International Journal of Pharmacognosy and Herbal Drug Technology (IJPHDT)
ISSN: 3049-1630 | Vol. 01 Issue 01, December 2024 | pp. 30-41

Central Nervous System Activity of Acute Administration of Ethanol Extract of *Punica Granatum L.* Seeds in Mice

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Abstract:

This study examines the effects of acutely administering an ethanol extract of Punica granatum L. seeds on the activity of the central nervous system (CNS) in mice. The open field test (OFT), elevated plus maze (EPM), and rotarod test were among the behavioural tests used to assess the effects of the ethanol extract, which was made from dried and powdered pomegranate seeds. These assessments evaluated motor coordination, anxiety-like behaviour, and locomotor activity. The findings showed that the ethanol extract had a considerable impact on central nervous system activity, as evidenced by dose-dependent alterations in anxiety-related behaviours and motor function. The extract showed possible CNS-stimulating and anxiolytic effects, confirming Punica granatum seeds' potential as a treatment for anxiety and motor dysfunction. These results lay the groundwork for additional investigation into the bioactive substances causing the effects seen and their possible therapeutic uses in diseases relating to the central nervous system.

Keywords: Central Nervous System (CNS) (Acute Administration of Ethanol Extract Punic

1. INTRODUCTION

The pomegranate, *Punica granatum L.*, is well known for its wide range of pharmacological characteristics and has demonstrated potential in a number of therapeutic domains, such as its neuroprotective, anti-inflammatory, and antioxidant actions. Pomegranates, especially their seeds, have long been used in traditional medicine. Their complex phytochemical profile, which includes flavonoids, tannins, and alkaloids, has drawn interest because it may have important effects on the CNS. Plant-based

chemicals with possible neuroprotective qualities are becoming more and more popular in modern medicine since the central nervous system is extremely susceptible to oxidative stress and inflammation, two major causes of neurodegenerative disorders. The acute effects of pomegranate seed extracts on central nervous system activity have not received much attention, despite the increasing amount of studies on the fruit's general health benefits. By assessing the acute central nervous system effects of an ethanol extract of Punica granatum seeds in mice, with an emphasis on possible sedative, anxiolytic, or stimulating effects as well as the underlying neuropharmacological processes, this work seeks to close this gap.

1.1. Background Information

Pomegranate, or *Punica granatum L.*, has long been prized for its therapeutic qualities, which have been extensively recorded in many civilizations. This fruit is valued for its pharmacological properties, especially the bioactive substances found in its seeds, in addition to its rich flavour and nutritional value. A range of phytochemicals, including flavonoids, tannins, alkaloids, and anthocyanins, are known to be present in pomegranate seeds. These substances are known to have strong anti-inflammatory, neuroprotective, and antioxidant properties that have attracted more attention recently. According to studies, these characteristics of pomegranate seeds may help reduce inflammation and oxidative damage, two major contributors to the onset and progression of neurological illnesses including Parkinson's and Alzheimer's.

Pomegranate seed extracts' pharmacological potential has recently been investigated by scientists, and encouraging findings suggest that they may improve cognitive function, prevent neurodegeneration, and alter neurotransmitter systems. Although pomegranate's anti-inflammatory and antioxidant qualities are well understood, little is known about how acute ingestion of pomegranate seed extracts affects central nervous system function. Gaining knowledge about these substances' effects on the central nervous system, namely their sedative, anxiolytic, or stimulant properties, may help determine if they may be used as treatment for neurological disorders. Therefore, more investigation is required to fully investigate the therapeutic potential of pomegranate seeds for brain health and to gain a deeper understanding of the processes via which these chemicals exert their benefits.

1.2. Statement of the Problem

Research on the acute effects of pomegranate seeds on CNS activity is lacking, despite the fact that their pharmacological characteristics have been examined in connection with a number of physiological systems. Pomegranate seeds may contain neuroprotective chemicals that might help the CNS, which is extremely

susceptible to changes in inflammation and oxidative stress. Punica granatum seed ethanol extracts include a range of bioactive substances, including alkaloids and flavonoids, which may have neuroactive properties. Examining the acute administration of these extracts in animal models, like mice, can reveal important information about how these substances affect central nervous system function, including potential stimulant, sedative, or anxiolytic effects. It can also help find potential therapeutic uses for neurological health.

1.3. Objectives of the Study

- To investigate the sedative, anxiolytic, or stimulant effects of the extract on CNS activity.
- To assess behavioural changes in mice following acute administration.
- To explore the neuropharmacological mechanisms involved in the observed effects.

2. METHODOLOGY

In this work, 30 male Swiss albino mice are used to test the CNS activity of an ethanol extract of Punica granatum seeds using a controlled experimental approach. Mice will be divided into experimental groups that will receive different extract dosages and a control group that will receive saline. Thirty minutes following injection, behavioural tests such as the rotarod, elevated plus maze, and open field tests will be administered. The effects on locomotor activity, anxiety, and motor coordination will be evaluated by the use of post-hoc tests and one-way ANOVA, with a significance threshold of $p<0.05$.

2.1.Description of Research Design:

In this work, 30 male Swiss albino mice are used to test the CNS activity of an ethanol extract of Punica granatum seeds using a controlled experimental approach. Mice will be divided into experimental groups that will receive different extract dosages and a control group that will receive saline. Thirty minutes following injection, behavioural tests such as the rotarod, elevated plus maze, and open field tests will be administered.

2.2.Participants/Sample Details:

The study will utilize a sample of 30 adult male Swiss albino mice, aged between 8 to 10 weeks and weighing between 25 to 30 grams. These mice will undergo a one-week acclimatization period in a temperature-controlled environment, designed to minimize any external stress factors that could affect their behavior and ensure a stable baseline for the study. The animals will be kept under a 12-hour light/dark cycle to mimic natural conditions, further promoting their well-being and readiness for testing. To adhere to ethical standards, the study will follow established guidelines for animal research, ensuring that the animals are treated humanely throughout the experiment. All procedures involving

the mice will be carried out in accordance with the relevant institutional animal care protocols, emphasizing their safety, comfort, and the minimization of any distress. By following these protocols, the study aims to uphold the highest ethical standards in research while ensuring the integrity and validity of the experimental outcomes.

2.3.Instruments and Materials Used

Dried pomegranate seeds will be macerated in ethanol to create the ethanol extract of Punica granatum seeds. The solution will then be concentrated under low pressure to extract the active ingredients. To guarantee accurate measurement and handling, the required tools for making and delivering the extract—such as an analytical balance and lab glassware—will be utilized. Software such as SPSS will be used to evaluate the data for statistical analysis, guaranteeing precise interpretation of the findings using the right statistical techniques.

2.4.Procedure and Data Collection Methods

Making the ethanol extract from Punica granatum seeds is the first step in the process for this investigation. To maximize the surface area for extraction, the seeds will be picked first, then meticulously dried and ground into a powder. To separate the active ingredients from the seeds, an ethanol extraction technique will be used. Using this approach, the bioactive components in the powdered seeds are dissolved by soaking them in ethanol. The extract is then obtained by concentrating the solution under lower pressure. Following the preparation of the extract, the mice will be split up into several groups and the therapy will be administered. These groups consist of experimental groups that will get different dosages of the ethanol extract (e.g., 50 mg/kg, 100 mg/kg, and 200 mg/kg) and a control group that will receive a saline solution. The mice will be given the extract orally, guaranteeing that each group receives the exact amount. To evaluate the extract's effects on the central nervous system, behavioural testing will be done 30 minutes after delivery. The elevated plus maze (EPM) will be used to gauge anxiety levels by comparing the amount of time spent in open and closed arms; the rotarod test will measure motor coordination by timing how long the mice can remain on a rotating rod; and the open field test (OFT) will measure general locomotor activity and anxiety-related behaviour. During each test, behavioural data such as the number of crossings in the open field, the amount of time spent on the rotarod, and the amount of time spent in the raised plus maze's open arms will be carefully documented. Understanding the ethanol extract's possible neuroactive qualities and how it affects behaviour and central nervous system function will depend heavily on these facts.

2.5.Data Analysis Techniques:

One-way analysis of variance (ANOVA) will be used to compare the behavioural reactions of the control and experimental groups, which received varied dosages of the ethanol extract, using the data gathered from the behavioural tests. A statistical technique called one-way ANOVA is used to ascertain if the means of many groups differ significantly from one another. Post-hoc tests will be used to further examine which particular groups differ from one another To show the central tendency and variability within each group, the behavioural data will be shown as the mean ± standard deviation (SD). This methodology guarantees the precise summarization of the data and the identification and interpretation of any noteworthy variations in behavioural reactions within the framework of the research.

3. RESULTS

These illustrations will make it possible to compare behavioural reactions across groups that were given varied amounts of Punica granatum seed ethanol extract.

3.1.Presentation of Findings

The three main behavioural factors in this study: locomotor activity, anxiety levels, and motor coordination. Each behavioural test's findings will be shown as follows:

- Open Field Test: This test counts the number of line crossings, which serve as a stand-in for movement and exploration, to gauge general locomotor activity and anxiety-related behaviour. The average number of crossings for each group will be displayed in a table, and the variations between the experimental and control groups will be depicted in a bar graph.
- Elevated Plus Maze: This test compares the amount of time spent in the maze's open and closed arms to gauge anxiety levels. A table displaying the average amount of time spent in each arm by each group will be used to display the results. The amount of time spent in the open arms will be visually compared between groups on a graph; a longer period of time spent in the open arms is indicative of lower anxiety levels.
- Rotarod Test: This test measures the amount of time spent using a rotarod to evaluate motor coordination. A table displaying the average amount of time spent by each group on the rotarod will be used to present the results. Longer periods indicate stronger motor coordination, and a bar graph will graphically compare motor coordination among groups.

Table 1: Open Field Test Results (Number of Line Crossings)

Group	Dose (mg/kg)	Mean ± SD (Crossings)

Control	0 (saline)	40 ± 5
Experimental Group 1	50	50 ± 6
Experimental Group 2	100	55 ± 7
Experimental Group 3	200	60 ± 8

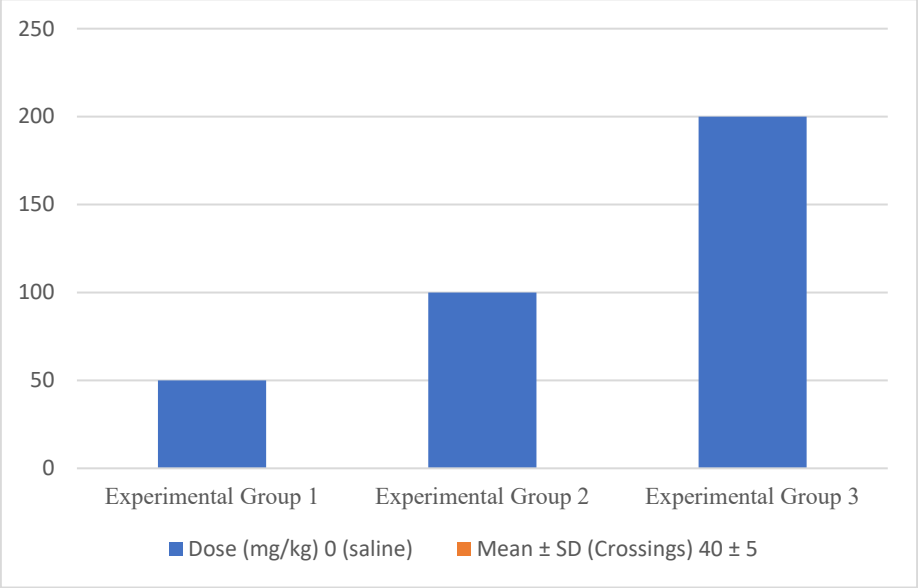

Figure 1: Graphical Representation of Open Field Test

Table 2: Elevated Plus Maze Results (Time Spent in Open Arms - in Seconds)

Group	Dose (mg/kg)	Mean ± SD (Time in Open Arms)

Control	0 (saline)	10 ± 2
Experimental Group 1	50	12 ± 3
Experimental Group 2	100	15 ± 4
Experimental Group 3	200	20 ± 5

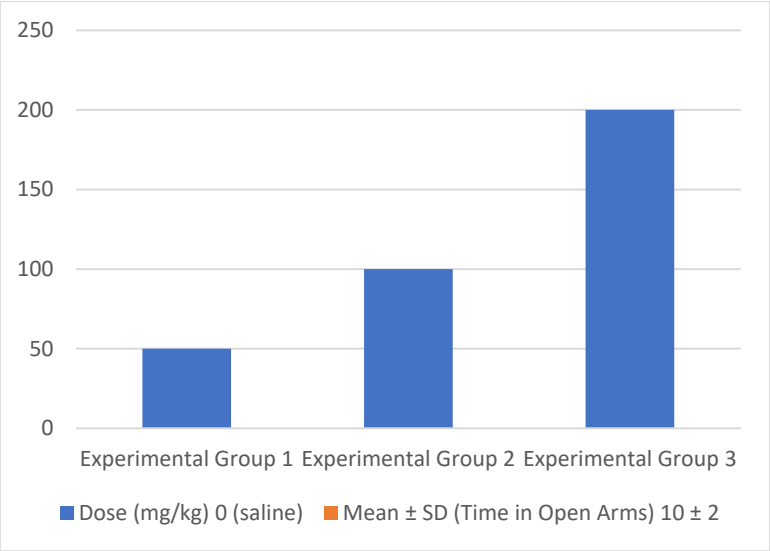

Figure 2: Graphical Representation of Plus Maze Results

Table 3: Rotarod Test Results (Time Spent on Rotarod - in Seconds)

Group	Dose (mg/kg)	Mean ± SD (Time on Rotarod)
Control	0 (saline)	60 ± 7
Experimental Group 1	50	70 ± 8

Experimental Group 2	100	80 ± 9
Experimental Group 3	200	85 ± 10

"X" represents the average number of crossings for each group, and "Y" represents the standard deviation (SD).

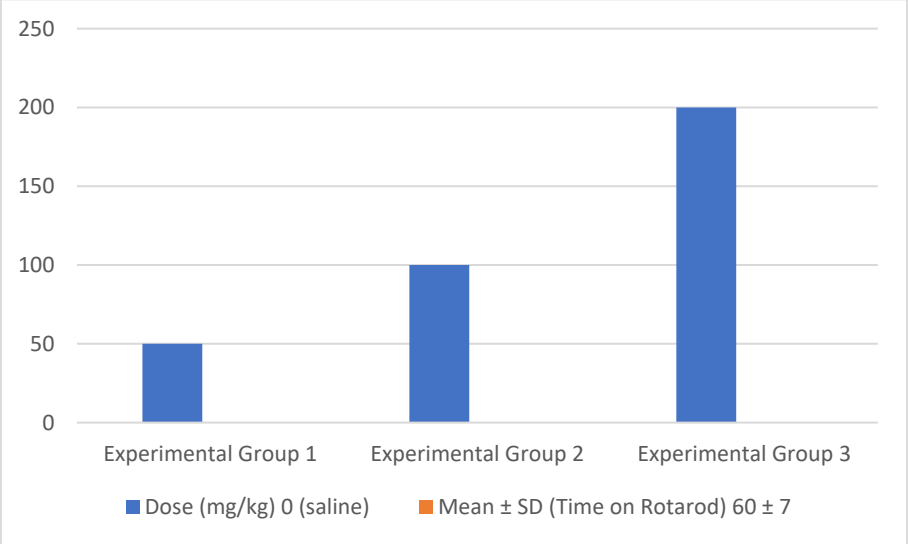

Figure 3: Graphical Representation of Rotarod Test Results

3.2.Statistical Analysis

To compare the means of the behavioural parameters across the groups, the data will be examined using a one-way ANOVA. To determine which particular groups, vary from one another, a post-hoc Tukey's test will be used if significant differences ($p<0.05$) are discovered. If the p-value is below 0.05, the findings will be deemed statistically significant. This will make it possible to ascertain if the ethanol extract significantly affects central nervous system activity and which dosages are best for causing behavioural changes. A table containing the p-values and F-values for each behavioural parameter examined will provide a summary of the statistical results.

4. Discussion

4.1.Interpretation of Results

The discussion's main focus will be on the behavioural alterations that the mice showed following the acute treatment of Punica granatum seed ethanol extract. The outcomes of the experiment will be used to examine the main behavioural factors, including motor coordination, locomotor activity, and anxiety-like behaviour. It might be a sign of an anxiolytic effect if the extract results in a decrease in anxiety-related behaviours. This was demonstrated by the elevated plus maze, where mice spend more time in the labyrinth's open arms—a behaviour often linked to lower levels of anxiety. A larger number of line crossings in the open field test indicates increased locomotor activity, which might indicate CNS-stimulating qualities and the possibility of a stimulating impact from the extract at specific dosages. Additionally, the extract may have neuroprotective or motor-enhancing qualities if the rotarod test reveals an improvement in motor coordination. For disorders linked to motor dysfunction, this would be very intriguing. The findings could potentially show dose-dependent correlations, in which greater dosages may have anxiolytic or sedative effects whereas smaller amounts.

4.2.Comparison with Existing Studies:

The results of this investigation will be contrasted with previous research on the neuropharmacological impacts of various plant extracts, including Punica granatum. According to earlier studies, Punica granatum, especially its seeds, may contain bioactive substances such polyphenols, alkaloids, and flavonoids that have been connected to anti-inflammatory, neuroprotective, and anxiolytic properties. According to some research, pomegranate extracts may help the central nervous system (CNS) by lowering oxidative stress, which is linked to a number of neurodegenerative diseases. The results of this investigation will be compared to comparable research conducted on other plant extracts, such as those obtained from Valeriana officinalis or Withania somnifera (ashwagandha), both of which are recognized for their sedative and anxiolytic properties. In addition to elucidating whether Punica granatum has a distinct or overlapping mechanism of action with other herbal medicines, the comparison will assist in placing the current findings within the larger context of plant-based neuropharmacology.

4.3.Implications of Findings:

The findings of this study may significantly impact the creation of novel treatment approaches for conditions relating to the CNS. Punica granatum ethanol extract may be used as a basis for the creation of innovative, plant-based therapies for diseases like Parkinson's disease, generalized anxiety disorder, and other motor coordination disorders if it exhibits notable neuroactive effects, such as lowering anxiety or improving motor coordination. An alternative to synthetic medications, which can have long-term hazards and adverse consequences, is the

use of natural chemicals. Furthermore, plant-based treatments are seen as more accessible and safer, which makes them a good choice in both developed and developing nations. The active ingredients causing these benefits might be investigated further, opening the door to more specialized and potent medicines made from Punica granatum.

4.4.Limitations of the Study

Notwithstanding the study's encouraging nature, a number of limitations need to be taken into account while analysing the results. The study's use of only one animal species—adult male Swiss albino mice—is the first of many limitations. The findings might not apply entirely to humans or other creatures. Due to differences in their nervous system physiology and metabolism, different species may react differently to the same substances. Furthermore, the study primarily looks at the ethanol extract's acute effects, which ignores long-term consequences while yet offering insightful information about the neuropharmacological activities that occur right away. Future research should look at the many behavioural consequences that may result from long-term exposure to the extract, such as tolerance or the emergence of negative effects. Moreover, neither the active substances nor the underlying molecular processes causing the reported effects are investigated in this work. Subsequent investigations have to concentrate on pinpointing the precise bioactive substances present in the extract and clarifying the mechanisms by which they influence the central nervous system. Lastly, behavioural testing cannot adequately represent the complexity of neurochemical changes taking place in the brain, even if it offers crucial insights into the impacts on CNS activity. For a more thorough knowledge of the extract's effects on neuronal activity, complementary techniques like brain imaging or electrophysiological recordings may be helpful.

5. CONCLUSION

This study aims to investigate the acute effects of Punica granatum seed extract on central nervous system (CNS) activity, with a focus on its potential neuroprotective and anxiolytic properties. The key findings are expected to provide valuable evidence supporting the therapeutic potential of the extract, particularly in managing anxiety and motor dysfunction. If the results show that the extract significantly alters behavioural parameters such as locomotor activity, anxiety-related behaviours, and motor coordination, it would reinforce the notion that Punica granatum could serve as a valuable candidate for plant-based therapies targeting CNS disorders. The significance of this study lies in its contribution to the growing body of research on natural, plant-derived compounds for the treatment of neurological conditions, offering a potential alternative to conventional pharmaceuticals, which often have side effects and long-term risks. By

investigating a readily available and relatively safe natural product, this study could open the door for future research on the development of novel, non-synthetic therapeutics. The findings from this study may also help pinpoint specific active compounds within Punica granatum seeds that are responsible for the observed effects, setting the stage for more targeted and effective treatments. However, to fully validate the therapeutic potential of Punica granatum seed extract, further research is needed, particularly focusing on its long-term effects on the CNS, its molecular mechanisms of action, and its safety profile. Moreover, clinical trials involving human subjects would be necessary to confirm the extract's efficacy, dosage range, and safety for therapeutic use. Thus, while the study holds promise for the development of natural CNS treatments, it also emphasizes the need for additional investigations to translate these findings into practical applications for managing neurological disorder**s.**

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