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Research Article

A Comparison Between Ethanolic and Aqueous Extracts of *Amorphophallus paeoniifolius* Tuber in Amelioration of Experimentally Induced Anxiety in Swiss Albino Mice

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Abstract

Objective: Anxiety is an unpleasant emotional state. When it becomes excessive, it needs medical intervention. The intent of this study was to assess and to compare the potential of aqueous and ethanolic extracts obtained from the tuber of Amorphophallus *paeoniifolius* to alleviate anxiety in Swiss albino mice.

Method: A. *paeoniifolius* tuber was procured, shed dried and ground to powder. Then it was extracted using water and ethanol by cold maceration and soxhlation technique respectively. The extracts were underwent phytochemical screening. The aqueous and ethanolic extract was subsequently examined for its anxiety-reducing effects at various doses (100mg/kg, 150mg/kg and 250mg/kg) employing different animal anxiety models such as the open field test, elevated plus maze test, light and dark box test.

Result: The extracts were found to contain alkaloid, flavonoid, reducing sugar, carbohydrate, tannin, steroid and triterpenoids when they underwent phytochemical screening. Both the extract shows potent anxiolytic activity in all three animal models of anxiety when compared to control in Swiss albino mice. However, ethanolic extract shows more significant anxiolytic activity than aqueous extract. 250mg/kg ethanolic extract showed highest anxiety amelioration when compared to all other doses.

Conclusion: Amorphophallus *paeoniifolius* aqueous and ethanolic extract is effective in ameliorating anxiety in Swiss albino mice.

Keywords: Amorphophallus *paeoniifolius*; Anxiolytic activity; Animal behavioural tests; Medicinal plants

1. INTRODUCTION

Anxiety is a distressing psychological state associated with worries, tension, unease, and a feeling of unease about future health risks. Some anxiety is acceptable in everyday life, but when it becomes excessive, disproportionate to the situation, and disabling, treatment is required[1]. Numerous subtypes of anxiety, including social anxiety, panic disorder, general anxiety disorder, agoraphobia, posttraumatic stress disorder, and obsessive-compulsive disorder (OCD), exists in the present day and age[2,3]. The dysfunction of gamma-aminobutyric acid (GABA) has long been associated with anxiety[4-6] and several researches indicates that both in animals and humans, positive modulators of GABA receptors exhibit anxiolytic properties, whereas negative modulators demonstrate anxiogenic properties[7,8].

In underdeveloped nations, such as India, traditional medicine remains the predominant approach for disease treatment among the majority of people. Despite the significant breakthroughs of contemporary scientific medicine, the number of individuals using alternative or complementary medicine in one form or another is quickly rising globally, especially among those who have access to western care. Throughout history, plants have served as the primary source of medicinal substances. It is noteworthy that over 50% of medicinal agents are obtained from sources found in nature[9]. Despite notable progress in modern medicine, alternative medicine continues to be the primary approach to treating various chronic conditions in developing countries. The recent upswing in the acceptance of traditional medicine offers India a remarkable chance to delve into new therapeutic compounds inherent in its own traditional medical framework, Ayurveda.

The Elephant foot yam, scientifically known as Amorphophallus *paeoniifolius*, is a highly promising tuber crop cultivated in tropical regions and belonging to the Araceae family. This plant is distinguished by its subterranean, hemispherical, dark brown corm[10] and is widely cultivated and commonly consumed in countries located in Southeast Asia, such as India, Indonesia, Malaysia, and the Philippines. In India, it is commonly known as suran or jimmikand and is cultivated in Kerala, Tamil Nadu, Uttar Pradesh, Maharashtra, Gujrat, Andhra Pradesh, and West Bengal. The tubers are consumed as food and also commonly used in many ayurvedic formulations. The corms have traditionally been employed for their stomach-soothing, carminative, digestive, pain-relieving, aphrodisiac, anti-inflammatory and anthelmintic properties. They have been effective in the treatment of elephantiasis,

haemorrhoids, haemorrhages, cough, asthma, anorexia, bronchitis, vomiting, dysmenorrhoea, amenorrhoea, fatigue, anaemia, lethargy, arthralgia, tumours[11].

The pharmacological activities of different extracts derived from the tuber of *Amorphophallus paeoniifolius* have been demonstrated including antimicrobial activity[12,13], anthelmintic activity[14], analgesic activity[15,16], anti-inflammatory activity[17] and hepatoprotective activity[18].The pet ether extract of the tuber shows significant CNS depressant activities[19]. Researchers in 2022 found similar CNS depressant activities in aqueous and ethanolic extracts of the plant[20]. According to some studies done in 2013 the petroleum ether extract of *A. paeoniifolius* has anti-anxiety activity in mice[21]. Here we show that the phytochemical constituent of pet ether extract is totally different from aqueous and ethanolic extracts and several studies supported our findings[22, 23].The pet ether extract fetch only non-polar constituents of the tuber but here we compare the polar fractions i.e. the ethanolic and aqueous extracts of *A. paeoniifolius* in amelioration of experimentally induced anxiety in Swiss albino mice.Many researchers also showed that A. *paeoniifolius* extracts have flavonoid quercetin as a phytocontituent[18]. Quercetin is a well-known natural compound which has anti-anxiety activity[24-26]. That is why we delve into the experiment where we will study whether A. *paeoniifolius* extracts have anti-anxiety activity. This study explores the potential therapeutic effects of the extracts on anxiety-related behaviours in animal models. Through controlled experiments and observations, we aim to evaluate its potential as a novel treatment for anxiety disorders. Such investigations contribute valuable insights into the pharmacological profile of *Amorphophallus paeoniifolius*.

2. MATERIALS & METHODS

2.1. Collection of the plant part

The fresh corm of the A. *paeoniifolius* plant was procured from the nearby market in Asansol, West Bengal, India, and subsequently verified at the Botanical Survey of India, Botanical Garden, Shibpur, Howrah, West Bengal, India, with the assignment of a unique specimen number BSI/PLANT CHEM/00020-2022/.

2.2. Preparation of extracts

The tuber was cut into thin slices and shade dried. The dried pieces were powdered with the help of a mixer grinder. The powdered tuber was subjected to soxhlation with ethanol to prepare ethanolic extract of A. *paeoniifolius*. Extracting the tuber also involved a cold maceration process using distilled water to yield the aqueous extract. Both the extracts were subjected to phytochemical screening using various phytochemical tests[27].

2.3. Animals

For the study, we utilized male Swiss Albino mice with a body weight ranging from 18 to 25 grams. The mice were kept in colony cages and subjected to controlled environmental conditions, which included a temperature maintained at 25 ± 2°C, a 12-hour each light and dark phase, and a specific relative humidity level ranging from 45% to 55%. Throughout the duration of the experiment, they had unfettered access to water and food. Prior to the experiment, the mice were fasted overnight. The protocol for the animal study received approval from the Institutional Animal Ethical Committee of Gupta College of Technological Sciences, and it was assigned a specific protocol number GCTS/IAEC/2022/SEPT/01.

2.4. Drugs and chemicals:

As the standard anxiolytic agent, Diazepam (Ranbaxy Laboratories Ltd.) was used for the study. Ethanol and Tween 80 was obtained from Merck. 5% Tween 80 was used to dissolve ethanolic extract in distilled water before oral administration.

2.5. Evaluation of anxiolytic activity:

Evaluation of anxiolytic effect was conducted through the utilization of the elevated plus maze (EPM) test, light and dark box, open field test. The experiments were conducted under controlled and quiet conditions. Great care was taken to minimize any potential discomfort experienced by the animals. Throughout the experiments, strict adherence to ethical guidelines was observed. The LD50 (lethal dose for 50% of the population) of the ethanolic extract was determined to be 2000mg/kg[28] and the reported LD50 for the aqueous extract was estimated to be greater than 2500 mg/kg[29]. For the experiments, doses equivalent to approximately 1/10th and 1/20th or less of the LD50 were administered.

2.6. Experimental design

The subjects were segregated into eight groups, each consisting of six animals

Group A: 5% Tween 80 (per oral route)

Group B: Diazepam (1 mg/kgper oral route)

Group C: A. *paeoniifolius* aqueous extract (100 mg/kg, in water, per oral route)

Group D: A. *paeoniifolius* aqueous extract (150 mg/kg, in water, per oral route)

Group E: A. *paeoniifolius* aqueous extract (250 mg/kg, in water, per oral route)

Group F: A. *paeoniifolius* ethanolic extract (100 mg/kg, in 5% Tween 80, per oral route)

Group G: A. *paeoniifolius* ethanolic extract (150 mg/kg, in 5% Tween 80, per oral route)

Group H: A. *paeoniifolius* ethanolic extract (250 mg/kg, in 5% Tween 80, per oral route)

2.7. Experimental approaches for assessing anxiolytic effects

2.7.1. Elevated plus maze (EPM) test

Swiss albino mice were administered different doses of A. *paeoniifolius* (100,150, 250 mg/kg both aqueous and ethanolic extracts; orally), diazepam (1 mg/kg; orally) or a control substance according to their respective groups. This administration took place 30 minutes before the mice placed one by one at the center of the maze, orienting their heads in the direction of the open arm. Their activities in each of the open and enclosed arms were observed and noted for duration of 10 minutes. The test also involved measuring the frequency of entries made and the average time spent in both the open and closed arms. An entry was defined as ensuring that the animal's four paws are present within the arms[30].

2.7.2. Open field test

Animals were administered different doses of A. *paeoniifolius* (100, 150, 250 mg/kg both aqueous and ethanolic extracts; orally), diazepam (1 mg/kg; orally) or a vehicle according to their respective groups. Thirty minutes following drug administration, each animal was individually positioned in one of the corner squares of the open field apparatus and then observed for duration of five minutes[31]. Time spent in middle boxes, frequency of rearings and the count of squares traversed by individual animal after drug treatment were assessed.

2.7.3. Light and dark box test

Animals were administered different doses of A. *paeoniifolius* (100, 150, 250 mg/kg both aqueous and ethanolic extracts; orally), diazepam (1 mg/kg; orally) or a control substance according to their respective groups. Thirty minutes later, the mice were individually positioned in the center of the light box, and then the lid was closed. They were observed for five minutes[32].The number of transitions and latency to move in the dark box from the light box was measured. We also evaluate the cumulative duration spent in the light box.

2.8. Statistical analysis

The results from behavioural experiments were expressed as Mean±SD, and statistical analysis was conducted utilizing Student t test. Values of probability less than 0.05 (P<0.05) for with each group consisting n=6 mice were considered to be statistically significant.

3. RESULTS

3.1. Phytochemical screening

The phytochemical screening of A. *paeoniifolius* aqueous extract revealed the existence of flavonoids, alkaloids, reducing sugar, carbohydrates, and tannins. In contrast, the A. *paeoniifolius* ethanolic extract exhibited the presence of flavonoids, alkaloids, steroids, triterpenoids, reducing sugar, carbohydrates, and tannins during the screening process.

3.2. Elevated plus maze (EPM) test

Amorphophallus *paeoniifolius* demonstrated a notable rise in the frequency of passages into the open arm. The ethanolic extract showed a rise in the frequency of passage into the open arm, which was dependent on the dosage.At 150mg/kg and 250mg/kg frequency of entries were notably higher (p<0.05, each group consisting n=6 mice) compared to the control animals. However standard drug diazepam (1mg/kg) demonstrated higher anxiolytic potency (p<0.001, n=6) as revealed in Figure 1. The aqueous extract did not exhibit any significant activity in three test doses (100mg/kg, 150mg/kg and 250mg/kg) when compared to control.

A. *paeoniifolius* aqueous and ethanolic extracts showed significant Anti-anxiety effects as measured by the average time mice spent in the open arm. At a dosage of 100mg/kg, the aqueous extract did not demonstrate significant activity in comparison to the control group.However, the aqueous extract at 150mg/kg displayed a significant (p<0.05, n=6) enhancement in the average time spent in the open arm. This significance further increased in dose dependent manner for 250mg/kg (p<0.001 n=6). Ethanolic extract in all the test doses (100mg/kg, 150mg/kg and 250mg/kg) indicated a significant rise in the average duration mice spent in the open arm. At a dosage of 100mg/kg, there was a noteworthy increase (p<0.05, n=6) in the average time spent in the open arm compared to the control group. While at higher doses the significance further increased. At highest dose (250mg/kg) the response by the animals was significantly greater than control (p<0.001 n=6) and equivalent to the standard drug diazepam (1mg/kg) (p<0.001 n=6) as shown in Figure 2.

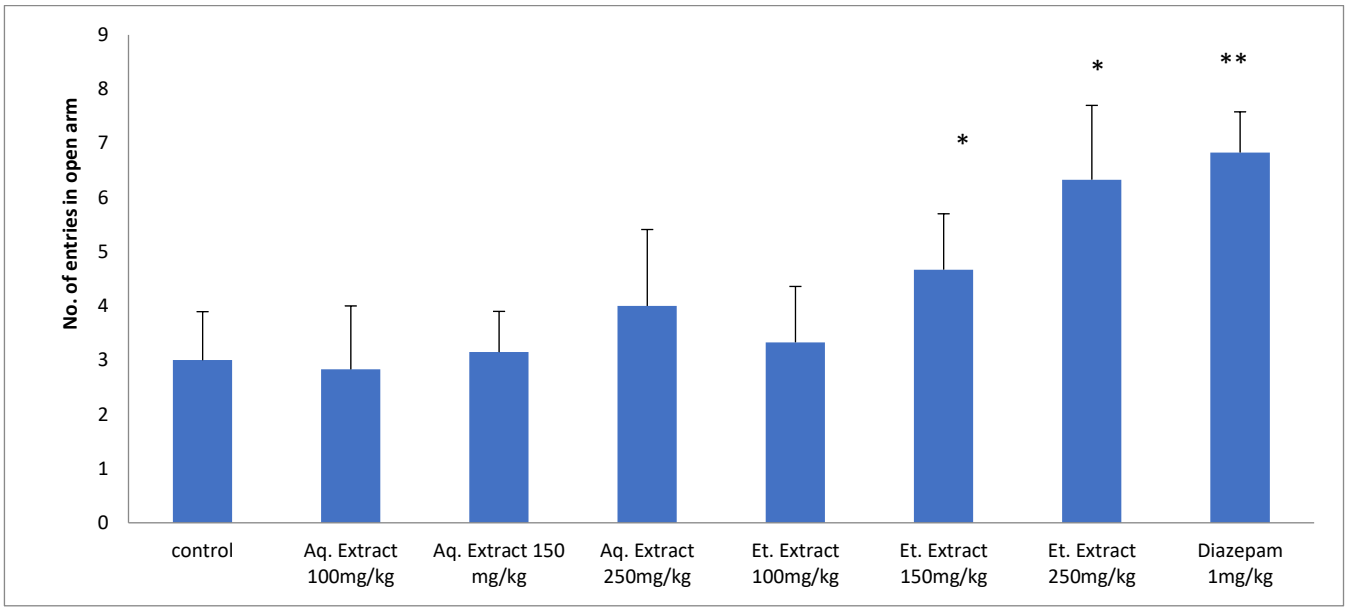

Figure 1: Impact of A. *paeoniifolius* on frequency of passages to the open arm of the elevated plus maze. The ethanolic extract (150mg/kg p<0.05 where n=6 and 250mg/kg p<0.05 where n=6) demonstrated a significant increase in the frequency of passages into the open arm when compared to the control. Standard drug diazepam (1mg/kg) also exhibited a comparable effect (p<0.001 where n=6). The aqueous extract and lower doses of ethanolic extract showed little effect. The values are presented as Mean±SD. *p<0.05, **p<0.001, aqueous extract (Aq. Extract), ethanolic extract (Et. Extract)

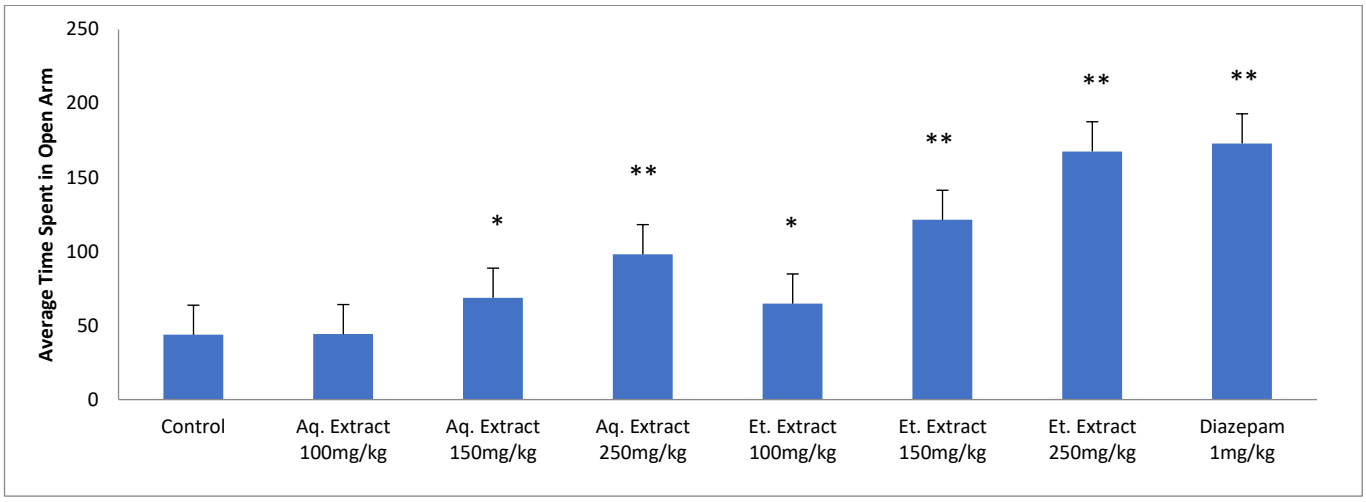

Figure 2: Impact of A. *paeoniifolius* on the mean duration spent in the open arm of the elevated plus maze. The aqueous extract (150mg/kg p<0.05 where n=6, 250mg/kg p<0.001 where n=6) and ethanolic extract (100mg/kg p<0.05 where n=6 150mg/kg p<0.001 where n=6, 250mg/kg p<0.001 where n=6) exhibited significant increase in average time passed in open arm. Standard drug diazepam (1mg/kg) demonstrated a comparable impact (p<0.001 where n=6). Lower dosages of the aqueous extract exhibited minimal effect. These values presented as the Mean±SD.*p<0.05, **p<0.001, aqueous extract (Aq. Extract), ethanolic extract (Et. Extract)

3.3. Open field test

In this test, the extracts demonstrate a significant rise in the number of squares crossed when compared to control. At 100mg/kg the aqueous extract did not significantly increase. The count of squares traversed in an open field apparatus. However, aqueous extract 150mg/kg and 250mg/kg showed significant increase (p<0.001, n=6) in the count of squares traversed in comparison to the control group. The count of square crossed for ethanolic extract at 100mg/kg and 150mg/kg showed a significant increase (p<0.05, n=6) in comparison to the control group. At 250 mg/kg the ethanolic extract shows potent anxiolytic activity which is significantly higher (p<0.001, n=6) than control and comparable to standard drug diazepam (1mg/kg) which also showed significant increase (p<0.001, n=6) in number of square crossed as shown in Figure3.

Another parameter we had checked in open field test was number of rearing which was increased in dose dependent manner with extract and diazepam treatment. Aqueous extract (100mg/kg, 150mg/kg, 250mg/kg) and ethanolic extract (100mg/kg, 150mg/kg) both exhibited a substantial elevation (p<0.05, n=6) in the number of rearing instances when compared to the control group. This number of rearing further increased for ethanolic extract 250mg/kg (p<0.001, n=6) which is comparable with standard drug diazepam (1mg/kg) (p<0.001, n=6) as shown in Figure 4.

Anxiolytics prolong the duration spent in middle boxes by the mice. The aqueous extract (250mg/kg) and ethanolic extract (150mg/kg) of A. *paeoniifolius* significantly increase (p<0.05, n=6) the duration spent in middle compartments in comparison to the control treatment. The response further significantly increased (p<0.001, n=6) with ethanolic extract 250mg/kg. Standard drug diazepam also demonstrated strong anxiolytic effects at the specified dosage of 1mg/kg (p<0.001, n=6) as shown in Figure5.

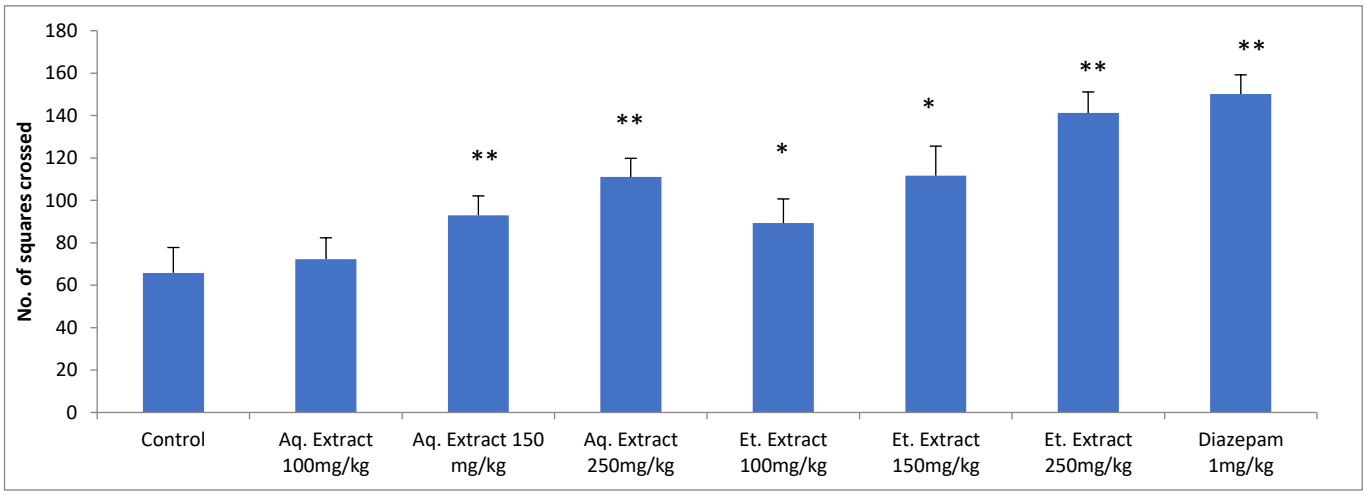

Figure 3: The impact of A. *paeoniifolius* on the count of squares crossed in the open field test. The aqueous extract (150mg/kg p<0.001 where n=6; 250mg/kg p<0.001 where n=6) exhibited a significant increase in the number of squares crossed in comparison to the control group. Similarly, the ethanolic extract (100mg/kg p<0.05 where n=6; 150mg/kg p<0.05 where n=6; 250mg/kg p<0.001 where n=6) also demonstrated a significant increase in the count of squares crossed in the open field test. Diazepam (1mg/kg p<0.001 where n=6) exhibited a similar effect compared to the vehicle treated control group. The values are presented as Mean±SD. *p<0.05, **p<0.001, aqueous extract (Aq. Extract), ethanolic extract (Et. Extract)

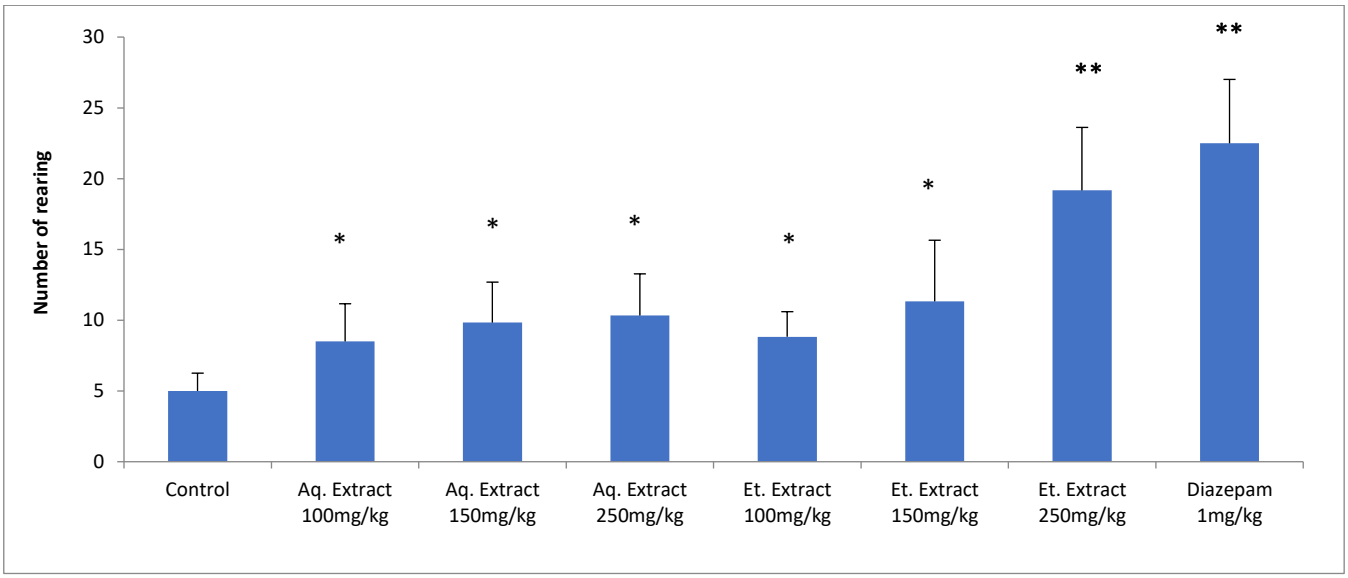

Figure 4: The impact of A. *paeoniifolius* on count of rearing in open field test. The aqueous extract (100mg/kg, 150mg/kg, 250mg/kg) and ethanolic extract (100mg/kg, 150mg/kg) showed substantial increase ($p<0.05$ where n=6) in number of rearing in comparison to control. The ethanolic extract (250mg/kg $p<0.001$ where n=6) and diazepam (1mg/kg $p<0.001$ where n=6) showed similar effect. The values are presented as Mean±SD. *$p<0.05$, **$p<0.001$, aqueous extract (Aq. Extract), ethanolic extract (Et. Extract)

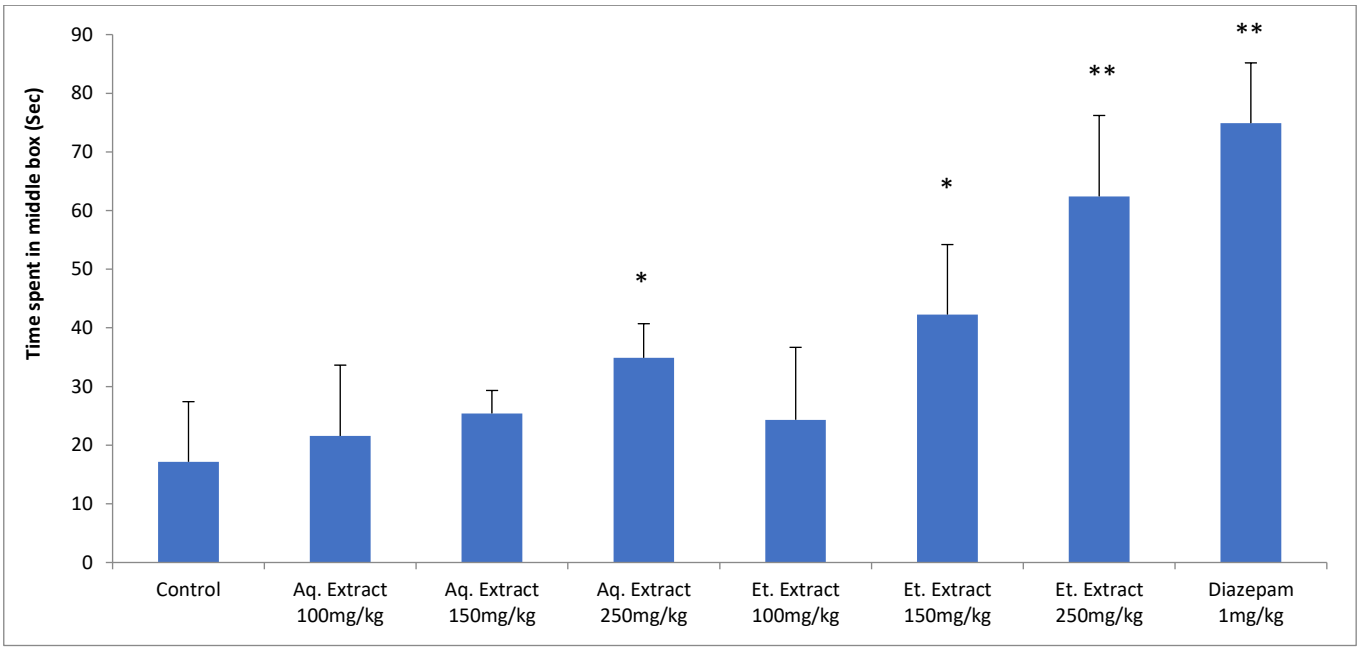

Figure 5: The impact of A. *paeoniifolius* on duration spent in middle box in open field test. The aqueous extract (250mg/kg $p<0.05$ where n=6) and ethanolic extract (150mg/kg $p<0.05$ where n=6 and 250mg/kg $p<0.001$ where n=6) showed increase in duration spent in middle box in comparison to the control. Diazepam (1mg/kg $p<0.001$ where n=6) showed similar effect. Lower doses of extracts shows little effect. The values are presented as Mean±SD. *$p<0.05$, **$p<0.001$, aqueous extract (Aq. Extract), ethanolic extract (Et. Extract)

3.4. Light and dark box test

A. *paeoniifolius* ethanolic extract 150mg/kg, 250mg/kg and diazepam 1mg/kg showed notable ($p<0.05$, n=6) increase in latency to move to dark box in comparison to control in light and dark box test. However all three test doses of aqueous extract and 100mg/kg ethanolic extract failed to show significant results when compared to control as shown in Figure6.

The standard drug diazepam shows significant ($p<0.001$ where n=6) rise in duration spent in light box while among all the test samples only 250mg/kg ethanolic extract shows significant ($p<0.001$ where n=6) rise in duration spent in light box in comparison to control as shown in Figure7.

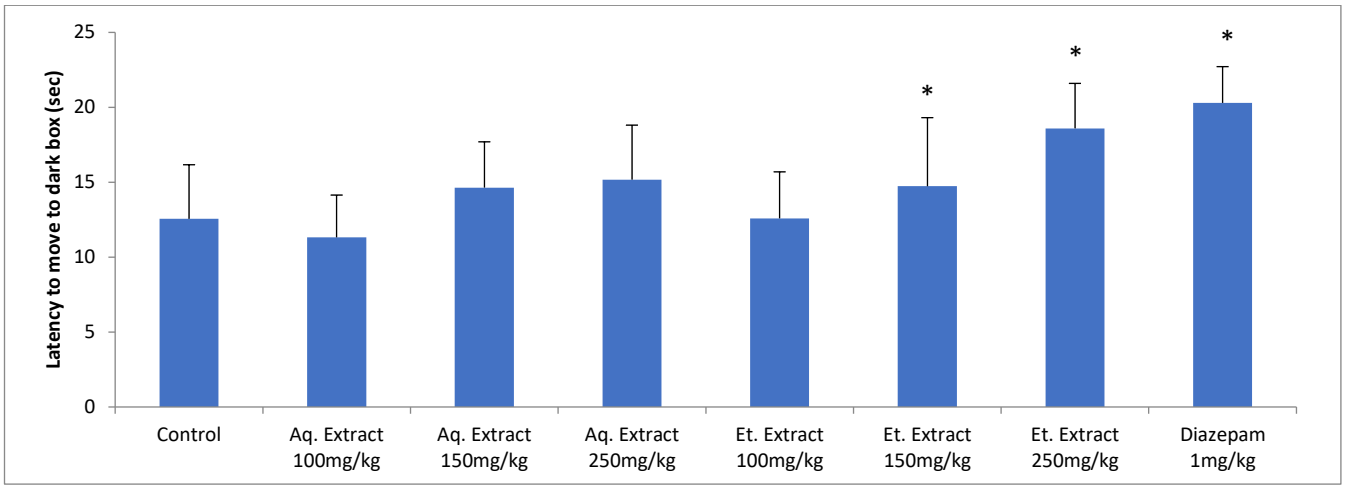

Figure 6: Effect of A. *paeoniifolius* on latency to move to dark box in light and dark box test. Ethanolic extract of the tuber showed significant increase (150mg/kg $p<0.05$ where n=6 and 250mg/kg $p<0.05$ where n=6) in latency to move to dark box in comparison to control. Diazepam (1/mg/kg) showed similar effect($p<0.05$ where n=6). Aqueous extract and lower doses of ethanolic extract showed very little effect. The values presented as Mean±SD. *$p<0.05$, **$p<0.001$, aqueous extract (Aq. Extract), ethanolic extract (Et. Extract)

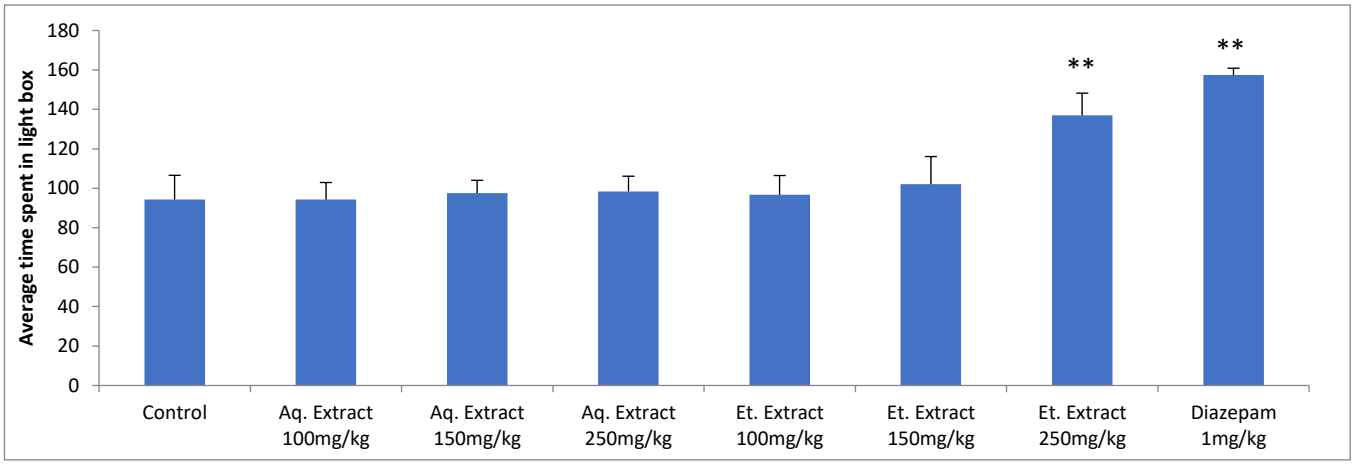

Figure 7. Effect of A. *paeoniifolius* on average duration spent in light box in light and dark box test. Only ethanolic extract (250mg/kg $p<0.001$ where n=6) and diazepam (1mg/kg $p<0.001$ where n=6) showed significant increase in time spent in light box. All other doses of aqueous extract and ethanolic extract showed little effect when compared to control. The values presented as Mean±SD. *$p<0.05$, **$p<0.001$, aqueous extract (Aq. Extract), ethanolic extract (Et. Extract)

4. DISCUSSION

The current research illustrates that both the extracts of A. *paeoniifolius* (aqueous and ethanolic) exhibit anxiolytic properties, as evidenced by behavioural assessments such as the Elevated Plus Maze (EPM), open field test, light and dark box test. The phytochemical tests reveal that the aqueous extract has alkaloid, flavonoid, reducing sugar, carbohydrate and tannin while the ethanolic extract of A. *paeoniifolius* indicates the presence of flavonoids, alkaloids, steroids, triterpenoids, reducing sugars, carbohydrate, and tannin.

All rodents have natural aversion for open space which induce anxiety and increase plasma corticosterone level in them. This is the working principle behind the elevated plus maze model which has two open arms and two closed arms. Rodents make significantly fewer entries to open arms than in closed arms. Time spent in open arms is also less than closed arms. Anxiolytics like benzodiazepins significantly increase in percentage of time spent on the open arms and also increase the number of entries in the open arms[33].

So in EPM the most indicative measures of anxiolytic activity are regarded as the rise in the frequency of entries and the time used in the open arm. Here mice typically exhibit a preference for spending a significant portion of their given time in the enclosed arms. This inclination seems to indicate reluctance in the direction of the open arm due to a fear of exposed areas. Substances that enhance exploration of the open arm are regarded as having anxiolytic effects, whereas those that have the opposite effect are considered anxiogenic[34]. In this study, A. *paeoniifolius* aqueous (150mg/kg, 250mg/kg) and ethanolic (100mg/kg, 150mg/kg, 250mg/kg) extracts exhibited a notable rise in the time passed in open arms and the frequency of entrances in the open arm in comparison to the vehicle treated group i.e. control. Therefore, both the extracts show significant anxiety amelioration in mice. One of the most commonly applied animal models to assess anxiety in rodents is open field test. The time fraction spent by the rodent in the perimeter (thigmotaxis) versus the center is measured here. Increased thigmotaxis is interpreted as increased anxiety in rodents[35]. Thus, the open field test model assesses anxiety-related behaviour, which is characterized by the typical avoidance of

the animal towards an open area that is brightly illuminated[36]. Here also the aqueous and ethanolic extract shows significant anxiolytic property by increasing the number of square crossed, time spent in middle boxes and number of rearing when compared to control i.e. vehicle treated group. The light and dark box test is built upon the inherent aversion of mice to well-lit areas. Here mice are placed in an arena with two chambers, one is dark and another one is brightly illuminated. Elevated avoidance to well lit chamber interpreted as increased anxiety[37]. Anxiolytic substances diminish this innate light aversion and prolong the time spent in the brightly illuminated compartment. Here also we can see that ethanolic extract of A. *paeoniifolius* showed significant anxiolytic property by increasing latency to move to dark box and total time spent in light box by the mice when compared to control. Thus both the extracts show amelioration of anxiety in mice.

Benzodiazepines possess significant importance among GABA modulating drugs due to their effects that reduce anxiety, prevent seizures, relax muscles, and induce sleep and calmness. *A. paeoniifolius* exhibits a synergistic effect with diazepam[38] suggesting that its mechanism for the anxiolytic effects could be comparable to those of benzodiazepines involving the modulation of the inhibitory neurotransmitter GABA. Moreover, possible presence of flavonoid quercetin[18, 24] may be behind the anxiety amelioration as quercetin also have anxiolytic-like activity and act through GABA receptor interaction pathway[25].

5. CONCLUSION

The outcomes from this study indicate that the aqueous extract of *A. paeoniifolius* contains alkaloids, flavonoids, reducing sugars, carbohydrates, and tannins, while the ethanolic extract also includes alkaloids, flavonoids, steroids, triterpenoids, reducing sugars, carbohydrates, and tannins. Both aqueous and ethanolic extracts exhibit significant anxiolytic effects compared to the vehicle treated group, with the ethanolic extract demonstrating even more significant and potent activity than the aqueous extract when tested with EPM and open field test. However in light and dark box test aqueous extracts in most of the doses failed to show significant activity but ethanolic extracts in higher doses show significant anxiety amelioration. It is crucial to emphasize that this study utilized raw extracts. Further investigation is necessary to pinpoint the exact phytoconstituents accountable for these pharmacological effects. Subsequent research endeavours should focus on unravelling the molecular mechanisms that underlie the anxiolytic activity of A. *paeoniifolius*.

6. ACKNOWLEDGEMENT

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Conflict of Interest

There are none to declare for all the authors.

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