Anxiolytic effect of ethanolic extract of Piper methysticum plant leaves in experimental animals mice by actophotometer elevated plus maze and light-dark model in mice. Present study also determines the plasma corticosterone level in mice post experiment.

2. Experimental

2.1. Materials and Methods

The data will be collected, based on laboratory animal experimentation. The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC) and was carried out as per the guidelines of Committee for the purpose of Control and Supervision of Experimental Animals (CPCSEA), Ministry of environment and Forests, Government of India (Reg. No. 2011/PO/Re/S/ 18/CPCSEA and date of registration is 1/5/2018) for the use and care of experimental animals. Adequate measures were taken to minimize pain or discomfort with animal's experimental procedure. Research protocol is duly approved by IAEC/CPCSEA (IAEC/SSIP/2023/PR-038).

2.2. Methods of collection of data

All chemicals of analytical grade will be procured from Sigma chemical, USA and S. D. Fine Chem. Ltd., India.

2.2.1. Collection and Preparation of plant material:

The ethanolic extract of leaves part of plant Piper methysticum was procured from Shreedha Phyto Extract, Jaipur. The same group also provided a certification of the plant's identity and quality (Certificate of Analysis).

2.2.2. Preparation of the extract:

The leaves part of plant Piper methysticum were separated and dried between 55 º to 60º C and then pulvirized to very fine powder. The powder was extracted using soxhlet apparatus (Fig. 1) using ethanol and aqueous as a solvent. The % yield was found to be 7.2%W/W.

Figure 1: Soxhlet apparatus

2.2.3. Animals: Healthy, adult Swiss albino mice of either sex weighing (25-40 g), maintained under standard laboratory conditions, at temperature 25 ± 2°C and a 12 h light-12 hdark period will be employed for the experimentation. Food and water will be provided ad libitum.

2.2.4. Acute oral toxicity study: Acute toxicity study for the ethanolic extract of Piper methysticum leaves extract will be done according to the OECD guidelines No: 423 and low, medium and high dose will be selected for treatment.

2.3. Acute oral toxicity study

An acute toxicity study of the ethanolic extract of *Piper methysticum* leaves was conducted following OECD guidelines No. 423. Overnight-fasted mice were divided into four groups of three animals each, and the extract was administered orally at doses of 5, 50, 300, and 1000 mg/kg. The mice were observed for behavioral changes, tremors, convulsions, salivation, diarrhea, lethargy, and other toxic symptoms for 2 hours, at 24 hours, and up to 14 days. No mortality or severe toxicity was observed, and surviving mice were rehabilitated for further experimentation. The study was conducted on Swiss mice (30-35 g) at the St. Soldier Institute of Pharmacy, Jalandhar, Punjab, under standard laboratory conditions. All procedures were approved by the Institutional Animal Ethics Committee (IAEC) and followed CPCSEA guidelines (Reg. No. 2011/PO/Re/S/18/CPCSEA, registered 1/5/2018) to ensure animal welfare[24-27].

2.4. Preliminary phytochemical screening

The different qualitative chemical tests were performed for establishing the profile of the plant extracts for its chemical composition. The following tests were performed to detect various phytoconstituents present in them.

2.4.1. Test for alkaloids

Dragendorff's test: To 1 ml of the extract, add 1 ml of Dragendorff's reagent (Potassium Bismuth iodide

solution). An orange-red precipitate indicates the presence of alkaloids.

Mayer's test: To 1 ml of the extract, add 1 ml of Mayer's reagent (Potassium mercuric iodide solution). Whitish yellow or cream coloured precipitate indicates the presence of alkaloids.

Hager's test: To 1 ml of the extract, add 3ml of Hager's reagent (Saturated aqueous solution of picric acid), yellow coloured precipitate indicates the presence of alkaloids.

Wagner's test: To 1 ml of the extract, add 2 ml of Wagner's reagent (Iodine in Potassium Iodide), Formation of reddish brown precipitate indicates the presence of alkaloids.

2.4.2. Test for Cardiac Glycosides

Legal test: Dissolve the extract in pyridine and add sodium nitroprusside solution to make it alkaline. The formation of pink red to red colour shows the presence of glycosides.

Baljet test: To 1ml of the test extract, add 1ml of sodium picrate solution and the yellow to orange colour reveals the presence of glycosides.

Keller-Killani test: Five ml of each extract was treated with 2 ml of glacial acetic acid containing one drop of ferric chloride solution. This was underlayed with 1 ml of concentrated sulphuric acid. A brown ring of the interface indicates a deoxysugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer, a greenish ring may form just gradually throughout thin layer [28-30].

2.4.3. Tests for Anthraquinones:

Borntrager's test: Add a few ml of dilute Sulphuric acid to1ml of the extract solution. Boil, filter and extract the filtrate with chloroform. The chloroform layer was treated with 1ml of ammonia. The formation of red colour of the ammonical layer shows the presence of anthraquinones glycosides.

2.4.4. Test for carbohydrates and sugars

Molisch's test: To 2 ml of the extract, add 1ml of α-napthol solution, add concentrated sulphuric acid through the side of the test tube. Purple or reddish violet colour at the junction of the two liquids reveals the presence of Carbohydrates.

Fehling's test: To 1 ml of the extract, add equal quantities of Fehling solution A and B, upon heating formation of a brick red precipitate indicates the presence of sugars.

Benedict's test: To 5 ml of Benedict's reagent, add 1ml of extract solution and boil for 2 minutes and cool. Formation of red precipitate shows the presence of sugars.

2.4.5. Test for flavonoids

Shinoda's test: The alcoholic extract of powder treated with magnesium foil and concentrated HCl give intense cherry red colour indicates the presence of flavonones or orange red colour indicates the presence of flavonols.

Alkaline reagent test: Test solution when treated with sodium hydroxide solution shows increase in the intensity of yellow Colour, which becomes colorless on addition of drops of dilute acid.

2.4.6. Test for steroids

Libermann-Burchard test: 1gm of the test substance was dissolved in a few drops of chloroform, 3ml of acetic anhydride, 3ml of glacial acetic acid were added, warmed and cooled under the tap and drops of concentrated sulphuric acid were added along the sides of the test tube. Appearance of bluishgreen colour shows the presence of sterols.

2.5. Salkowski test: Dissolve the extract in chloroform and add equal volume of conc. H2SO4. Formation of bluish red to cherry colour in chloroform layer and green fluorescence in the acid layer represents the steroidal components in the tested extract.

2.6. Mouse as a model for anxiety

Major advantages of using mouse as a model, is their remarkable similarity to human in genetics, anatomy and physiology. More than 95% of the mouse genome is similar to human beings, making mouse genetic research specifically appropriate to human disease. Anxiety related to the Central Nervous System and brain is accomplished using animals as experimental models. Animal models form the backbone of preclinical research on the neurobiology of psychiatric disorders, and are employed as screening tools in the search for novel therapeutic agents. Rodents especially mice have proven to be helpful in research as mice and humans share more than 90% of their genes in common. Furthermore, animal models are particularly helpful in situations when the impact of stress cannot be studied in humans because of ethical and other reasons Other advantages of selecting mice as a model are, cost-effectiveness, small dose in relation to body weight, easy to handle and an accelerated breeding time, these points will definitely make the research easily manageable for the researcher. In addition, animal models of anxiety are crucial for identifying novel therapies for anxiety[31-33]. The detail of experimental design is given in Table 1.

Table 1: Experimental design of mice treatment with synthesized extract for 14 days

Grouping of Animals		
Groups	**Treatment**	**No. of animal**
Group-I	Control group, normal saline 0.9% NaCl (10 ml/kg, or 1 ml/100 g body weight p.o.)	06
Group-II	PMELE 300 mg/kg (p.o)	06
Group-III	PMELE 600 mg/kg (p.o)	06
Group-IV	APZ 0.25mg/kg p.o.	06

PMELE- Piper methysticum Ethanolic Leaves Extract (Test drug) APZ- Alprazolam (Standard drug)

Total No. of animals required:
No. of the animal in each group (n) = 6
No. of groups (N) = 4
Total no. of animals required = 24

Note: All the parameters will perform with suitable time interval to prevent unwanted stress in animals.

2.7. Parameters for anxiety

The behavioural effects of an acute or sub-acute (14-day course) will be orally administered. "ethanolic extract of "Piper methysticum" leaves (300 AND 600 mg/kg) ethanol extract will be evaluated in either sex Swiss mice by Locomotors activity, Light Dark Model (LDM) and Elevated Plus Maze (EPM). The effects of Alprazolam (APZ; 0.25 mg/kg) will also assess.

Laboratory models for testing anti-anxiety activity are locomotors activity (actophotometer), Light – Dark Model (LDM) and Elevated Plus Maze (EPM)[34-36].

2.6.1. Locomotors activity

Most of the central nervous system acting drugs influence the locomotors activities in man and animals. The CNS depressant drugs such as barbiturates and alcohol reduce the motor activity. In other word, the locomotor activity can be an index of wakefulness (alertness) of mental activity. The locomotor activity can be easily measured using an actophotometer which operates on photoelectric cells which are connected in circuit with a counter. When the beam of light falling on the photocell in cut off by the animal, a count is recorded. An actophotometer could have either circular or square across in which the animal moves. Both mice and rats may be used for testing in this equipment (Fig. 2).

Figure 2 : Actophotometer

2.6.2. Light-dark model: A simple behaviour model in mice to detect compounds with anxiolytic effects. Mice tend to explore a novel environment, but to retreat from the aversive properties of a brightly-lit open field (Fig. 3). In a two chambered system, where the animals can freely move between a brightly-lit open field and a dark corner, they show more crossings between the two chambers and more loco motor activity after treatment with anxiolytic. The numbers of crossings between the light and dark sites are recorded.

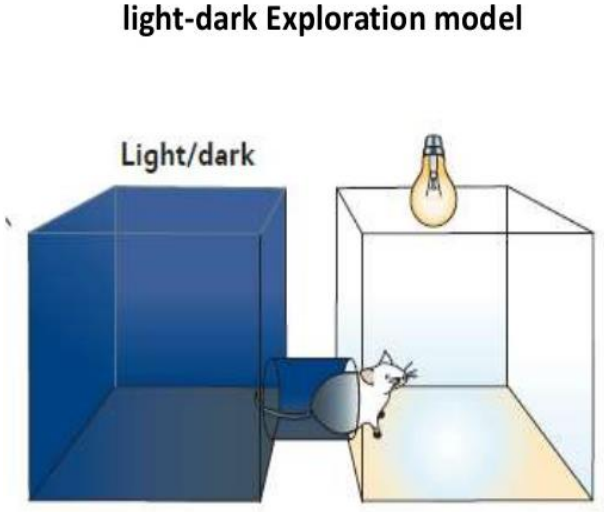
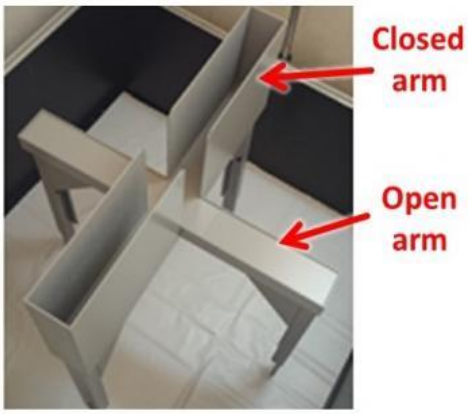


Figure 3: - Light Dark model

2.6.3. Elevated plus maze : This test comprises two opposing open arms (50 × 10 cm) and two opposing closed arms (50 × 10 × 40 cm), connected by a common central platform (10 × 10 cm). The entire apparatus is elevated 50 cm above floor level. To prevent falls, both open arms will be fitted with a 1 cm Plexiglas edge. At the beginning of the test, the animal will placed on the central platform facing an enclosed arm and will allowed to explore the maze freely for 5 min. The following parameters will be scored: Number of open and enclosed arm entries and time spent on the central platform and in the open and enclosed arms (Fig. 4).



Endpoint	Anxiety Level	
	High	Low
Time spent in the open arms (%)	↓	↑
Distance traveled in the open arms (cm)		

Figure 4: - Elevated plus maze

2.8. Biochemical estimation:

2.8.1. Collection of Blood Samples

On 15th day, blood (0.3 ml) was withdrawn from tail vein from all groups of mice. Blood samples were centrifuged at 2500 rpm for 10 min using refrigerated centrifuge (Paramount scientific works, Ambala cantt , India) to separate the plasma, which was used for estimation of corticosterone levels.

2.8.2. Estimation of plasma corticosterone levels

The quantitative estimation of corticosterone levels in the blood plasma was performed by the method of Bartos and Pesez, 1979. To 1.0 ml of sample in ethanol, 0.50 ml of 0.10 % solution of p-nitroso-N,N-dimethylaniline in ethanol was added and the tubes were immersed in ice water for 5 min, and then 0.50 ml of 0.10 N sodium hydroxide was added. The tubes were plugged with cotton-wool, and were let to stand at 0°C for 5 h, protected against light. To the above solution, 2.0 ml of buffer for pH 9.8, 5.0 ml of 0.10 % solution of phenol in ethanol and 0.50 ml of 1.0 % aqueous solution of potassium ferricyanide were added. The tubes were kept in a water bath at 20±2°C for 10 min. The solution was read at 650 nm using UV-visible spectrophotometer (UV 3200 UV-VIS Spectrophotometer, Somajiguda, Hyderabad).

During the same time of study, following analysis was also be recorded on weekly basis.

1. Body weight analysis
2. Feed intake
3. Water intake

2.9. Statistical Analysis

All the results were expressed as Mean ± SEM. The data of all the groups were analyzed by one way ANOVA followed by Turkey's test using software Graph pad prism In Stat (Graph Pad Software Inc., USA). A value of $p<0.05$ was considered to be significant.

3. RESULTS

3.1. Effect of Piper methysticum extract on body weight (g) of mice

The mice treated group with "Ethanolic extract of "*Piper methysticum*" leaves (300 &600 mg/kg/p.o) showed significantly ($p<0.05$) increased in body weight as compared to the control group. Treatment with APZ (0.25 mg/kg p.o.) the body weight significantly increased as compared to normal group (Fig. 5).

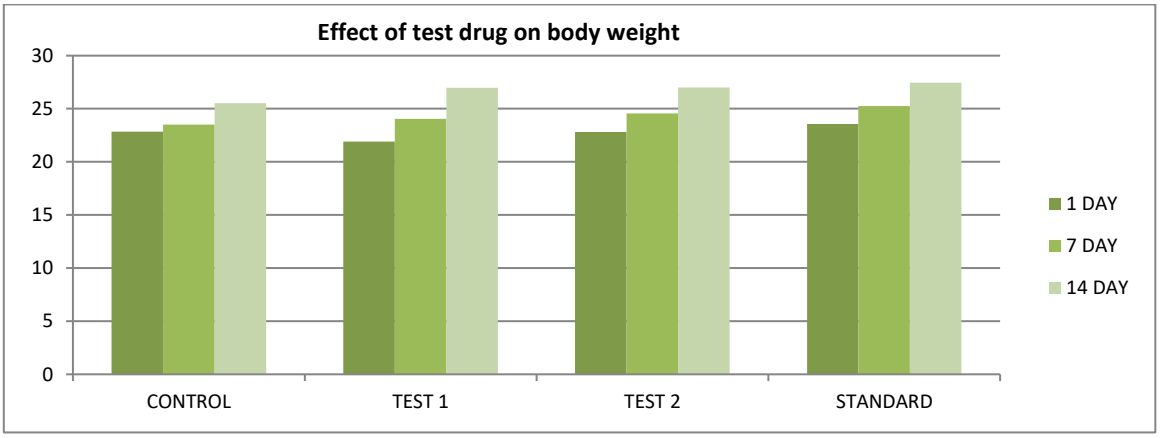


Figure 5: Effect of Piper methysticum extract on body weight (g) of mice.

Values are expressed as mean ± S.E.M. a denotesp<0.05 as compared with to normal control group and b denotes p<0.05 as compared to APZ (0.25 mg/kg p.o.) treated group (One way ANOVA followed by Tukey's test).

3.2. Effect of *Piper methysticum* extract on Feed intake (g) of mice

The mice treated group of *Piper methysticum* extract (300 &600 mg/kg/p.o) showed significantly ($p<0.05$) increased in feed intake as compared to the control group. Treatment with APZ (0.25 mg/kg i.p)the feed intake significantly ($p<0.05$) increased as compared to control group group.

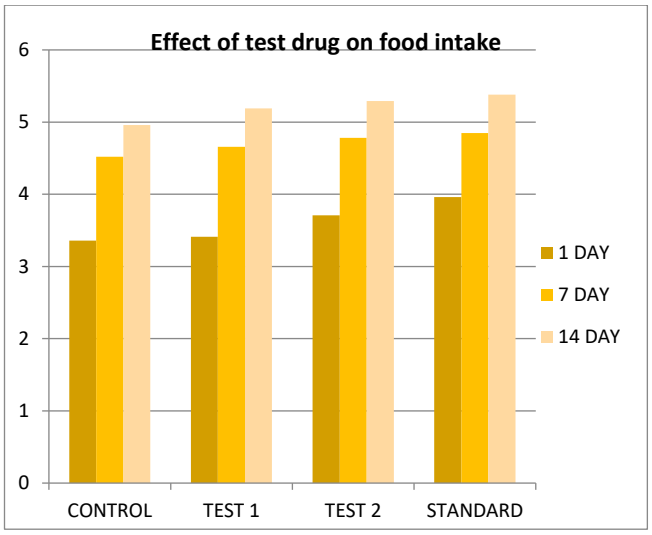


Figure 6: Effect of Piper methysticum extract on Feed intake (g) of mice

Values are expressed as mean ± S.E.M. a denotesp<0.05 as compared with to normal control group and b denotes p<0.05 as compared to APZ (0.25 mg/kg p.o.) treated group (One way ANOVA followed by Tukey's test).

3.3. Effect of Piper methysticum extract on Water intake (ml) of mice

The mice of Piper methysticumextract (300 &600 mg/kg/ p.o.) treated group showed significantly ($p<0.05$) increased in body water intake as compared to the control group. Treatment with APZ (0.25 mg/kg p.o.) the water intake significantly ($p<0.05$) increased as compared to another group (Fig. 7).

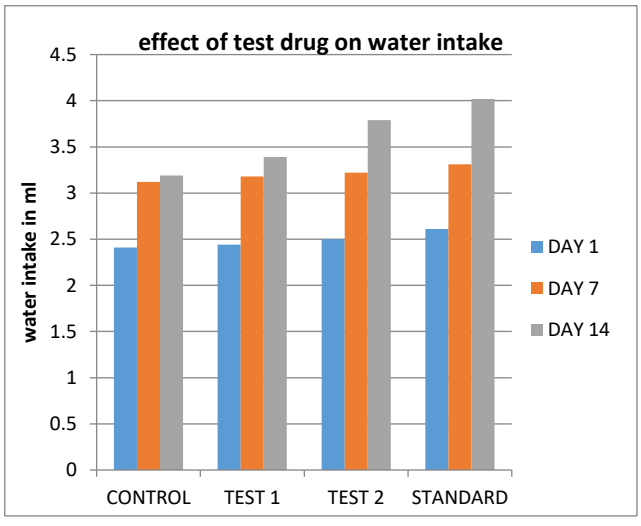


Figure 7: Effect of Piper methysticum extract on Water intake (ml) of mice

Values are expressed as mean ± S.E.M. a denotesp<0.05 as compared with to normal control group and b denotes p<0.05 as compared to APZ (0.25 mg/kg p.o.) treated group (One way ANOVA followed by Tukey's test).

3.4. Effect of Piper methysticum extract on locomotors activity of mice

The mice treated group of Piper methysticumextract (300 & 600 mg/kg/p.o) showed significantly ($p<0.05$) difference in locomotors activity as compared to control group. Treatment with APZ (0.25 mg/kg p.o.) also showed more significant change as compared to control group (Fig. 8).

When compared with the control group. All values represent = Mean ± SEM, n = 6 in each group.

*P<0.05, **P<0.01, ***P<0.001

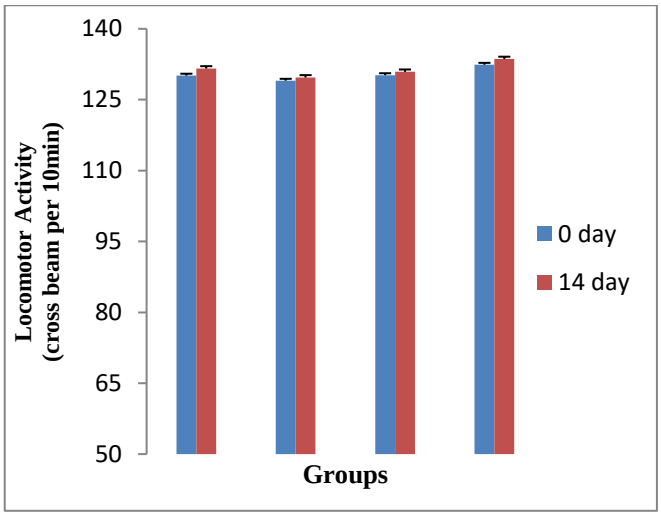


Figure 8: Effect of Piper methysticum extract on locomotors activity of mice

3.5. Effect of Piper methysticum extract on Elevated Plus-maze Test activity of mice

When compared with the control group. All values represent = Mean ± SEM, n = 6 in each group.

*P<0.05, **P<0.01, ***P<0.001

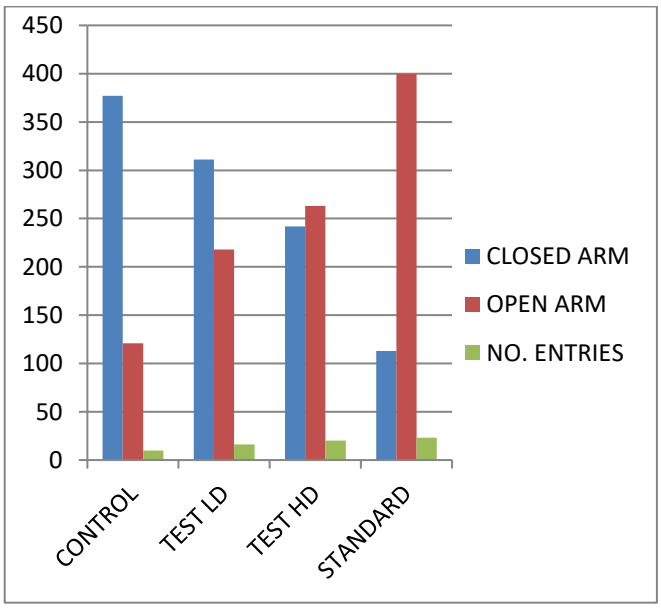


Figure 9: Effect of Piper methysticum extract on Elevated plus Maze of mice.

The vehicle-treated mice (Normal control) had spent more time in closed arm and showed less entries in open arm compared to closed arm of the maze during 10 min.

Group 1: In this group animals were treated with normal saline solution in which mice spent 122.8±2.34 sec in open arm and 378.6±1.53 sec in closed arm & no. of crossings were 10±2.02.

Group 2: In this group animals were treated with low dose of Ethanolic extract (300mg/kg) solution in which mice spent 218.1±1.99 sec in open arm and 312.2±1.22 sec in closed arm & no. of crossings were 161.59±.

Group 3: In this group animals were treated with high dose of Ethanolic extract (600mg/kg) solution in which mice spent 264.6±2.31 sec in open arm and 242.2±0.56 sec in closed arm & no. of crossings were 20±0.22.

Group 4: In this group animals were treated with Alprazolam (0.25mg/kg) solution in which mice spent 402.6±0.22 sec in open arm and 114.6±0.21 sec in closed arm & no. of crossings were 23±0.22.

Animals treated with Alprazolam (Standard) showed significant ($p<0.001$) increase in the percentage of open arms entries as well as time spent in open arm. All the doses of Piper methysticum(300 and 600mg/kg) Showed a significant increase in the time spent and number of entries into open arms ($P < 0.05$) in dose dependent manner.

3.6. Effect of Piper methysticum extract on Light/Dark Model Test activity of mice

When compared with the control group. All values represent = Mean ± SEM, n = 6 in each group.

*P<0.05, **P<0.01, ***P<0.001

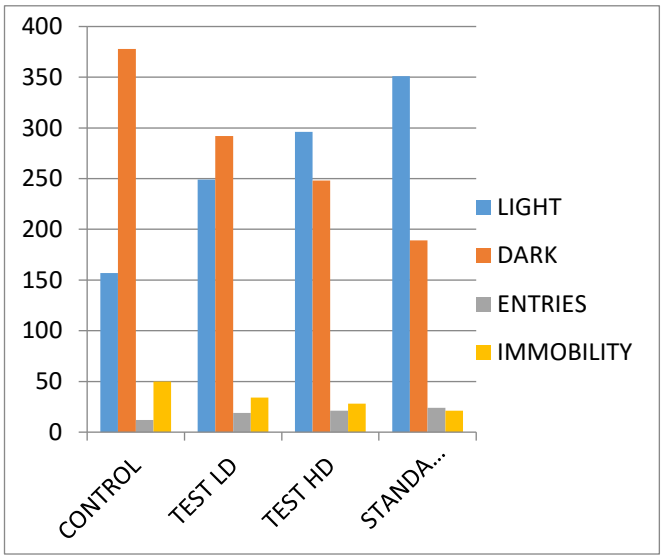


Figure 10: Effect of Piper methysticum extract on Light / Dark Model in mice

The low dose of Piper methysticumextract shows less effect as compared to ethanolic extract of high dose.

Group 1: In this group animals were treated with Normal saline solution in which mice spent 158.6±1.02 sec in light chamber and 378.8±0.24 sec in dark chamber & no. of crossings were 12±0.12. Immobility period was 50.6±1.65 sec.

Group 2: In this group animals were treated with low dose of Ethanolic extract (300mg/kg) solution in which mice spent 249.4±2.56 sec in light chamber and 292.2±0.55 sec in dark chamber & no. of crossings were 19±1.94. Immobility period was 34.3±0.30 sec.

Group 3: In this group animals were treated with high dose of Ethanolic extract (600mg/kg) solution in which mice spent 296.8±1.06 sec in light chamber and 248.2±2.01 sec in dark chamber & no. of crossings were 21±1.02. Immobility period was 28.3±0.12 sec.

Group 4: In this group animals were treated with Alprazolam (0.25mg/kg) solution in which mice spent 352.6±0.26 sec in light chamber and 189.5±0.25 sec in dark chamber & no. of crossings were 24±2.02. Immobility period was 21.3±0.18 sec.

Treatment with Alprazolam significantly increased the time spent ($P < 0.001$) in light box as well as the number of crossings between the light and dark boxes, but duration of immobility was significantly reduced. Ethanolic extract of Piper methysticumtreated mice also exhibited dose dependent significant increase in the time spent in light box and the number of crossings between light and dark boxes. The duration of immobility was also significantly reduced as compared to the vehicle treated group (Fig. 10).

But there is no significant difference between Piper methysticumextract treated animals & Alprazolam treated animal. The above observation suggests that Piper methysticumhas anxiolytic activity.

3.7. Plasma corticosterone levels:

Groups 1 to 4 were tail bled on day 14th and then corticosterone levels were combined to obtain the average levels in tail blood. For treatment of Groups see their respective experimental design.

*P < 0.05, # P < 0.01, ##P < 0.001

When compared with the control group. All values represent = Mean ± SEM, n= 6 in each group

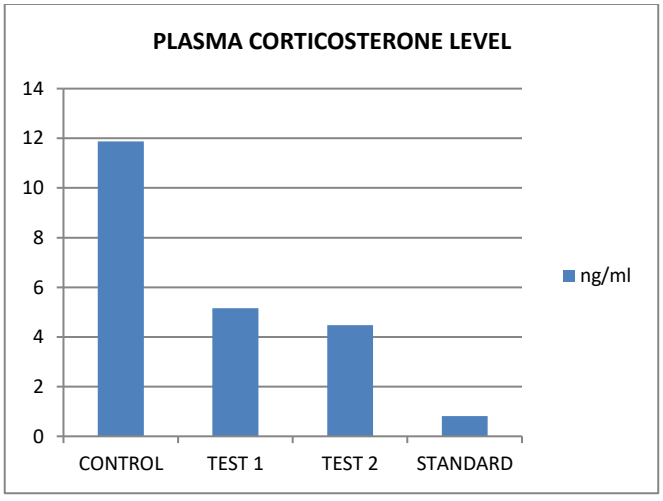


Figure 11: Corticosterone levels in Post Test Experiments in Mice

Group 1: In this mice were treated with Normal saline and corticosterone level is measured which is 11. 88±0.54 ng/ml.

Group 2: In this mice were treated with low dose of Ethanolic extract and corticosterone level is measured which is 5.16±1.28 ng/ml.

Group 3: In this mice were treated with High dose of Ethanolic extract and corticosterone level is measured which is 4.48±0.32 ng/ml.

Group 4: In this mice were treated with Standard (Alprazolam) and corticosterone level is measured which is 0.82±0.36 ng/ml (Fig. 11).

It is known that stress enhances the activity of the hypothalamus-pituitary-adrenal (HPA) axis and results in increased secretion of corticosteroids from the adrenal cortex. Cortisol and corticosterone are thus often used as biomarkers for stress and depressive disorders. Although corticosterone is considered the main glucocorticoid involved in regulation of stress responses in rodents, researchers often choose to detect cortisol for stress indicators in consideration of convenience and kits availability.

4. DISCUSSION

Anxiety is a normal response to stress, but anxiety disorders involve excessive, unrealistic worry and discomfort. Studies show high global prevalence, with 6% of men and 13% of women in the U.S. experiencing symptoms in a six-month period, often alongside other psychiatric conditions like depression. The present study investigates the anxiolytic effect of *Piper methysticum* (kava) leaf extract in Swiss albino mice. The extract was administered orally at two doses (300 & 600 mg/kg) for 14 days. Results showed increased food and water intake and enhanced locomotion. In the elevated plus maze test, the extract reduced anxiety by increasing open-arm exploration, and similar effects were observed in the light-dark model. Additionally, plasma corticosterone levels, elevated due to anxiety, decreased with extract treatment, with the ethanolic extract being more effective than the aqueous one. The highest anxiolytic effect was observed with the standard drug Alprazolam. The study suggests that *Piper methysticum* extract may have potential as an anti-anxiety remedy, possibly due to its effects on GABA/benzodiazepine or 5-HT receptors.

CONCLUSION

The ethanolic extract of Piper methysticum can increase the duration of action. It can also increase the time spent in open arm, entry in open arm (in elevated plus model) as well as increase the time spent in light field (in Light Dark field) thus we can conclude that it can also possesses Anxiolytic action. The ethanolic extract of Piper methysticum possesses an anxiolytic like activity without sedative side effect. However, further neurochemical studies will be necessary to clarify its mechanism of action and to characterize the active principle.

However, while recent research in OCD has revealed substantial endophenotypic differences between OCD and anxiety disorders, depression, schizophrenia, and addictions (Fineberg et al., 2007; Chamberlain et al., 2005) the identification of reliable endophenotypes in GAD, social anxiety, PTSD and panic disorder remains a goal for future research. In doing so, improvements in our understanding of anxiety may mirror those observed in the psychiatric disorders for which endophenotypes have been already been proposed. This may in turn aid diagnosis, classification, treatment, clinical research and the development of refined preclinical models of anxiety, by reducing the complexity of symptoms and behaviors into units of analysis that are more readily modeled in animals.

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