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ORIGINAL ARTICLE

The anticonvulsant and antioxidant effect of Rosa moschata (J) fruit extract in pentylenetetrazole induced epileptic mouse model

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ABSTRACT

Background: *Rosa moschata (J)* is widely used as a traditional medicine in different ailments including central nervous system diseases, hepatic and gastrointestinal disease. The current study focused on the anticonvulsant effects and memory improvement property of *Rosa moschata (J)* fruit extract by the modulation of oxidative stress markers and GABAA receptor in Pentylenetetrazole (PTZ) induced mice epileptic model. **Methods:** The epileptic mice model was developed using Pentylenetetrazole (35 mg/kg). The extract was used at doses of 50 mg/kg, 100 mg/kg and 150 mg/kg. The seizure was evaluated according to the Racine Scale. Cognitive functions were evaluated using the Y Maze and Morris Water Maze behavioral tests. The antioxidant effect of *Rosa moschata (J)* extract was measured by assessing the level of lipid per oxidation (LPO), superoxide dismutase (SOD), glutathione (GSH), and catalase (CAT). Additionally, the effect of RM (J) extract on the expression of GABAA receptors was evaluated using the qPCR. **Results:** *Rosa moschata (J)* fruit extract exhibited a significant ($P<0.5$) dose-dependent improvement in memory compared to the mice treated with PTZ. Furthermore, the *Rosa moschata (J)* extract significantly increased the levels of GSH and CAT and reduced LPO level compared to the PTZ group. The onset time of seizure was prolong while duration of seizure was significantly short in *Rosa moschata (J)* extract treated group compared to PTZ group. Interestingly, there was a significant increase in the expression of GABAA receptors in the groups treated with the *Rosa moschata (J)* extract, compared to the PTZ group. **Conclusions:** *Rosa moschata (J)* extracts showed anticonvulsant effect and memory improvement potential due to the reduction of oxidative stress markers and enhanced in the expression of GABAA receptors.

Keywords: Epilepsy, GABAA, Oxidative Stress Markers, Maze Test, Morris Water Model test, Pentylenetetrazole, Rosa moschata (J)

Introduction

Epilepsy is one of the most common and most disabling chronic neurologic disorders (50).

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Epilepsy has a heterogeneous cause but literature shows that abnormality in anatomical structures, functional connections, disrupted neurotransmission and neuroinflammatory processes are considered the main cause of epilepsy (51). Deciphering the pathophysiology of epilepsy has advanced the understanding of the cellular and molecular events initiated by pathogenetic insults that transform normal circuits into epileptic circuits

(epileptogenesis) and the mechanisms that generate seizures (ictogenesis) (50).

More than that brain needs elevated metabolic demand compared to other organs of the body. So, neurons consume high quantities of oxygen, generating significant amounts of reactive oxygen species (ROS) as a by-product (52). Reactive oxygen species have been implicated in the development of seizures under pathological conditions and linked to seizure (53). Neuroinflammation is closely related to oxidative stress processes that contributing to reduce epileptic threshold (54, 55).

Currently available anti-seizure medications (ASMs) are almost exclusively symptomatic drugs, thus they are able to prevent seizure's recurrence, but they do not act against the cause of epilepsy and they do not stop disease progression. Considering that the pharmacological treatment options for epilepsy are limited by the heterogeneity of the epilepsy. More, Some antiepileptic drugs induce oxidative stress and cognitive impairment which may limit their clinical applications (56). It has been found that pretreatment with ascorbic acid (57), and combination with Nox2 inhibitor or Nrf2 activator decrease epileptogenesis and severity of seizure using vitro model (58). We are interested in understanding how to optimize the existing therapy by using antioxidant combinations with ASMs. In vivo and in vitro models have significantly contributed to our current understanding of the mechanisms of drug response. Traditional medicine has a long history in the treatment of human being. Several natural compounds discovered from the medicinal plants,suchasalkaloids,terpenoids, flavonoids ,phenolicacids, lignans, tannins, quinones, co umarins, etc. exhibit significant antioxidant and other activities (59). Antioxidant rich photochemical and/or extracts are gaining importance as anti-stress therapy. From different studies, it has been evident that several medicinal plants and their extracts were used to protect neuronal damage. Polyphenols are considered among the most important antioxidants in human diet, and their presence in plant foods can protect consumers against oxidative stress, cardiovascular and chronic diseases (60-62). *Rosa moschata* belongs to the family rosaceae. More than 120 species of this genus are reported. Seven of them are reported to found in Malakand region of Pakistan (63). Gas chromatography-mass spectroscopy (GC-MS), revealing the presence of stearic acid, palmitic acid, oleic acid, margaric acid, linoleic acid, and linolenic acid in Rosa moschata. Additionally, R. moschata contains vitamins A, C, and E, flavonoids, and essential oils (64-66). More, *R. moschata* is a rich source of flavonoids. Flavonoids have been shown to reduce oxidative stress in different ailments (67). In traditional folk medicine, petals, fruit, and leaves of this genus are applied in the treatment of various diseases such as nephritis, common cold, flu, coughing, bronchitis, eczema, itching, and biliary diseases (68). *R. moschata* are rich in bioactive compounds responsible for the free radicals scavenging and cholinesterase inhibition and this plant may be a valuable candidate for the treatment of neuro-degenerative disorders, like Alzheimer's disease (69). The petals of Rosa species are used in the food industry and various traditional medicinal products (70). It has been found that Rosa species has high antioxidant property which support its use as beverages for various health benefits (71). It has found that methanolic extract of *R.moschata* has high flavonoids content and antioxidant activity (71).Approximately,129

chemical compounds have been isolated and identified from *R.moschata* which is important species of the rosaceae (72). The fruits extracts exhibit different pharmacological activities like antioxidant, antidiabetic, anti-hyperlipidaemic, antiinflammatory, antiarthritic, gastroprotective and anti-cancer (73). Taken together, data suggest that leaf extracts of different Rosa species should be used as a potential source of antioxidant phenolic, and R. sempervirens and R. moschata are a good alternative to R. canina especially in those regions where these species can be easily found (74).

We were interested in understanding that how seizure activity and oxidative stress drives in PTZ induced model can be treated with *R.moschata* methanolic extract. Finally, we also evaluated the effect of *R.moschata* on the GABAA receptor during seizure induced model.

Methods

The study was conducted at Institute of pharmaceutical sciences, Khyber Medical University from june 2022 to june 2023. This study was reviewed and approved by the Khyber Medical University ethics board, with the approval number: KMU/IBMS/IRBE/meeting/2022/8069 date 10-12-2022.

Chemicasl and reagents used in the experiment include Pentylenetetrazole (Sigma-Aldrich, St. Louis, MO, U.S.A), Sodium carbonate (Sigma-Aldrich, St. Louis, MO, U.S.A), Sodium bicarbonate (Sigma-Aldrich, St. Louis, MO, U.S.A MKCL4235), Sodium Phosphate dibasic dihydrate (Sigma-Aldrich, St. Louis, MO, U.S.A BCCC4108), Potassium phosphate dibasic (Sigma-Aldrich, St. Louis, MO, U.S.A SLCJ0423), Hydrogen peroxide solution (Sigma-Aldrich, St. Louis, MO, U.S.A 10F20013), Iron (111) chloride hexahydrate (SLCF2050), Ascorbic acid (BCCC1421), Thiobarbituric acid (Sigma-Aldrich, St. Louis, MO, U.S.A BCBK7728V) were obtained from (Sigma-Aldrich, St. Louis, MO, U.S.A). Potassium dihydrogen phosphate is anhydrous (BMP-132-X1 Sigma Aldrich). Trichloroacetic acid (2515, sigma Aldrich). Nitrobenzoic acid (00169-2019050901) was obtained from CHEM-IMPEX INT. Triazole reagent (18132201-USA), Isopropanol Alcohol (13216RD1), 2X TaqMan PCR Master Mix (Solarbio 20210610), cDNA Kit (Promega USA - 0000424526), Primer (Molecular biology product Lot No: 9081605).

The plant was collected from the medical flora of the Malakand division .It was authenticated by Professor Dr. Jehandar Shah, ex vice chancellor and plant taxonomist, University of Malakand, Pakistan. A voucher specimen (RM-2103) has been deposited in the Department of Pharmacology, Khyber Medical University, Peshawar (75). The plant was shade-dried and then repeatedly extracted (3 times) with commercial-grade methanol (80%) at room temperature. The combined extract was evaporated in a rotary evaporator at 35-40°C to a semisolid mass, the crude extract of *R.moschata*. The extract was completely dissolved in 0.9% normal saline using the vertex. The extract in a dose of 50, 100, and 150 mg/Kg were used in-vitro study (75, 76). Plant extracts was tested for various active principles i.e. Triterpenoids, Steroids, Glycosides, Saponins, Alkaloids, Flavonoids, Tannins, and Carbohydrate using different tests such as Liebermann Burchard test was used for steroids and Triterpenoids, Keller Killiani and Bromine water test for Glycosides, Foam test for Saponins, Hager's test for Alkaloids, Ferric chloride test,

Alkaline reagent test and Lead acetate solution test for Flavonoids, Gelatin test for Tannins, Biuret test for proteins and Benedict's test for carbohydrates described by Bhaddray, 2012 (77).

Albino mice weighing between 25-30 g were housed in cages under a 12-hour light/dark cycle, at a temperature of 22 ± 1 and a humidity level of 50% ± 10%. They had free access to food and water. The mice were housed and handled according to the ethical standards set by the university ethics board (Ethic No: KMU/IBMS/IRBE/meeting/2022/8069) at the animal house of Khyber Medical University, Institute of Pharmaceutical Sciences. The food for the mice was prepared in the IPS Lab according to NIH protocol. For behavioral and experimental purposes, the mice were randomly divided into six groups containing both genders with equal proportions, with each group consisting of 5 mice. Group 1 served as the negative control and received normal saline treatment; with 0.5M of 0.9% saline injected intraperitoneally. Group 2 served as the positive control, PTZ was administered intraperitoneally at a dose of 35 mg/kg on the 1st, 3rd, and 5th days (35). Group 3 was the standard group, receiving both PTZ (35 mg/kg) and valproic acid (10 mg/kg, intraperitoneally). Groups 4, 5, and 6 received PTZ (35 mg/kg) along with different doses of *R.Moschata* fruit extract intraperitoneally: 50 mg/kg, 100 mg/kg, and 150 mg/kg, respectively. The injection volume of the drug and extract was calculated using the formula: "Injection volume (ml) = Animal weight (kg) × Animal doses (mg/kg) / Concentration (mg/ml)".

During and after the administration of the treatments, each mouse was closely monitored, and seizures were recorded using a camera connected to a computer. Cognitive and memory behaviors were observed after three days of treatment. Following the completion of behavioral observations, the mice were euthanized in a CO_2 chamber, and brain tissue was isolated. The brain tissues were stored in 4% paraformaldehyde at -80°C for further experimentation. The experimental plan is presented in Figure 1.

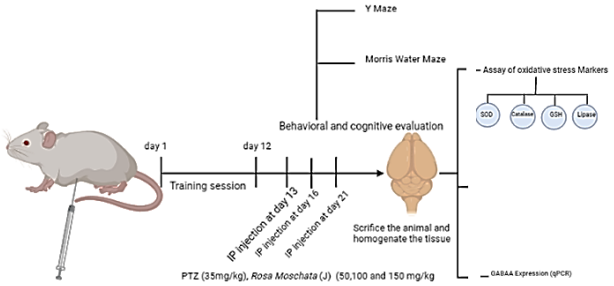


Figure 1: Experimental Plan

The severity of seizures was assessed and validated using the Racine's Scale (78). Racine scale was not justified initially due to some seizure behavioral differences when compared with EEG but with continuous effort and research a modified and advanced Racine scale was established which highly correlates with the EEG finding and dose administered. The Racine scale is mostly used for amygdale kindling models and is adequate for seizure intensity measurement of the PTZ model (79).

Spatial memory behaviors were assessed using a Y Maze assembly with dimensions of 30 × 15 × 15 cm. The mice were placed in the center of the Y maze and allowed to freely explore for 8 minutes. Entry into the alternate arm of the Y maze was considered spontaneous alternation, while entry into the adjacent arm was recorded as the total number of arm entries. The percentage of alternation behavior was calculated using the formula: (Number of successful entries into three different arms / Total number of arm

entries - 2) × 100 (80). A higher percentage of alternation behavior indicated enhanced spatial memory, whereas a lower percentage indicated the opposite.

To evaluate the acquisition and retention of memory in epileptic mice, the Morris water maze (MWM) test was conducted. The apparatus consisted of a circular plastic tank measuring 150 cm in width and 45 cm in height. The tank was filled with opaque water to a depth of 30 cm, and the water temperature was maintained at 28 ± 1 °C. A hidden platform was placed at the center of the tank, dividing it into four equal quadrants. During the training session, the platform was positioned 1 inch above the water surface, and the mice underwent daily training sessions lasting 10-15 minutes for 5 consecutive days. After completion of the training, the platform was submerged 1 inch below the water surface, and the water was made opaque to render the platform invisible. The entry point into the tank was changed for each trial. The probe test was conducted to measure the latency to reach the platform, swimming speed, time spent in the target quadrant, and the number of platform crossings. Video-capturing devices were employed to record the data. The platform remained in the same location throughout the learning trials.

The brain tissues were homogenized in PBS (pH 7.4). Subsequently, the homogenized brain tissues were centrifuged at 600 rpm for 10 minutes at 4 °C. The resulting clear supernatant was collected and used for the measurement of antioxidant enzyme activities, including catalase, lipid peroxidase, SOD, and glutathione peroxidase. The activity of these enzymes was determined using a spectrophotometer.

To the brain tissue sample (pH 10.2), 3 mL of 0.5 M EDTA-sodium carbonate buffer was added. Following that, 100 mL of epinephrine solution (30 M in 0.1 M HCl) was added to the mixture. The reaction's activity was assessed by measuring the absorbance at 480 nm for 4 minutes. The unit of SOD was determined as the amount of enzyme that inhibited the oxidation rate of epinephrine by 50% (81).

Thiobarbituric acid reactive substances (TBARSs) were utilized to measure the levels of malondialdehyde (MDA) in the homogenized brain samples of mice (82). The thiobarbituric acid solution was prepared by combining 0.66 g of thiobarbituric acid with 580 ml of 0.1M phosphate buffer and 20 ml of ferric chloride. This mixture was then mixed with 200 µl of ascorbic acid. The trichloroacetic acid solution was prepared by dissolving 10 g in 100 ml of water.

Next, the brain homogenate samples were incubated at 37ċ for 30 minutes. After incubation, the samples were mixed with 1 ml of 10% trichloroacetic acid and 1 ml of 0.67% thiobarbituric acid in a ratio of 1:10. The mixture was subjected to centrifugation (800 g; 5 min), and the absorbance at 535 nm was measured to determine the levels of thiobarbituric-acid-reacting compounds, which corresponded to the concentration of malondialdehyde (MDA) in nmol/g (83).

The method commonly employed for the measurement of GSH levels was developed by Ellman (84) and Beutler et al.(85), relies on the interaction between the Ellman reagent, DTNB, and compounds containing sulfhydryl groups. This interaction results in the formation of a mixed disulfide (GS-TNB) and 2-nitro-5-thiobenzoic acid (TNB). The spectrophotometric absorbance of GSH in the samples was determined at 412 nm (86).

To conduct the catalase assay, we prepared a 0.06 M phosphate buffer solution and added 2.0 ml of H_2O_2. A blank sample was prepared

using phosphate buffer in 100 ml, and its absorbance was recorded. The phosphate buffer alone served as a control. Next, we took 190 ml of H_2O_2 phosphate buffer and added 10 µl of the sample. The absorbance of H_2O_2 decreases as it is degraded by the enzyme catalase. The enzyme activity was calculated based on the decrease in absorbance at 240 nm (87).

For expression of GABAA Receptor brain tissue homogenate was incubated in 1 ml of triazole reagent per 50-100 mg of tissue for 2 hours. Incubation was done for 5 minutes at a temperature ranging from 15 to 30ċ to allow for the complete dissociation of nucleus protein complexes. Then, 200 µl of chloroform per ml of triazole reagent was added to the mixture. The sample was capped and vortexed for 2-3 minutes. Mixture was incubated for an additional 2 to 3 minutes at a temperature ranging from 15 to 30Ċ, and subsequently centrifuged at 12,000g for 15 minutes at 2 to 8Ċ. The sample was incubated at a temperature ranging from 15 to 30Ċ for 10 minutes and then centrifuged for 10 minutes at 12,000g at 2 to 8Ċ. The supernatant was removed, and the RNA pellet was washed once with 75% ethanol per ml of triazole reagent. The sample was mixed by vortexing and centrifuged again at 7,500g for 5 minutes at 2 to 8Ċ. The fluid was carefully removed from the pellet. Finally, the pellet was dried at 65Ċ for 5 minutes and mixed with RNAase-free water or TEA Buffer.

cDNA was synthesized using chemicals (Supplementary Table 2) and the PCR parameter is presented in Supplementary Table 3. The resulting cDNA was stored at -20Ċ and used for GABAA expression through a qPCR Cyber Green assay (Solarbio -20210610). The primers for the GABAA gene and GAPDH gene were designed using Primer-Primer 5 software. The sequence of the forward and reverse primers (Molecular biology product Lot No: 9081605) is presented in Table 1. GAPDH was used as a positive control during the expression of the GABAA receptor. The dry form of primers was diluted to completely resuspend them by adding 290 µl of RNAase-free water. The mixture was vortexed until the solution was equilibrated, and after 5 minutes, it was vortexed again to ensure complete resuspension.

The PCR mixture was diluted at a ratio of 1:10 due to the presence of an inhibitor that hindered the optimization process. For the amplification of the GABAA gene, a PCR mixture of 20 µl was prepared in PCR tubes (0.1 ml) from Axygen® (California, USA). The mixture included 4 µl of cDNA (50 ng/µl) sample, 5 µl of 2X TaqMan PCR Mixture Mix2X from Solarbio (life sciences), 1.5 µl of GAPDH forward primer (10 picomole/µl), 1.5 µl of GAPDH reverse primers (10 picomole/µl), 1.5 µl of GABAA forward primer (10 picomole/µl), 1.5 µl of GABAA reverse primers (10 picomole/µl), and 5 µl of RT-PCR grade water. The PCR mixture was then centrifuged for 30 seconds using a microcentrifuge from Edison (New Jersey, USA) for proper mixing (refer to Supplementary Table 4).

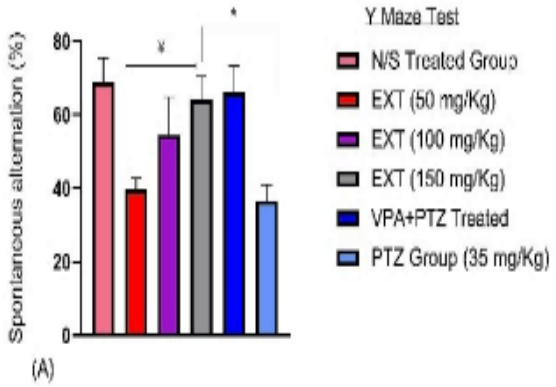
The data were entered into Microsoft Excel 365. Data analysis was performed using Graph Pad Prism 9 software (USA). The results were presented as Mean±SEM for three independent experiments. One-way ANOVA followed by post hoc Tukey's test was conducted. Additionally, correlation tests and linear regression analysis were performed for each group using Graph Pad Prism 9. The expression of GABAA was normalized to GAPDH expression using the $2^{-\Delta\Delta CT}$ method (88).

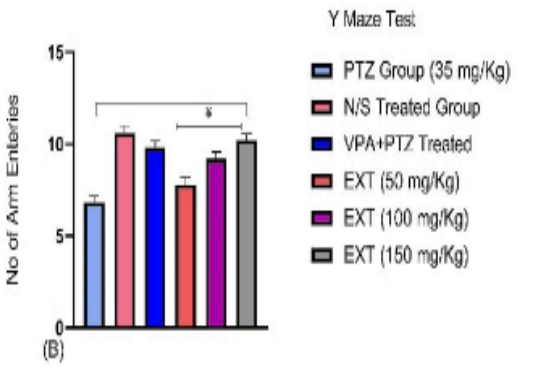
Results

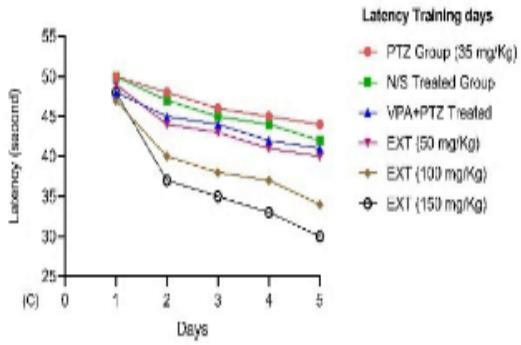
Based on our Y-maze findings, animals treated with PTZ + RM extract (150 mg/kg) exhibited a high percentage of spontaneous alternation compared to the PTZ-alone treated group. There was no significant difference in the percentage of alteration between epileptic animals treated with the extract (150 mg/kg) and the control group treated with valproic acid (VPA) (Figure 3A). However, a significantly higher percentage of alteration was observed in the group treated with PTZ + RM fruit extract (150 mg/kg) compared to the positive group (PTZ-alone) (Figure 2B, C).

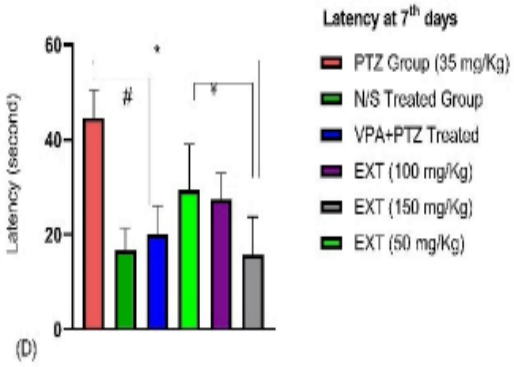
In the Morris water maze (MWM) test, it was observed that the latency to reach the hidden platform was significantly lower in the PTZ + RM fruit extract group (100 and 150 mg/kg) compared to the PTZ group (Figure 3D). The latency in mice treated with *R. moschata* fruit extract (50, 100, and 150 mg/kg) and PTZ co-treatment gradually decreased with increasing doses of RM (Figure 2D).

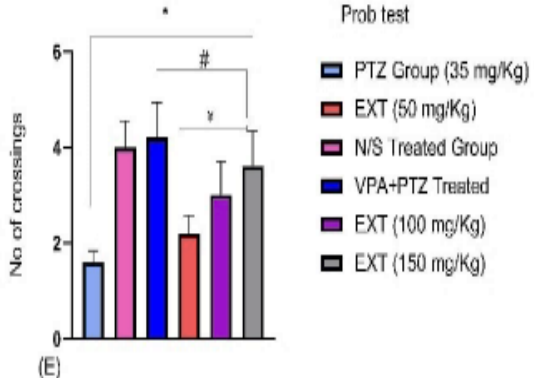
Furthermore, in the PTZ + *R. moschata* fruit extract group (100 and 150 mg/kg), the number of target crossings and the time spent in the target quadrant were significantly higher ($p < 0.05$) compared to the PTZ-alone group (Figure 2E).











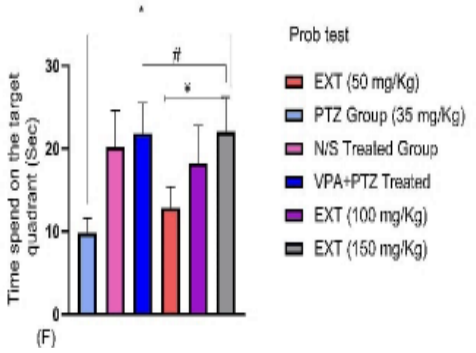


Figure 2: To show the effect of *Rosa moschata (J)* fruit extract on memory dysfunction due to oxidative stress. (A) Percentage of spontaneous alternations behaviors in the Y-maze test. (B) The total number of arm entries in the Y-maze test. (C) Mean escape latency to reach the hidden platform during training days. (D) Latency to reach the hidden platform on day 5th. (E) The number of platform crossings in the probe test. One-way ANOVA followed by post hoc Tukey's test was used to compare the mean of each group. The results have been presented in its units as mean ± SEM (n=3) for three independent experiments (5 mice/group). * (asterisk), indicating a significant difference from the PTZ group; # indicating a significant difference from PTZ+ VPA injected mice. ¥ is an indication difference among the *R. moschata* (J)fruit extract group (50,100 and 150 mg/kg). Significance: *p ≤ 0.05, # p≤ 0.05. PTZ = Pentylenetetrazol, VPA = Valproic acid, Ext= Fruit extract, N/S=Normal saline.

PTZ-induced epileptic animals exhibited a significant difference in the onset time and duration of seizures compared to mice treated with RM fruit extract in combination with PTZ ($p < 0.05$, Figure 3). The onset time of seizures increased with higher doses of RM fruit extract (50, 100, and 150 mg/kg) (Figure 3). Similarly, the duration of seizures (in minutes) decreased with the administration of *R. moschata* fruit extract (100 and 150 mg/kg) (Figure 3). These results demonstrate that the onset time and duration of seizures were dependent on the dose of RM fruit extract (100 and 150 mg/kg) (Figure 3).

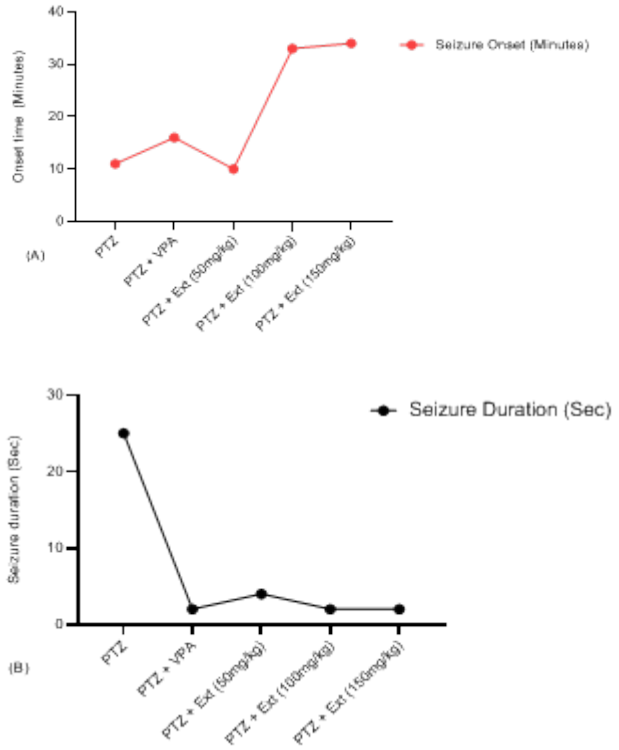


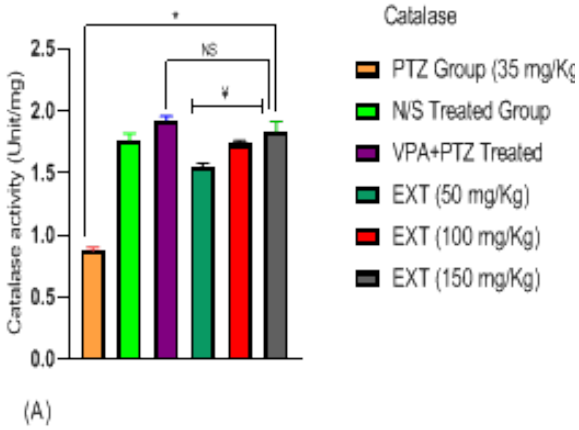
Figure 3: Effects of *Rosa moschata* (J) fruit extract on onset and duration of Seizure

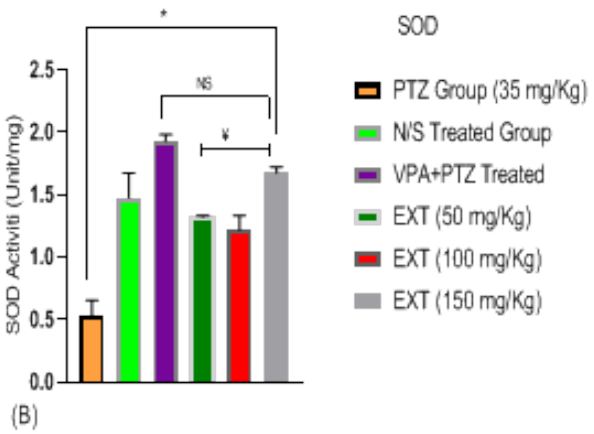
The antioxidant role of *R. moschata* fruit extract at doses of 50, 100, and 150 mg/kg in a PTZ-induced epileptic model is illustrated in Figure 5. The administration of RM fruit extract at doses of 50, 100, and 150 mg/kg resulted in an increase in the levels of oxidative stress markers compared to mice treated with PTZ alone. This increase in antioxidant enzyme expression in the brains of PTZ + *R. moschata* extract co-treated mice was dependent on the dose of the extract. The highest levels of oxidative stress markers were observed at a dose of 150 mg/kg of the test sample, which was comparable to the effect of valproic acid (the standard group) (Figure 4).

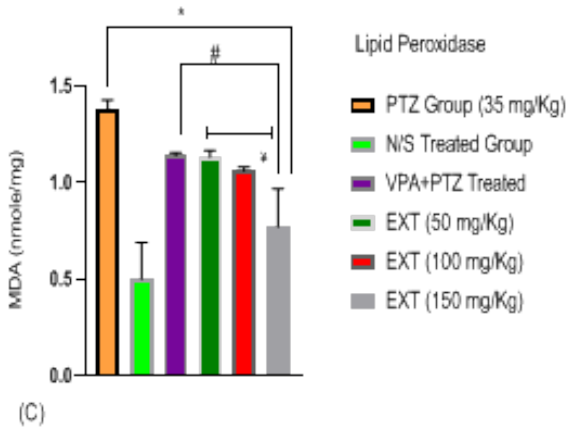
In the PTZ-treated group, the levels of catalase, SOD, and GSH were significantly lower compared to the control group. However, with the administration of RM

fruit extract, these levels were enhanced. Additionally, the level of MDA, a marker of oxidative stress, was significantly higher in the brains of PTZ-treated mice compared to the control group. However, it was reduced with the administration of RM extract (Figure. 4A–D).

We conducted qPCR analysis to measure the mRNA expression of GABAA in the experimental groups (Figure 5). We observed that the expression of the GABAA receptor was significantly higher in mice treated with RM fruit extract at doses of 50, 100, and150 mg/kg compared to mice injected with PTZ alone (Figure 3). The extent of the increase in GABAA levels among the mice treated with R. moschata fruit extract and PTZ depended on the dose of the extract (Figure 5).







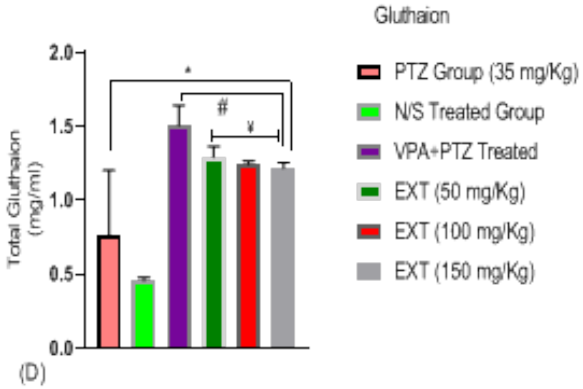


Figure 4: Effects of *Rosa moschata* (J) fruit extract against oxidative stress markers in PTZ-injected and treatment mice brains. (A & B)) Histograms showing Catalases SOD activity in experimental mice. (C & D) Histograms showing Lipid peroxidase and GSH assays in experimental mice. One-way ANOVA followed by post hoc Tukey's test was used to compare the mean of each group. The results have been presented in its units as mean ± SEM (n=3) for three independent experiments (5 mice/group). * (asterisk), indicating a significant difference from the PTZ group; #, indicating a significant difference from PTZ+ VPA injected mice. ¥ is an indication difference among the *R. moschata* fruit extract group (50, 100, and 150 mg/kg). Significance: *p ≤ 0.05, # p ≤ 0.05. PTZ = Pentylenetetrazol, VPA = Valproic acid, Ext= Fruit extract, N/S=Normal saline

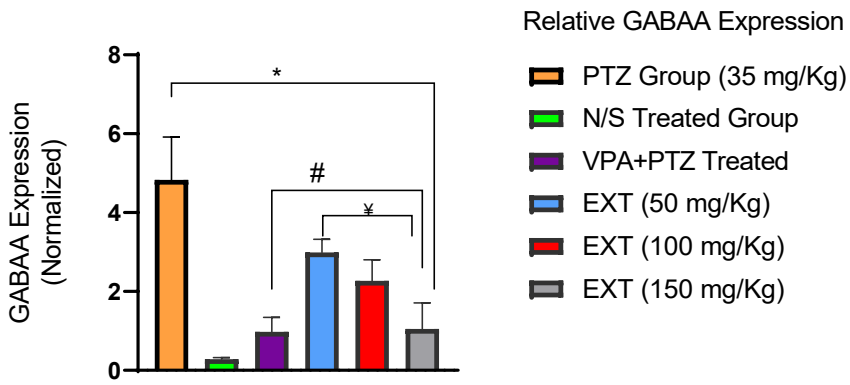


Figure 5: Effects of *Rosa moschata* (J) fruit extract against the GABAA receptor expression. The expression of GABAA was normalized to GAPDH expression using the 2-ΔΔCT method (88). One-way ANOVA followed by post hoc Tukey's test was used to compare the mean of each group. The results have been presented in its units as mean ± SEM for three independent experiments (5 mice/group). * (asterisk), indicating a significant difference from the PTZ group; #, indicating a significant difference from PTZ+ VPA injected mice. ¥ is an indication difference among the *R. moschata* fruit extract group (50,100 and 150 mg/kg). Significance: *p ≤ 0.05, #p ≤ 0.05. PTZ = Pentylenetetrazol, VPA = Valproic acid, Ext= Fruit extract, N/S=Normal saline

Discussion

The present study provides evidence that *R. moschata* fruit extract, administered at doses of 50, 100, and 150 mg/kg, improves cognitive behaviors in an animal model of PTZ-induced epilepsy. The extract exhibits a significant role in reducing oxidative stress by enhancing the levels of endogenous enzymes and reducing lipid peroxidation (LPO), it also significantly increases the expression of GABAA compared to mice treated with PTZ alone.

In a study conducted by Bara et al. in 2008, it was demonstrated that free radicals play a crucial role in epileptogenesis and neuronal cell death (89). Their findings suggested that PTZ impairs the antioxidant defense system in erythrocytes, liver, and brain of mice, leading to a decrease in oxidative stress markers such as catalase, SOD, lipid peroxidase, and GST (89). Our study aligns with the findings of Bara et al., indicating that the levels of oxidative stress markers were lower in the groups treated with *R. moschata* fruit extract. These findings are consistent with a recent study highlighting the role of PTZ in depleting the antioxidant defense mechanism.

Another study suggests that *R. moschata* fruit extract possesses beneficial effects on cognitive behaviors, reduces oxidative stress, and enhances GABAA expression in an animal model of PTZ-induced epilepsy (90). Camilla M. Hoyos et al. (2022) have emphasized the potential role of oxidative stress in cognitive dysfunction (91). Their study explores the possible link between oxidative stress and cognitive dysfunction (91).

In a study by Yong Feng Ren et al. in 2002, the antioxidant, anti-inflammatory, and anti-apoptotic properties of certain agents were highlighted as potential treatments for neurotoxicity and neurodegeneration (92). Furthermore, cognitive impairments observed in animal models have relevance to human data and play a crucial role in unraveling the pathogenesis of neurological disorders (93). Numerous studies suggest that oxidative stress influences the onset and

progression of epilepsy (94). In our present study, we observed that the mRNA levels of GABA were lower in PTZ-treated mice compared to the standard and extract-treated mice. These findings align with the observations made by Till Scheue et al. in 2022 (95). The author discusses how oxidative stress resulting from high oxygen exposure can lead to damage to GABAergic interneurons (95). Additionally, Jie Tu et al. in 2022 conducted a study showing that exogenous GABA improves antioxidant capacity in silkworms (96). Furthermore, we investigated the effect of oxidative stress and plant extract on the levels of dopamine, nor-epinephrine, and serotonin. Our findings revealed that the levels of dopamine, nor-epinephrine, and serotonin were lower in PTZ-treated mice compared to the standard group and the groups treated with R. moschata fruit extract. Existing literature suggests that oxidative stress plays a role in modulating the levels of monoamine neurotransmitters in seizures (97, 98). The available literature directly or indirectly supports our data, indicating that the levels of monoamine neurotransmitters are reduced in mice with a depleted antioxidant defense system compared to the treated groups. Plants play a crucial role as potential sources of drugs and can enhance the brain's protective mechanisms.

R. moschata is known to contain various fatty acids such as palmitic, linoleic, stearic, margaric, oleic, and linoleic acids (99). This species is also rich in vitamins A, C, and E, as well as flavonoids (100). Flavonoids have been proven to reduce oxidative stress levels in epilepsy (67). The Rosa genus is known for its abundance of phenolic compounds and other phytochemicals, which may contribute to antioxidant activity (74).

Conclusion

R. moschata fruit extract showed a good anticonvulsant effect by decreasing the oxidative stress marker and increasing the expression of GABAA in PTZ induced seizure mice model. The extract also showed a beneficial effect on the cognitive behaviors in the mice model.

Limitations of the Study:

The current study is carried out in whole brain tissues instead of different parts of the brain and lacks western blot.

Recommendation:

Fractionation is advised to isolate the new molecule with more antioxidant potential using a biological activity-guided approach. It is recommended that this effect should be explored in the hippocampus and cortex.

Abbreviations:

Rosa Moschata (RM), Catalase (CAT), Copper (Cu), Glutathione Peroxidase (GPx), Gamma Amino Butyric Acid (GABAA), Iron (Fe), Manganese (Mn), Morris Water Maze (MWM), Pentylenetetrazole (PTZ), Super Oxide Dismutase (SOD), Phosphate Buffer Solution (PBS) and Selenium (Se)

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