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AMELIORATIVE EFFECT OF SODIUM VALPROATE IN COMBINATION WITH *JUGLANS REGIA* FRUIT EXTRACT AGAINST PENTYLENETETRAZOLE INDUCED CONVULSIONS IN MICE

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ABSTRACT

Introduction: Epilepsy is a complex neurological disorder characterized by hypersecretion of excitatory neurotransmitters in the brain. Level of antioxidant enzymes in the brain changes during seizure. There are many herbal supplements that posses antioxidant potential and might help in prevention and recovery of seizures when used as an adjunct with modern antiepileptic drugs. **Objective:** The present work was undertaken to evaluate the Ameliorative effect of Sodium valproate in combination with *Juglans regia* extract against Pentylenetetrazole (PTZ) induced convulsions in mice. **Materials and Methods:** *Juglans regia* ethanolic extract (JREE) were administered at doses of (200mg/kg and 400 mg/kg p.o) and Sodium Valproate (500mg/kg p.o.) one hour before the administration of PTZ (80mg/kg i.p.). Brain tissues were screened for MDA, Catalase, GSH and protein estimation. **Result and Discussion:** Administration of JREE in the low and high dose prolongs the onset of myoclonic jerks dose-dependently and also showed protection against PTZ-induced convulsions. PTZ induced seizures caused a significant increase in MDA levels and a significant decrease in GSH and catalase levels in PTZ group as compared to the vehicle control group. *Juglans regia* pre-treatment prevented the oxidative stress as indicated by significant decrease in MDA levels and significant increase in GSH and catalse levels in comparison to PTZ group. **Conclusion:** The present study showed ameliorative effect of combination of Sodium Valproate in anticonvulsant activity of ethanolic extract of *Juglans regia* fruit against PTZ induced seizures and also its protective effect against seizure induced oxidative stress.

Keywords: *Juglans regia*, Pentylenetetrazole, Sodium Valproate, ameliorative effect

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INTRODUCTION

Epilepsy is a major communal health problem, affecting approximately 4% of individuals over their life time. [1] Antiepileptic drugs only provide symptomatic treatment, devoid of having any influence on the course of the illness. There is a great need for the development of alternative therapeutic approaches that prevent epilepsy. [2] Epilepsy is one of the most common diseases of the brain, affecting at least 50 million person's worldwide. [3] However, the challenge of trying to unravel the mechanisms of action on mood, humor and cognition led to an inconvenience: to ignore, or to face as low priority, the fact that plants could also have beneficial properties to treat mental disease and some psychic ailments. [4] [5] Till date very little attention was given by the scientific community to the benefits, as accepted by folk medicine and the medicinal properties of the natural product. [6]*Juglans regia* is a medicinal plant whose roots, leaves and fruit are well known in traditional medicine among the Hausa/Fulani tribe in Northern Nigeria in the treatment of several diseases including epilepsy. The walnut tree species is native to the old world. It is native in a region stretching from the Balkans eastward to the western Himalayan chain. [7] Walnuts are nutrient-rich food due to high content of fats, proteins, vitamins and minerals.

Walnut kernels are also good source of flavonoids, sterols, pectic substances, phenolic acids and related polyphenols. Several

phenolic compounds isolated from *J. regia* such as pyrogallol, p-hydroxybenzoic acid, vanillic acid, ethyl gallate, protocatechuic acid, gallic acid, 3,4,8,9,10-pentahydroxydibenzo pyran-6-one, tannins, adenosine, adenine, etc, could provide a chemical basis for some of the health benefits claimed for *J. regia* in foods and folk medicine. [8] [10] Indeed, the walnut is unique among the nuts due to the presence of antioxidants, and it is classified as second among foodstuffs for high amounts of antioxidants. [11] Wall nut kernels have high concentrations of phenolic compounds, which have beneficial effects on human health because of their antioxidant, anti-atherogenic and neuroprotective properties. [12] [13] The current study was designed to evaluate the anticonvulsant and antioxidative activity of *Juglans regia* fruit extract in combination of modern antiepileptic drug Sodium valproate against PTZ induced convulsions.

MATERIALS AND METHODS

Chemicals

5, 5'- dithiobis (2-nitrobenzoic acid) (DTNB), 2-thiobarbituric acid (TBA), 1,1,3,3 tetraethoxypropane (TEP), bovine serum albumin (BSA), were purchased from Sigma- Aldrich (St. Louis, U.S.A). Sodium carbonate, Cooper sulphate, Sodium Potassium tartarate, Folin-phenol reagent, Hydrogen Peroxide, Sodium lauryl sulphate, Acetic acid, n-butanol- pyridine, Sulphosalicylic acid were purchased from CDH.

Plant material

Juglans regia fruits were purchased from the local market of Jammu and Kashmir. The Fruits were authenticated Professor Vimla Y., Department of Botany, Chaudhary Charan Singh University, Meerut. The herbarium of the plant material has been deposited in the Department for future reference.

Preparation of Ethanolic extract

Juglans regia fruits were cracked then separated into two parts (Septum and kernel), then coarsely powdered and passed through mesh sieves. The powdered material (kernel) was extracted with ethanol using soxhlet apparatus at room temperature for 24hrs. The solvent was evaporated by rotary evaporation at 35°C, yielding a dark red colour mass (12.35g) and stored at 4°C. The yield of the ethanolic extract was found to be 15.43%.

Phytochemical Screening

Phytochemical screening of the prepared extract was conducted with various qualitative tests to identify the presence of chemical constituents by standard procedures. [8] [14] [15]

Experimental model selected for this work

Healthy Swiss male albino mice (25-30 g) were used for anticonvulsant activity and safety study respectively.

Animal care and selection

Adult male swiss albino mice were obtained from the Animal House of R.I.T. Greater Noida. They were housed in polypropylene cages in groups of six mice per cage and kept in a room maintained at 25±2 °C with a 12-h light/dark cycle. They were allowed to acclimatize for 1 week before the experiments and were given free access to standard laboratory feed and water ad libitum. Protocol is approved by Institutional animal ethics committee (385/PO/Re/S/2001/CPCSEA/2016).

Acute toxicity study

The acute toxicity study was performed according to OECD guideline 423, acute toxic class (three animals used). The adult Albino Swiss mice (25-30g) were randomly divided into five different groups containing three animals in each group. The animals were fasted overnight, extracts was administered orally at various dose levels (300, 500, 1,000, and 2,000 mg/kg, BW) suspended in 0.5% gum acacia. Fifth group was maintained as control and administered the vehicle only. The animals were observed continuously for 2 h, then occasionally for a further 24 h, and finally any mortality. The behavior of the animals and any other toxic symptoms were also observed for 72 h, and they were kept under observation for up to 8 days.

Experimental Design

Group 1: Control mice treated with vehicle of extract (0.5 % Gum Acacia).
Group 2: Pentylenetetrazole treated gp (80mg/kg, i.p) + Vehicle of extract (0.5 % Gum Acacia).
Group 3: Sodium Valproate treated group (500 mg/kg, p.o.) + PTZ (80 mg/kg)
Group 4: Sodium Valproate + JREE extract (200 mg/kg, p.o.) + PTZ (80 mg/kg)
Group 5: Sodium Valproate + JREE extract (400 mg/kg, p.o.) + PTZ (80 mg/kg)

To study ameliorative effect of sodium Valproate in combination with *Juglans regia* fruit extract against PTZ induced convulsions. Extracts were administered at doses of 200 and 400 mg/kg orally one hour before the administration of PTZ. Sodium Valproate was administered one hour before the administration of PTZ.

Anticonvulsant activity

Pentylenetetrazole Induced convulsions- Animals were randomly assigned into five groups. Group-1 was given (0.5% Gum Acacia) and serves as vehicle control group, group-2 received Pentylenetetrazole (80mg/kg, i.p.).

Group-3 served as positive control and received sodium valproate (500 mg/kg, p.o.) one hour before seizure induction with PTZ. Group-4 received sodium valproate + ethanolic extract of *Juglans regia* fruit at the dose of 200 mg/kg orally by gavage, 60 min before seizure induction with PTZ.

Group-5 received sodium valproate + ethanolic extract of *Juglans regia* fruit at the dose of 400 mg/kg orally by gavage, 60 min before seizure induction with PTZ. PTZ was prepared freshly by dissolving in normal saline. Vehicle/drugs were administered in a volume not exceeding 10ml/kg. Animals were observed for myoclonic jerk latency upto 30 minutes after PTZ injection. [16]

Brain tissue preparation

The mice were decapitated under ether anesthesia. The skull was cut, open and the brain was exposed from its dorsal side. The whole brain was quickly removed and cleaned with chilled normal saline on the ice. A 10% (w/v) homogenate of brain samples (0.03M sodium phosphate buffer, pH 7.4) was prepared by using a homogenizer. The homogenized tissue preparation was used to measure MDA, GSH, and Catalase.

Biochemical Estimation

Measurement of MDA

MDA, which is a measure of lipid peroxidation, was measured spectrophotometrically using reported method. [17] MDA is expressed as nM/mg protein. To 200µl of tissue homogenate in phosphate buffer (pH 7.4), 200µl of 8.1% sodium lauryl sulphate (SLS), 1500µl of 20% acetic acid and 1500µl 0.8% (w/v) 2-thiobarbituric acid (TBA) were added and volume was made upto 4ml with distilled water then the mixture was heated for one hour at 95°C. Then the mixture was cooled with tap water and 1ml of distilled water was added. Then 5ml of n-butanol: pyridine (15:1) mixture was added and shaken vigorously and centrifuged at 4000 rpm for 10 min. Pink colored supernatant was obtained, which was measured spectrophotometrically at 530 nm.

Measurement of GSH

GSH was determined by its reaction with 5, 5'-dithiobis (2-nitrobenzoic acid) to yield a yellow chromophore which was measured spectrophotometrically. [18] The brain homogenate was mixed with an equal amount of 4% sulfosalicylic acid and kept at 4°C and then centrifuged (Remi cold centrifuge) at 1200 rpm for 15 min. at 4°C. The supernatant was used for GSH estimation. To 0.1 ml of processed tissue sample, 2.7 ml of phosphate buffer (pH 8), 0.2 ml of 0.1mm of 5, 5'-dithiobis (2-nitrobenzoic acid) (DTNB) were added . The absorbance was read at 412 nm.

Catalase activity

The enzyme catalase is an endogenous antioxidant present in all aerobic cells helping to facilitate the removal of hydrogen peroxide. Catalase activity was measured by reported method. [19] A total of 0.1 ml of supernatant is added to cuvette containing 1.9 ml of 50 mM phosphate buffer (pH 7.0). The reaction started by the addition of 1 ml freshly prepared 30 mM H_2O_2. The rate of decomposition of H_2O_2 was measured spectrophotometrically from the changes in absorbance was recorded for 2 min at 30 s at 240 nm. The activity of catalase was expressed as U/mg protein.

Protein estimation

Protein was measured in all brain samples according to the method of Lowry using bovine serum albumin (BSA) (1 mg/ml) as standard. [20]

Statistical analysis

The results are expressed as mean ± S.E.M. Statistical analysis of anticonvulsant activity and biochemical values were performed by unpaired t-test and one-way analysis of variance (ANOVA) followed by Tukey test.

RESULTS AND DISCUSSION

Preliminary Phytochemical Screening

Preliminary qualitative phytochemical screening of *Juglans regia* ethanolic fruit extract showed the presence of alkaloids, phenols, tannins, steroids, fats and oils & Flavonoids as reported in Table 1.

Acute Toxicity Study

From the present study ethanolic extract of *Juglans regia* did not show any mortality and toxic manifestations upto the dose of 2000 mg/kg, orally. Based on acute toxicity studies, 1/10 of the safe dose (200 and 400 mg/kg) of the plant extracts had been selected as therapeutic doses.

Table 1: Chemical tests for Ethanolic extract of *Juglans regia* Fruit (kernel)

S. No.	Chemical Test	Obsevation
1.	Tannins and Phenolic compounds	+
2.	Alkaloids	+
3.	Carbohydrates	+
4.	Glycosides	-
5.	Proteins	-
6.	Aminoacids	-
7.	Steroids	+
8.	Flavonoids	+
9.	Fats and oils	+

(+) =Presence (-) = Absence

PTZ-induced seizures

All the animals showed complete phases of seizures in PTZ group. Pre- treatment with ethanolic extract and Sodium valproate (500 mg/kg) showed 83.3%, 100% and 83.3% protection against PTZ-induced convulsions in combination with Sodium Valproate at doses of 200 and 400 mg/kg, (JREE) respectively, (Table 2). Complete protection against seizures was observed with pre-treatment JREE (400 mg/kg). The onset of myoclonic seizures was increased significantly when extracts were administered at two doses (200 and 400 mg/kg), as compared to the PTZ group as reported in Table 2, Figure 1a and Figure 1b.

Table 2: Effect of *Juglans regia* ethanolic Fruit extract on PTZ induced seizures

Groups	Onset of seizures (Min.)	% Protection
PTZ (80 mg/kg)	1.95 ± 0.40	00
Sodium Valproate (500 mg/kg)	25.88 ± 1.10*	83.3
Sod. Valproate + JREE (200 mg/kg)	25.94 ± 1.18***	83.3
Sod. Valproate + JREE (400 mg/kg)	-------	100.0

***P<0.001, *P<0.05, as compared to PTZ group

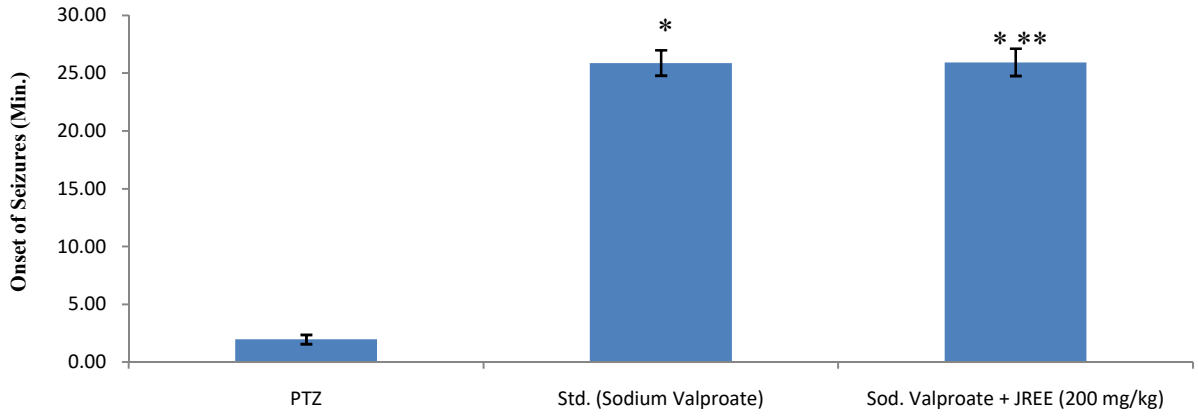

Figure 1a: Anticonvulsant activity of *Juglans regia* ethanolic fruit extract against PTZ induced convulsions. JREE: *Juglans regia* ethanolic extract ***P<0.001, *P<0.05, as compared to PTZ group.

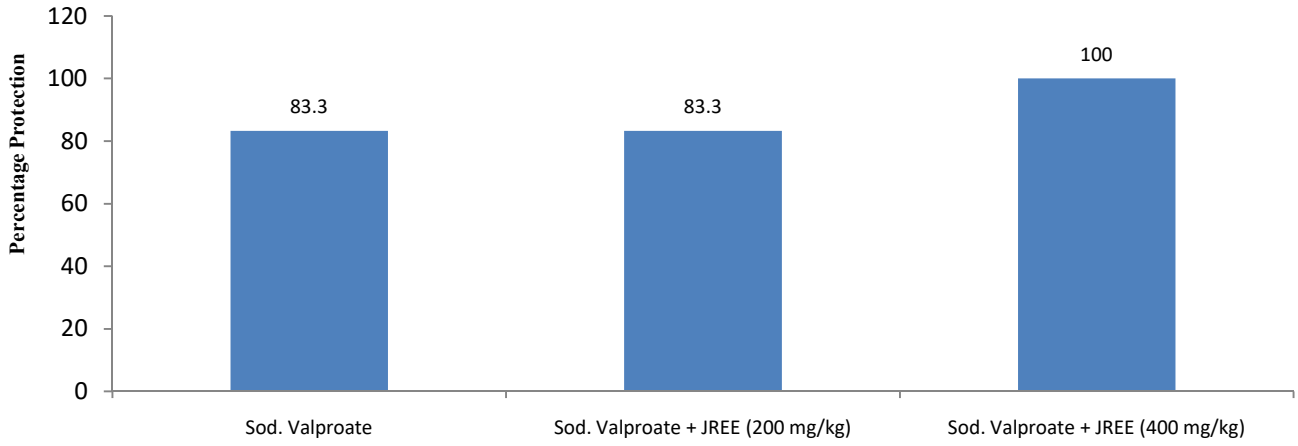

Figure 1b: Percentage protection of *Juglans regia* ethanolic fruit extract against PTZ induced convulsions.

Estimation of biochemical parameters

Measurement of MDA

The MDA level (nmol/mg protein) in the brain was measured. The level of MDA rise significantly in PTZ treated group ($P < 0.001$) as compared to control group. On the other hand, in combination Sodium valproate and JREE (200, 400 mg/kg) ($P < 0.001$) significantly decreased MDA level as compared to PTZ treated group as clearly seen in Table 3 and Figure 2.

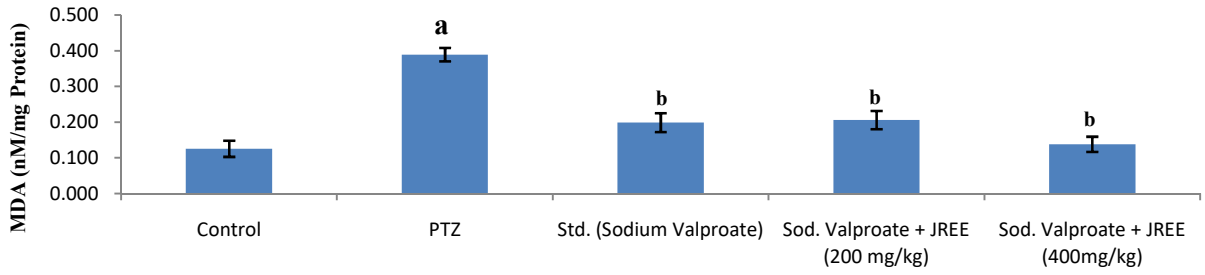

Figure 2: Effect of *Juglans regia* ethanolic fruit extract on MDA level, significant increase (p<0.001) vs. Control and significant decrease (p<0.05) vs. Pentylenetetrazole (PTZ) treated group. [a] P<0.001, as compared to Control group, [b] P<0.001, as compared to PTZ group.

Table 3: Effect of *Juglans regia* ethanolic fruit extract on oxidative stress markers.

Groups	MDA (nM/mg protein)	GSH (µg/mg protein)	Catalase (U/mg protein)
Vehicle Control	0.126±0.022	11.08±0.98	17.35±1.40
PTZ (80 mg/kg)	0.389±0.0187^{***a}	3.04±0.87^{***a}	7.03±0.86^{***a}
Sodium Valproate (500 mg/kg)	0.199±0.026^{***b}	10.63±1.71^{***b}	15.26±1.50^{***b}
Sod. Valproate + JREE (200 mg/kg)	0.206±0.025^{***b}	10.12±1.27^{***b}	16.86±2.30^{**b}
Sod. Valproate + JREE (400 mg/kg)	0.138±0.021^{***b}	10.81±1.25^{***b}	17.19±2.45^{**b}

^{a} P<0.001, as compared to Control group, ^{b} P<0.001, as compared to PTZ group. (**P<0.05, ***P<0.001)

Measurement of GSH

The GSH level (µg/mg protein) in the brain was measured. The level of GSH decrease significantly in PTZ treated group ($P < 0.001$) as compared to control group. On the other hand, in combination Sodium Valproate and JREE (200, 400 mg/kg) ($P < 0.001$) significantly increased GSH level as compared to PTZ treated group as presented in Table 3 and Figure 3.

Catalase activity

The Catalase level (U/mg protein) in the brain was measured. The level of Catalase decrease significantly in PTZ treated group ($P < 0.001$) as compared to control group. On the other hand, in combination Sodium valproate and JREE (200, 400 mg/kg ($P < 0.001$) significantly increased Catalase level as compared to PTZ treated group. The data is presented in Table 3 and Figure 4.

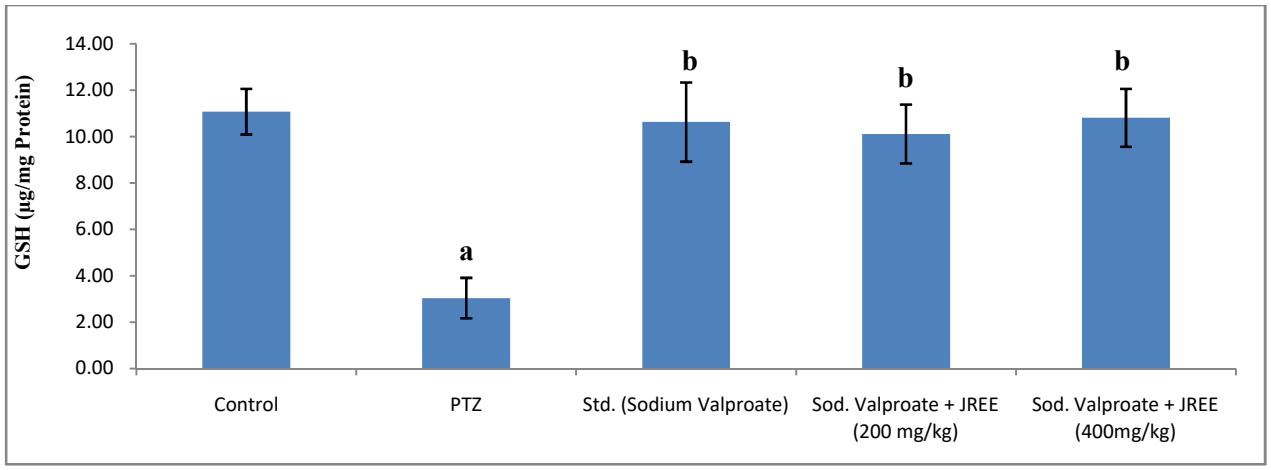

Figure 3: Effect of *Juglans regia* ethanolic fruit extract on GSH level, significant decrease (p<0.001) vs. Control and significant Increase (p<0.05) vs. pentylenetetrazole (PTZ) treated group. ^{a} P<0.001, as compared to Control group, ^{b} P<0.001, as compared to PTZ group.

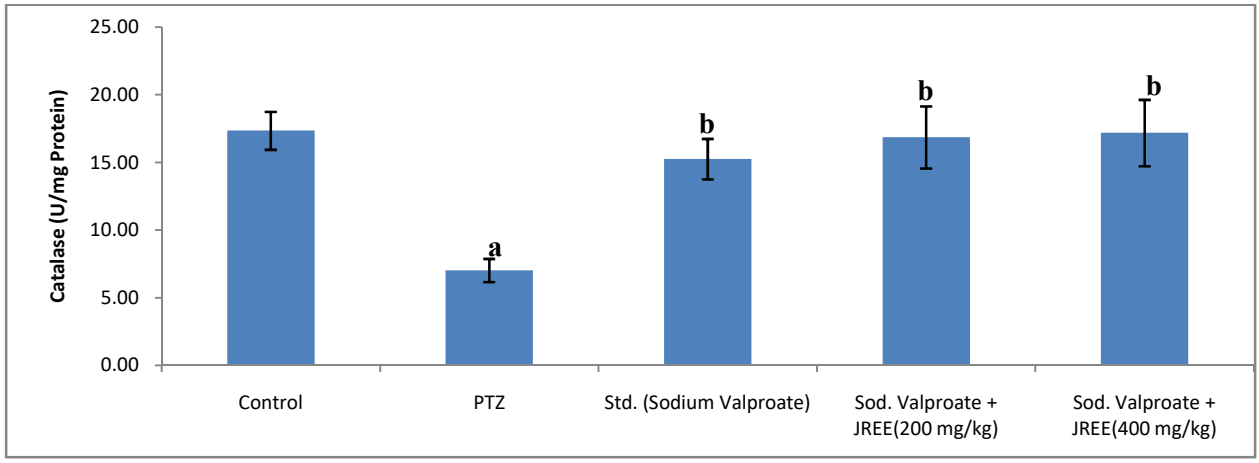

Figure 4: Effect of *Juglans regia* ethanolic fruit extract on Catalase level, significant decrease (p<0.001) vs. Control and significant Increase (p<0.05) vs. pentylenetetrazole (PTZ) treated group.

CONCLUSION

In the present study, protective effect of ethanolic extract of *Juglans regia* against seizures and seizure-induced oxidative stress was evaluated. Administration of extract in low and high dose prolongs the onset of myoclonic jerks dose-dependently and also showed protection against PTZ-induced convulsions. Seizures causes imbalance in oxidant, antioxidant system of brain which leads to oxidation of lipids, protein and DNA resulting into neurodegeneration. Thus, in the present study the seizure-induced oxidative stress was also evaluated. PTZ induced seizures caused a significant increase in MDA levels and a significant decrease in GSH and catalase levels in PTZ group as compared to the vehicle control group. *Juglans regia* pre-treatment prevented the oxidative stress as indicated by significant decrease in MDA levels and significant increase in GSH and catalse levels in comparison to PTZ group. Thus, the results of the present study showed ameliorative effect of Conbination of Sodium Valproate in anticonvulsant activity ethanolic extract of *Juglans regia* fruit against PTZ induced seizures and also its protective effect against seizure induced oxidative stress. This plant fruit may be beneficial for the future treatment of brain disorders and better serves for mankind. Further research is required to confirm the molecular mechanism of action.

CONFLICT OF INTEREST

The authors have no conflict of interest.

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