Neuroprotective Efficacy of Curcumin and Berberine with Gabapentin Pretreatment Against Chemically Induced Intractable Epilepsy in Albino Mice

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ABSTRACT

Background- Epilepsy is a chronic neurological disease characterized by recurrent seizure and a significant number of patients suffering from drug resistant (intractable) epilepsy against conventional antiepileptic drugs. The present study was designed to evaluate the neuroprotective potential of curcumin and berberine, alone and in combination, along with gabapentin pre-treatment on the pentylenetetrazole (PTZ)-induced intractable epilepsy in albino mice.

Methods- Intractable epilepsy was induced by repeated administrations of PTZ (40 mg/kg) on alternate days after a single administration of gabapentin (20 mg/kg) with one or more other treatment regimens. Assessment of behavioral activities including seizure severity, behavioural response, locomotor activity, anxiety-like behavior and cognitive performance was carried out using standard behavioral models. Biochemically determined oxidative stress markers viz.; malondialdehyde (MDA), reduced glutathione (GSH), superoxide dismutase (SOD) and catalase (CAT) were measured. Other parameters assessed included nitric oxide (NO), acetylcholinesterase (AChE) and total protein. Brain was removed for histopathological assessment of neuronal integrity.

Results- The repeated administration of PTZ induced frequent seizures, oxidative stress, impairment of learning and memory as well as substantial neuronal degeneration when compared to normal control. PTZ + gabapentin pre-treatments significantly increased the intensity of seizures whereas the concurrent administration of VPA ameliorated the PTZ-induced seizure effects and enhanced anticonvulsant activity. Curcumin and berberine provided a dose-dependent protection, improved behavioural parameters, attenuated oxidative damage and reduced lipid peroxidation with significant preservation of neuronal integrity. In combination, curcumin and berberine (100 mg/kg each) with gabapentin and VPA showed excellent neuroprotection against PTZ induced intractable epilepsy as evident from superior seizure protection and normalization of various parameters examined. **Conclusion**- Curcumin and berberine can be employed as promising adjunctive neuroprotective agents along with gabapentin in the treatment of chemically induced intractable epilepsy. These study suggest that curcumin and berberine can be used as effective adjunctive neuroprotective agents in combination with gabapentin in the treatment of chemically induced intractable epilepsy.

Keywords: PTZ kindling, Intractable epilepsy, Curcumin, Berberine, Gabapentine, Valproic acid, Histopathology.

1. Introduction

The most frequent chronic neurological disorder is epilepsy; 50 million people around the globe suffer from this illness. Unprovoked, recurring seizures are the main characteristic of this disorder; this is caused by abnormally high electrical brain activity of neurons. There are many prescribed anticonvulsants, but approximately 30% of individuals fail to achieve a good seizure control despite using several medications. These patients, the ones with intractable or drug-resistant epilepsy, develop significant psychiatric disorders, cognitive impairments, and are at risk for increased mortality [1]. The search for a new drug that offers seizure control with the added benefit of neuro protection, has lead scientists to new treatment avenues [2]. The Pentylenetetrazole (PTZ) kindling animal model mimics quite realistically the chronic epilepsy process found in humans, due to PTZ induces an exaggerated neuronal activity which produces progressive neuronal damage and finally, recurrent seizure generation. The PTZ kindling model induces many of the pathological processes observed in human epilepsy such as oxidative stress, neuro-inflammation and neuronal loss [3,4]. Gabapentin is the 2ndgeneration AED and causes its anticonvulsant actions via an affinity to the 2- subunit of voltage-dependent calcium channels reducing glutamate and other excitatory neurotransmitters release [5]. Valproic acid Another example is broad-spectrum antiepileptic drug with multiple action mechanisms (enhance GABA neurotransmission, modify the sodium and calcium channel activities) that nevertheless might be ineffective against seizure management in some patients suffering from drug-resistant epilepsy thus the demand to additional medication with targeting mechanisms is necessary. [6] Curcumin is a natural polyphenolic compound that was isolated from Curcuma longa and characterized for its antioxidant, anti-inflammatory and neuroprotective activity. Similarly, [7] berberine is an isoquinoline alkaloid from various plants with reported anticonvulsant, antioxidant, anti-inflammatory and anti-apoptotic activity in different experimental models of brain disorders. Both phytochemicals exhibited protection of neuronal integrity, reduced

oxidative stress and improved cognitive function, thus be good candidates for epilepsy adjuvant therapy [8]. The present study was therefore planned to evaluate neuroprotective potential of curcumin and berberine singly and in combination with Gabapentin pre-treatment and Valproic acid against Pentylenetetrazole (PTZ)- induced intractable epilepsy in albino mice, we will also assess the effects of them on severity of seizure and altered behavior as well as oxidative stress markers and histopathological changes in epileptic brain.

2. Materials And Methods

2.1. CHEMICALS AND DRUGS

The ethanolic extract of curcumin and berberine was procured from Vital Herbs, Add; - Z-26/27 Commercial Enclave, Mohan Garden, Uttam Nagar, Delhi, 110059 (GSTN: 07CIRPD7950H1ZV). The extract was stored at cool and dry place and kept safe from bright light and heat. In present study the chemicals namely; Pentylenetetrazole (PTZ), gabapentin, valproic acid, curcumin and berberine were used. PTZ was used to induced the intractable epilepsy whereas gabapentin and valproic acid are established standard antiepileptic drugs, whereas; curcumin and berberine were tested for the neuroprotective activity. All the chemicals and reagent for the estimation of biochemical markers were of analytical grade and were obtained from certified commercial agents.

2.2. EXPERIMENTAL ANIMALS

Animals The adult healthy albino mice of either sex, weighing between 25-30 g, were purchased from the animal house of a licensed animal breeding facility, Subharti University, Meerut, Uttar pradesh, India. The mice were housed in polypropylene cages with proper bedding material in the standard laboratory conditions i.e., 22 ±2°C temperature, 55–65% relative humidity and 12 h light/dark cycle. Animals were fed with standard pellet diet and were allowed to take water ad libitum. Animals were allowed to acclimatise to the laboratory conditions for 1 week prior to the start of experiment.

2.3. ETHICAL APPROVAL

The experimental protocol was reviewed and approved by the Institutional Animal Ethics Committee (IAEC) (1024/PO/ReBiBt/S/2008/CCSEA/25-7) and conducted in accordance with the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India.

2.4. EXPERIMENTAL DESIGN

The animals were randomly divided into nine groups containing six animals in each group (n = 6).

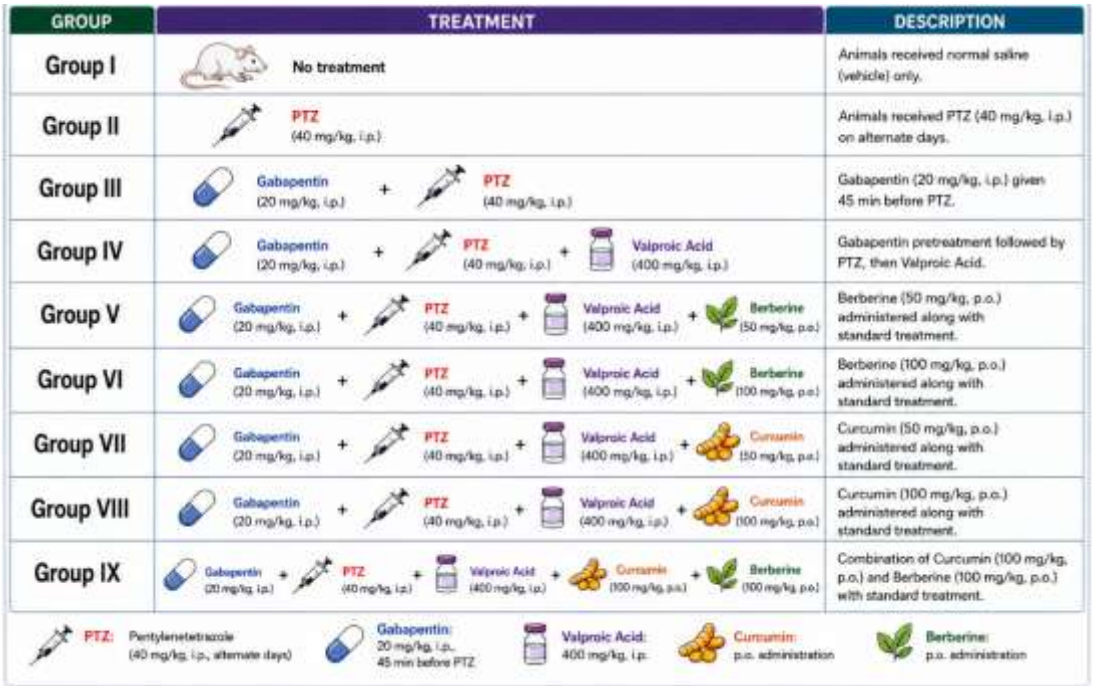


Table 1 Exprimental groups and treatment protocol

2.5. INDUCTION OF INTRACTABLE EPILEPSY

Intractable epilepsy was induced in animals by repetitive i.p. PTZ administration (40 mg/kg) every alternate day for 37 days according to PTZ kindling protocol. Gabapentin pre-treatment and valproic acid were administered before PTZ administration as standard therapy. Curcumin and berberine were given orally at indicated doses during whole course of treatment. The animals were examined after each PTZ injection for occurrence of seizures, seizure severity, behavioural changes, and motor deficit. The score of seizure severity was rated on modified Racine scale [9]

2.6. GABAPENTIN PRETREATMENT AND PTZ-INDUCED DRUG-RESISTANT EPILEPSY

Drug-resistant epilepsy was developed using repeated pentylenetetrazole (PTZ) kindling. Briefly, albino mice were pre-treated with gabapentin (20 mg/kg) 45 min before i.p. Injection of PTZ (40 mg/kg) on alternative days. The mice received the injections till they reached a fixed level of kindling defined as obtaining 3 consecutive stage 5 seizures or 19 PTZ injections, (around 37 days) were achieved, whichever happened earlier. After every PTZ injection, the mice were placed alone in a transparent Plexiglas observation chamber (40 23 23 cm) and observed continuously for 30 min. The behavior of the seizures was scored according to the modified Racine scale, [10,11].

2.7. BEHAVIOURAL ASSESSMENT

2.7.1. RACINE SCALE

Seizure severity was scored by using the modified Racine scale (9,10) that is a reliable measure of behavioural output and the most used behavioural scoring in experimental epilepsy studies. After the injection of each dose of PTZ, mice were individually tested for a period of 30 minutes in an open, transparent Plexiglas chamber and the highest score was reported. Stages on the modified Racine scale were as follows: Stage 0, No response; Stage 1, Hyperactivity and exploratory behaviour (e.g. Grooming, scratching, jumping); Stage 2, Myoclonus (jerking), twitching of whiskers and ears, nodding or shaking of head, stereotypic behaviour (pacing) and salivation; Stage 3, Unilateral forelimb flexion or extension; Stage 4, Forelimb clonic seizures and rearing on hindlimbs; Stage 5, Generalised tonic clonic seizures with loss of posture and collapse and Stage 6, Generalised tonic clonic seizure and tonic extension of the hindlimbs. [11,12]

2.7.2. ACTOPHOTOMETER TEST

The effects of the treatments on spontaneous motor function were assessed by locomotor activity with digital actophotometer (about 30 cm × 30 cm × 30 cm). Every mouse was singly introduced in the actophotometer chamber and its locomotory pattern was recorded by photoelectric sensors during the whole 5 min experimental period. The total scores of locomotive actions were measured in each mouse. Higher locomotor score indicates enhanced locomotive function while lower activity reveals motor dysfunction. [13,14]

2.7.3. ELEVATED PLUS MAZE (EPM)

The Elevated Plus Maze (EPM) test The EPM test, as used here to determine the anxiety-like behavior in mice is shown in the fig. The apparatus was composed of two open arms and two closed arms (18 cm 7 cm) connected by a central rectangular approximately 6 cm 5 cm (fig.) that constitutes another animal model of neurobehavioral alterations and this model was used for cognitive impairments. This was located 28 cm above ground, each arms having 12-cm-high walls above the ground. Each animal was placed in the centre of the maze, facing one open arm, and had access to all areas of the maze for 5 min. The number of visits to the open and closed arms and the time spent in them were measured. Greater proportion of visits to and time spent in open arms indicated decreased anxiety-like behaviour [15,16].

2.7.4. MORRIS WATER MAZE (MWM)

To determine spatial learning and memory, Morris water maze (MWM) test was performed in mice. MWM was a circular pool with the dimensions of 120 cm diameter, 50 cm in height which was filled with water up to approximately 30 cm height with 22 ± 2 °C temperature. A circular platform (10 cm in diameter) was located in the centre of the target quadrant about 1 cm below the surface of the water. During training trials mice swam freely in the pool to find the submerged platform and time of reaching the platform (escape latency) was recorded. The escape platform was removed and the time spent by the mice in the target quadrant during probe trial showed the spatial learning ability and retention [17, 18].

2.7.5. BRAIN AND HOMOGENIZATION ISOLATION

After completion of experimental period, animals were sacrificed by cervical dislocation after anaesthesia. The whole brain was dissected out, washed with ice-cold normal saline for complete removing of blood, dried by blotted tissue and weighed. The brain tissues were homogenized with ice-cold 0.1 M phosphate buffer (pH 7.4) and made 10% (w/v) homogenate with the help of Glass-Teflon homogenizer. Homogenate was centrifuged at 10,000 rpm for 15 min at 4C and supernatant obtained was stored at 20C for subsequent biochemical estimation of oxidative stress markers.19,20

2.8. BIOCHEMICAL ESTIMATION

At the end of experiment animals were anesthetized and then sacrificed and tissues were isolated and quickly homogenated for the biochemical study. Oxidative stress markers such as:

2.8.1. ESTIMATION OF MALONDIALDEHYDE (MDA)

To determine MDA levels as markers for lipid peroxidation, the thiobarbituric acid (TBA) method was carried out as described by [21] with minor modifications. Brain homogenate (500 µL) was homogenized in 10% trichloroacetic acid (TCA), mixed with thiobarbituric acid (TBA) reagent, and heated in a boiling water bath for 15 min. After cooling and centrifugation (3000 rpm, 15 min), the absorbance of the supernatant was read at 532 nm in a UV-Visible spectrophotometer 1800 (Shimadzu). MDA levels were expressed as nmol/mg protein. Higher MDA levels are associated with greater oxidative stress and lipid peroxidation in brain tissues.

2.8.2. ESTIMATION OF REDUCED GLUTATHIONE (GSH)

Contents of reduced glutathione (GSH) were measured using Ellman's method Brain homogenate was homogenized in trichloroacetic acid (TCA) and centrifuged to get the supernatant which was then incubated in the phosphate buffer with 5,5-dithiobis-(2-nitrobenzoic acid) (DTNB) to get a yellow complex and the absorbance was measured at 412 nm by a UV-Visible spectrophotometer [UV spectrophotometer 1800 (Shimadzu)]. Results are expressed as mol/mg protein; higher values for GSH suggest better antioxidant defence [22,23]

2.8.3. ESTIMATION OF SUPEROXIDE DISMUTASE (SOD)

SOD activity estimation: The SOD activity was determined based on the reduction of nitro blue tetrazolium (NBT) method. The reaction mixture, consisting of sodium pyrophosphate buffer, nitro blue tetrazolium (NBT), phenazine methosulfate (PMS), and nicotinamide adenine dinucleotide (NADH), was mixed with the brain homogenate and incubated. The reaction was stopped by the addition of glacial acetic acid, and the absorbance was measured at 560 nm in a UV-Visible spectrophotometer [UV spectrophotometer 1800 (Shimadzu)]. The activity was expressed as U/mg protein, and high enzyme activity showed improved defence against superoxide radicals. [24]

2.8.4. ESTIMATION OF CATALASE (CAT)

Assessment of Catalase (CAT) activity Brain homogenate was added to phosphate buffer containing hydrogen peroxide and decrease in absorbance caused by HO decomposition was monitored at 240 nm at UV-Visible spectrophotometer (UV spectrophotometer 1800 (Shimadzu)).6-7Catalase activity were expressed as U/mg protein. Catalase activity were more means better antioxidant protection provided by breakdown of hydrogen peroxide, decreased oxidative stress. [25]

2.8.5. ESTIMATION OF NITRIC OXIDE (NO)

Nitric oxide levels were determined indirectly by measuring nitrite levels by the Griess reagent assay. Brain homogenate was centrifuged, and the supernatant was combined with Griess reagent and incubated to generate a pink azo dye. This coloured compound was analysed for its absorbance at 540nm by a UV-Visible Spectrophotometer [UV spectrophotometer 1800 (Shimadzu)]. Nitric oxide was quantitated as mol/mg protein, wherein the increased amount of NO reflected greater nitrosative stress and neuronal damage to brain tissue [26].

2.8.6. ESTIMATION OF ACETYLCHOLINESTERASE (ACHE)

The AChE activity was estimated using Ellman's colorimetric method. 0.1 mL of brain homogenate was incubated in 0.1 M phosphate buffer, with acetylthiocholine iodide as the substrate, and 5,5-dithiobis-(2-nitrobenzoic acid) (DTNB) as the chromogenic reagent. The formation of a yellow color was estimated spectrophotometrically at 412 nm using a UV-Visible spectrophotometer [UV spectrophotometer 1800 (Shimadzu)]. The AChE activity was reported as mol of acetylthiocholine hydrolyzed/min/mg protein. Increased AChE activity is generally thought to reflect cholinergic dysfunction and decreased AChE activity reflects improvement in the neuronal function after treatment [27].

2.8.7. ESTIMATION OF TOTAL PROTEIN

Total Protein Content Total protein was quantified by Lowry's method using bovine serum albumin (BSA) as the standard. Brain homogenate was added to alkaline copper reagent, and the addition of Folin-Ciocalteu reagent was followed by incubation, after which the intensity of blue coloured complex was measured using UV-Visible spectrophotometer [UV spectrophotometer 1800 (Shimadzu)] at 660 nm wavelength. Total protein concentration was expressed as mg/g tissue (or mg/mL homogenate depending on protocol of the laboratory). Protein was estimated in order to normalise the values of biochemical parameters to assess the changes in brain tissue during PTZ induced epilepsy. [28]

2.9. HISTOPATHOLOGICAL EXAMINATION

The brain tissues were fixed in 10% neutral buffered formalin and embedded in paraffin after following the standard procedure and were then cut in to section of 4-5 m thick. The sections were stained with Haematoxylin and eosin (H&E) stains and were viewed under a light microscope to study the degree of neuronal degeneration, inflammation, oedema and other changes.

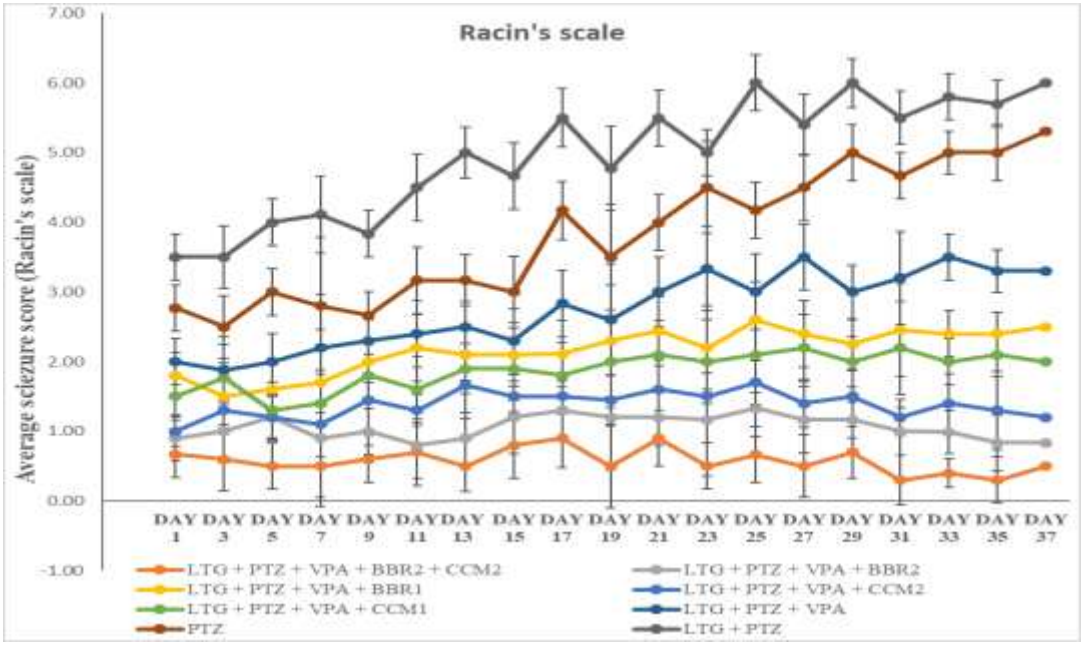
2.10. STATISTICAL ANALYSIS

The results were reported as mean standard error of the mean (SEM). Statistical analysis was carried out using one-way ANOVA followed by Tukey's multiple comparison test. Statistical analysis was done using the software GraphPad Prism. Values of $p < 0.05$ were considered to be significant.

3. Results And Discussion

3.1. INFLUENCE CURCUMIN AND BERBERINE ON PTZ-INDUCED INTRACTABLE SEIZURE SCORE

PTZ (40mg kg, i p.) administered on alternate days along with pre- treatment with GPT (20mg kg) for 45 minutes prior to administration of PTZ (40mg kg, ip) in alternate days until the animal reaches the stable kindled state(three consecutive stages and seizures) or till 19 injections of PTZ (for a period of 37 days) results in continuous escalation of convulsant potential(with the rise of score up to average of 4- 6). A significantly lower score was found for all Curcumin, Berberine and valproic acid [$F(8, 45) = 44.64$, $p< 0.05$] score compare to PTZ- treated animals. High dose of Curcumin and Berberine produced more seizures score attenuation and neuro protection than the low dose (50 mg kg).



Figure; -1 Effect of Curcumin and Berberine on PTZ-induced intractable seizures score.

Animals treated recurrently with the PTZ (40 mg kg, i.p.) in a daily alternate manner have resulted in intractable seizures/epilepsy. Pre-treatment of the mice with GPT (20 mg/kg, i.p) 45 minutes prior to the administration of PTZ (40mg/kg, i.p) on every alternate day up to that the stable kindled state of seizure severity was observed for hour following every administration of PTZ. Treatment of mice with Curcumin and Berberine (50 and 100 mg kg; p.o) and Valproic acid (200 mg kg; i.p) resulted in significant ($p<0.05$) reduction in seizures score in mice. Data have been presented as mean SEM; One way ANOVA followed by Tukey's multiple Comparison Tests. VPA (Valproic Acid), PTZ (Pentylene Tetrazole), LTG(Lamotrigine), CCM1 (Curcumin 50 mg), CCM2 (Curcumin 100 mg), BBR1 (Berberine 50 mg), BBR2 (Berberine 100 mg)

3.2. ELEVATED PLUS MAZE

The effect of the treated on learning and memory performance in the Elevated Plus Maze (EPM) test was evaluated by measuring the Transfer Latency (TL) significantly increase the acquisition and retention time when the compare to control group [$F(8, 45) = 11.23$, $p < 0.05$; $^{ab}p<0.50$ vs control. However, compared treatment group with PTZ and GPT+PTZ groups significantly decreased [$F(8, 45) = 11.23$, $p < 0.05$, $^{c}p<0.50$ vs PTZ and GPT+PTZ], The dose dependent effect has been observed.

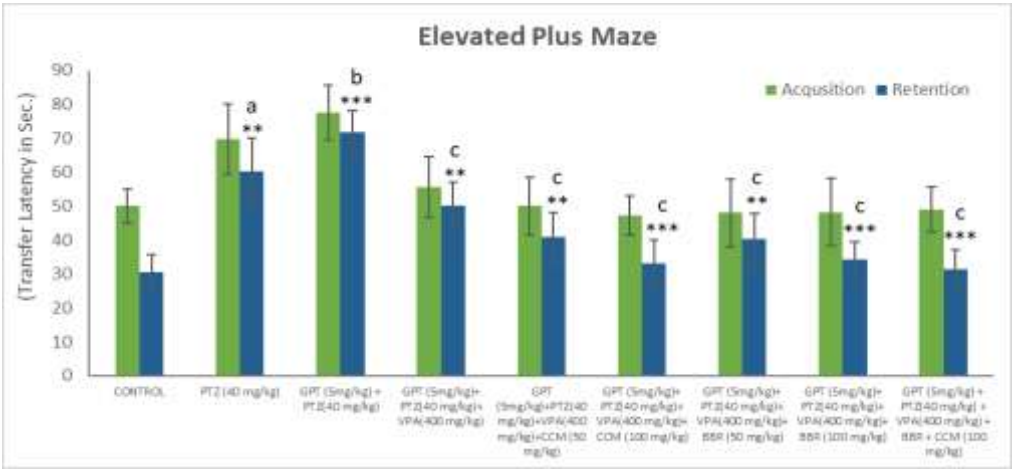


Figure: -2 Effect of Berberine and Curcumin on TL in PTZ and PTZ+GPT induced experimental animals

Results are expressed as mean ± SEM (n = 6). Statistical analysis was performed using one-way ANOVA followed by Tukey's multiple comparison test. Transfer Latency (TL) [$F(8, 45) = 11.23$, $p<0.05$, $^{ab}p<0.50$, vs control; $^{c}p<0.50$ vs PTZ and GPT + LTG] was considered statistically significant. EPM (Elevated Plus Maze Test), VPA (Valproic Acid), PTZ (Pentylene Tetrazole), GPT (Gabapentin), CCM (Curcumin), BBR (Berberine)

3.3. ACTOPHOTOMETER

The effect of the treatment on learning and memory performance in the Actophotometer test was evaluated by measuring the locomotor activity significantly decrease the locomotor activity time, when the compare to control group [$F(8, 45) = 10.23$, $p < 0.05$, $^{ab}p<0.50$, vs control], However the compared treatment group with PTZ and

GPT+PTZ groups significantly increased ELT [F (8, 45) = 10.23, P<0.05); [ab]p<0.05 vs control: [c]p<0.05 vs PTZ and PTZ+GPT) The dose dependent effect has been observed.

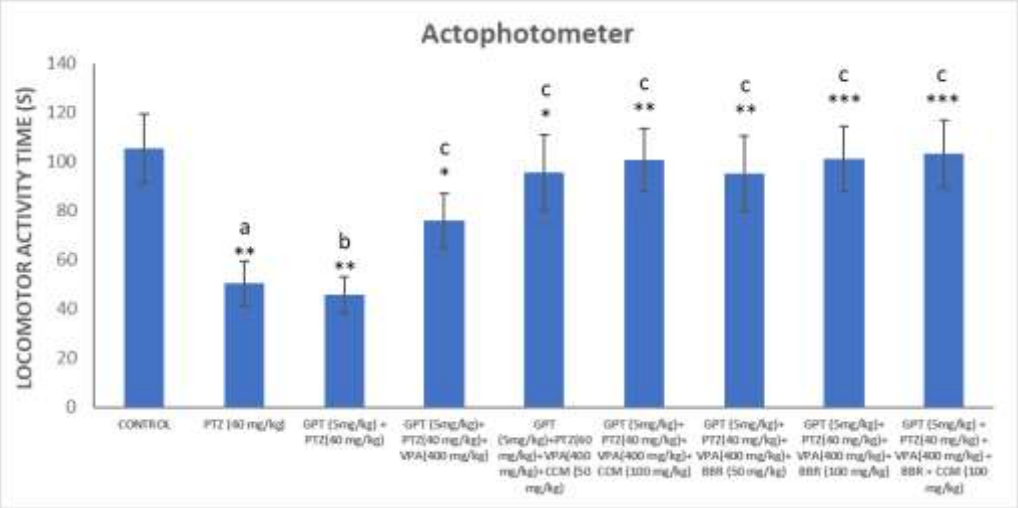


Figure:- 3 Effect of Berberine and Curcumin on locomotor activity time in PTZ and PTZ+GPT induced experimental animals

Results are expressed as mean ± SEM (n = 6). Statistical analysis was performed using one-way ANOVA followed by Tukey's multiple comparison test. Locomoter activity time [F (8, 45) = 11.23, *p*<0.05, [ab]p<0.50, vs control, [c]p<0.50 vs PTZ and PTZ+GPT was considered statistically significant. Actophotometer, VPA (Valproic Acid), PTZ (Pentylenetetrazole), GPT (Gabapentin), CCM (Curcumin), BBR (Berberine)

3.4. MORRIS WATER MAZE

The effect of the treatment on learning and memory performance or acquisition is indicated by the higher day 4 ELT in MWM trails compared to day 1st escape latency time (ELT). However, a decreased TSTQ on the fifth day indicate memory loss. The control group showed a remarkable (p<0.050) drop-in day 4 ELT compared to the first day of ELT Fig4, and a significant increase in the fifth day TSTQ (in the Q4 quadrant) compared to the other Q1, Q2, Q3 quadrants, which indicate typical learning and memory. On day 4[th], PTZ and PTZ+LTG showed that a significant increase in ELT [F (8, 45) = 11.30, p<0.05) [ab]p<0.05 vs control] and a significant decrease in TSTQ [F (8, 45) = 11.30, p<0.05) [c]p<0.05 vs PTZ and PTZ+GPT)] on day five. Fig4, However, the effect of PTZ and PTZ+GPT were considerably reversed in mice administered by the Valproic acid, Curcumin and Berberine. The dose dependent effect has been observed.

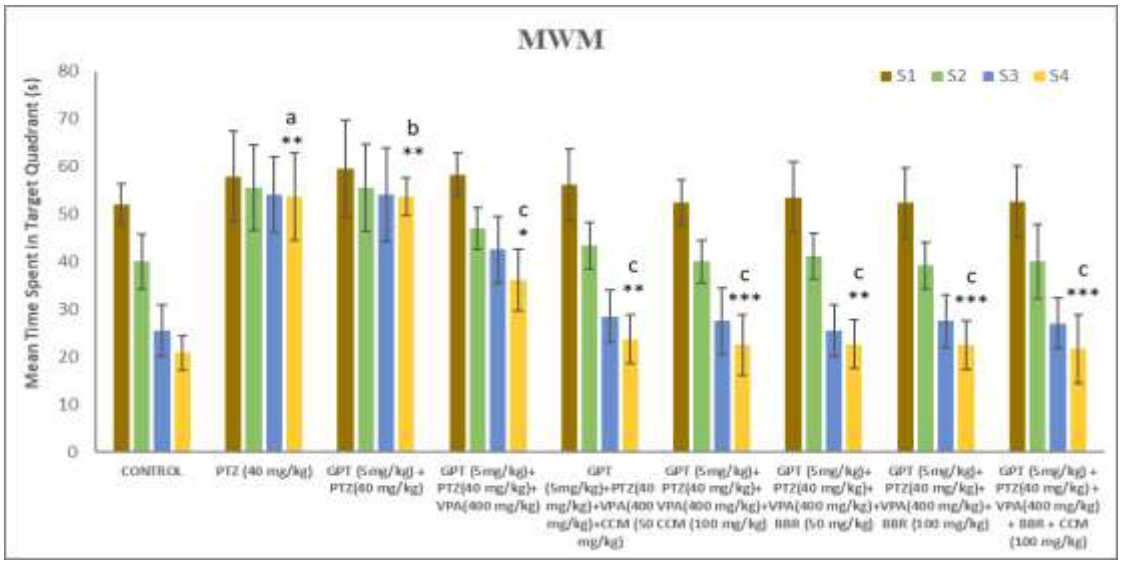


Figure:-4 Effect of Berberine and Curcumin on locomotor activity time in PTZ and PTZ+GPT induced experimental animals

Results are expressed as Mean ± SEM (n=6), one-way ANNOVA followed by Tukey's multiple comparison test. ELT [F (8, 45) = 11.30, P<0.05; [ab]p<0.05 vs control and [c]p<0.05 vs ELT in PTZ and PTZ+GPT). MWM (Morris Water Maze), ELT (Escape latency test), VPA (Valproic Acid), PTZ (Pentylenetetrazole), GPT (Gabapentin), CCM (Curcumin), BBR (Berberine)

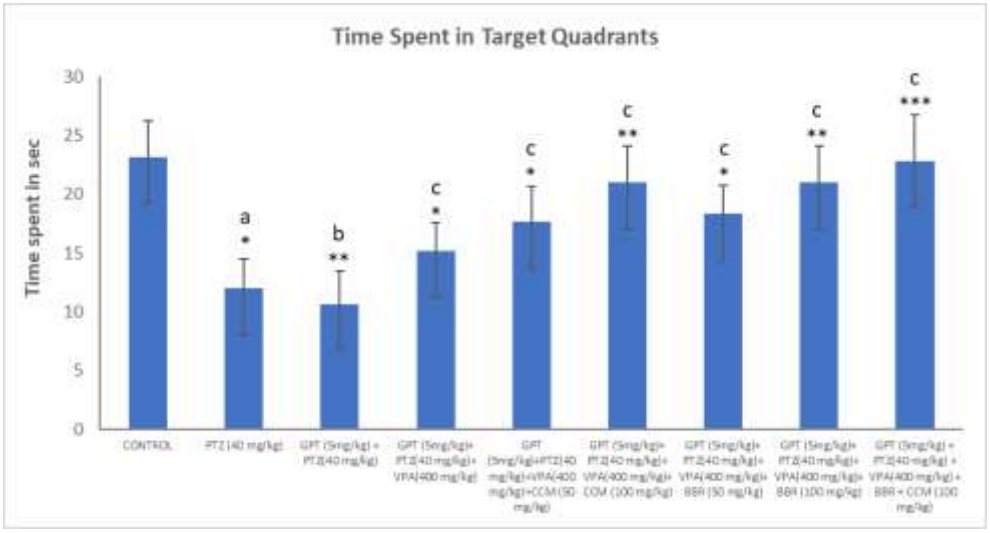


Figure:-5 Effect of Berberine and Curcumin on locomotor activity time in PTZ and PTZ+GPT induced experimental animals

Results are expressed as Mean ± SEM (n=6), one-way ANNOVA followed by Tukey's multiple comparison test. ELT [F (8, 45) = 11.23, P<0.05; ^{ab}p<0.05 vs control and ^{c}p<0.05 vs PTZ and PTZ+GPT]. MWM (Morris Water Maze), ELT (Escape latency test), VPA (Valproic Acid), PTZ (Pentylenetetrazole), GPT (Gabapentin), CCM (Curcumin), BBR (Berberine)

3.5. ESTIMATION OF REDUCED GLUTATHIONE (GSH)

When compared to the control group, the level of GSH was significantly decreased in the PTZ and GPT+PTZ treated group [F (8, 45) = 12.12, P<0.05; ^{ab}p<0.05 vs control]. When the compared treatment group with PTZ and GPT+PTZ groups significantly increased the level of GSH [F (8, 45) = 12.12, P<0.05; ^{c}p<0.05 vs PTZ and GPT+PTZ], the dose dependent effect has been observed.

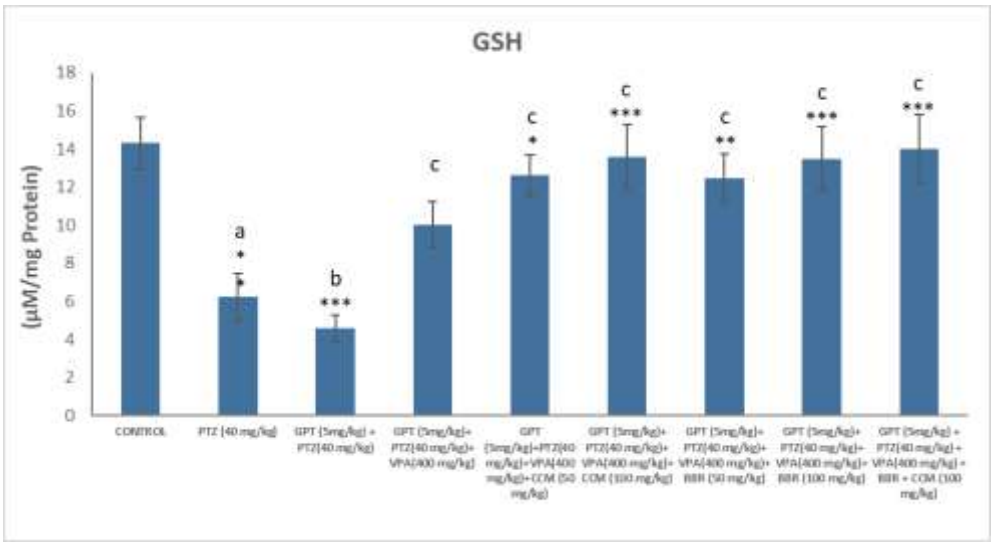


Figure:-6 Effect of Berberine and Curcumin on reduced glutathione (GSH) levels in PTZ+GPT induced experimental animals

Results are expressed as mean ± SEM (n = 6). Statistical analysis was performed using one-way ANOVA followed by Tukey's multiple comparison test [F (8, 45) = 12.12, P<0.05; ^{ab}p<0.05 vs control and ^{c}p<0.05 vs PTZ and PTZ+GPT] was considered statistically significant. GSH (Reduced Glutathione), VPA (Valproic Acid), PTZ(Pentylenetetrazole), GPT(Gabapentine), CCM(Curcumin), BBR(Berberine).

3.6. ESTIMATION OF MALONDIALDEHYDE (MDA)

When compared to the control group, the level of Malondialdehyde (MDA) was significantly increased in the PTZ and GPT+PTZ treated group the level of MDA [F (8, 45) = 9.112, P<0.05; ^{ab}p<0.05 vs control]. When the compared treatment group with PTZ and GPT+PTZ groups significantly decreased [F (8, 45) = 9.112, P<0.05; ^{c}p<0.05 vs PTZ and GPT+PTZ], the dose dependent effect has been observed.

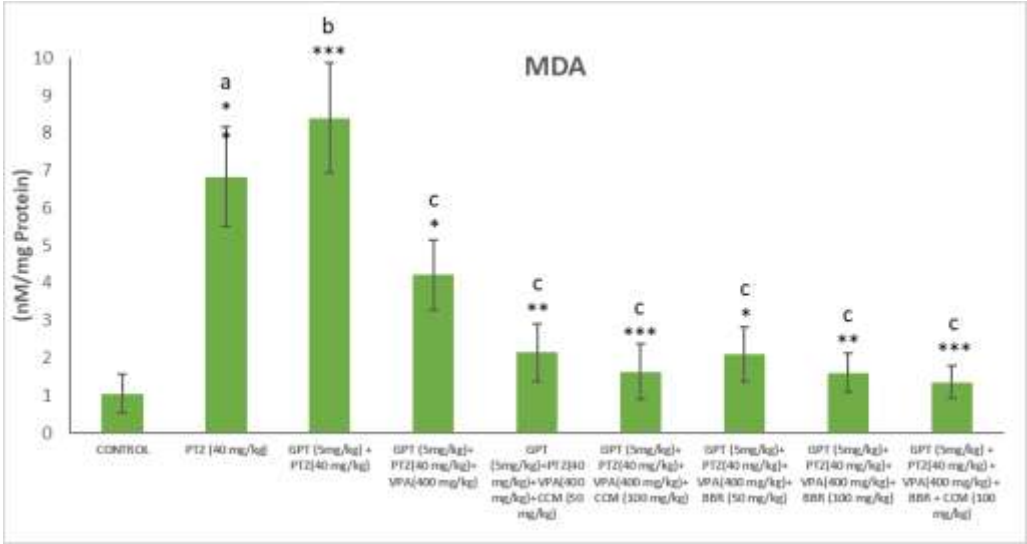


Figure:-7 Effect of Berberine and Curcumin on MDA (levels in PTZ+GPT induced experimental animals

Results are expressed as mean ± SEM (n = 6). Statistical analysis was performed using one-way ANOVA followed by Tukey's multiple comparison test [F (8, 45) = 9.112, P<0.05; ^{ab}p<0.05 vs control and ^{c}p<0.05 vs PTZ and PTZ+GPT] was considered statistically significant. MDA(Malondialdehyde**),** VPA(Valproic Acid), PTZ(Pentylene Tetrazole), GPT(Gabapentine), CCM(Curcumin), BBR(Berberine).

3.7. ESTIMATION OF NITRIC OXIDE (NO)

When compared to the control group, the level of Nitric Oxide (NO) was significantly increased in the PTZ and GPT+PTZ treated group [F (8, 45) = 9.232, P<0.05; ^{ab}p<0.05 vs control]. When the compared treatment group with PTZ and GPT+PTZ groups significantly decreased [F (8, 45) = 9.232, P<0.05; ^{c}p<0.05 vs PTZ and GPT+PTZ], the level of Nitric Oxide, dose dependent effect has been observed.

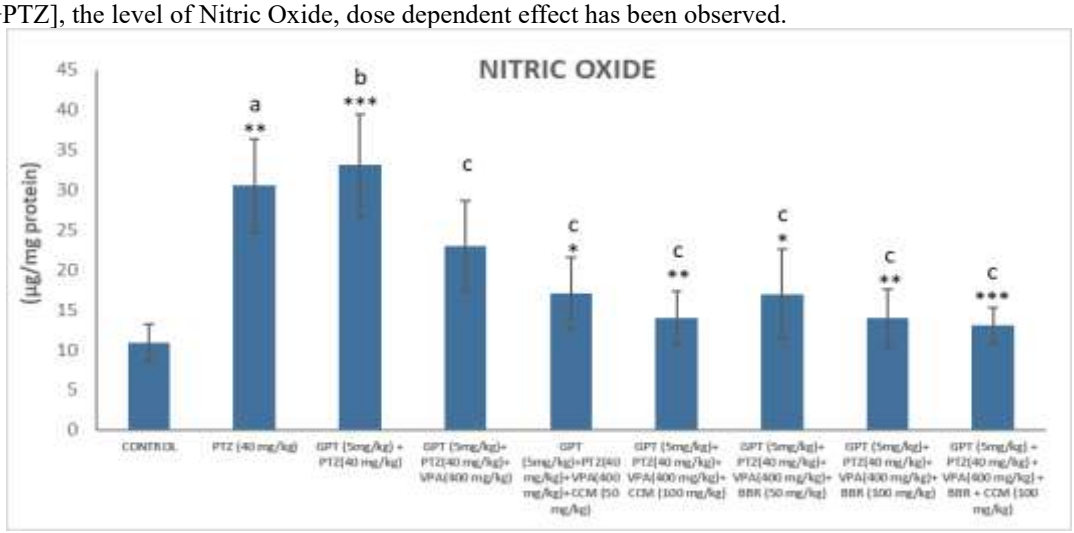


Figure: 8 Effect of Berberine and Curcumin on Nitric Oxide (levels in PTZ+GPT induced experimental animals

Results are expressed as mean ± SEM (n = 6). Statistical analysis was performed using one-way ANOVA followed by Tukey's multiple comparison test [F (8, 45) = 9.232, P<0.05; ^{ab}p<0.05 vs control and ^{c}p<0.05 vs PTZ and PTZ+GPT] was considered statistically significant. NO (Nitric Oxide), VPA(Valproic Acid), PTZ(Pentylenetetrazole), GPT(Gabapentine), CCM(Curcumin), BBR(Berberine).

3.8. ESTIMATION OF CATALASE (CAT)

When compared to the control group, the level of Catalase (CAT)was significantly decreased in the PTZ and GPT+PTZ treated group [F (8, 45) = 9.999, P<0.05; ^{ab}p<0.05 vs control]. When the compared treatment group with PTZ and GPT+PTZ groups significantly increased the level of Catalase [F (8, 45) = 9.999, P<0.05; ^{c}p<0.05 vs PTZ and GPT+PTZ], the dose dependent effect has been observed.

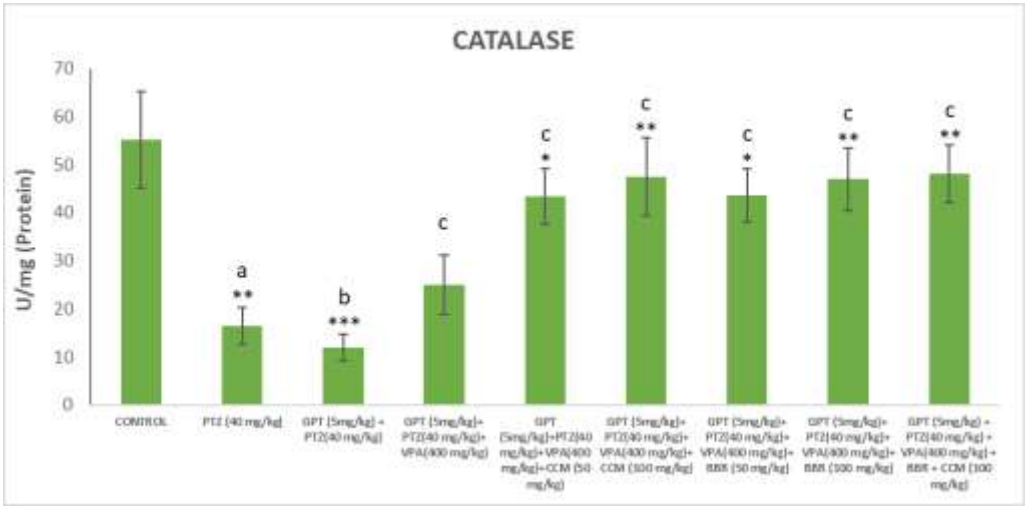


Figure:-9 Effect of Berberine and Curcumin on Catalase (CAT) levels in PTZ+GPT induced experimental animals

Results are expressed as mean ± SEM (n = 6). Statistical analysis was performed using one-way ANOVA followed by Tukey's multiple comparison test [F (8, 45) = 7.999, P<0.05; ^{ab}p<0.05 vs control and ^{c}p<0.05 vs PTZ and PTZ+GPT] was considered statistically significant. CAT(Catalase), VPA(Valproic Acid), PTZ(Pentylene Tetrazole), GPT(Gabapentine), CCM(Curcumin), BBR(Berberine).

3.9. ESTIMATION OF SUPEROXIDE DISMUTASE (SOD)

When compared to the control group, the level of Superoxide Dismutase (SOD) was significantly decreased in the PTZ and GPT+PTZ treated group [F (8, 45) = 9.932, P<0.05; ^{ab}p<0.05 vs control]. When the compared treatment group with PTZ and GPT+PTZ groups significantly increased level of Superoxide Dismutase [F (8, 45) = 9.932, P<0.05; ^{c}p<0.05 vs PTZ and GPT+PTZ], the dose dependent effect has been observed.

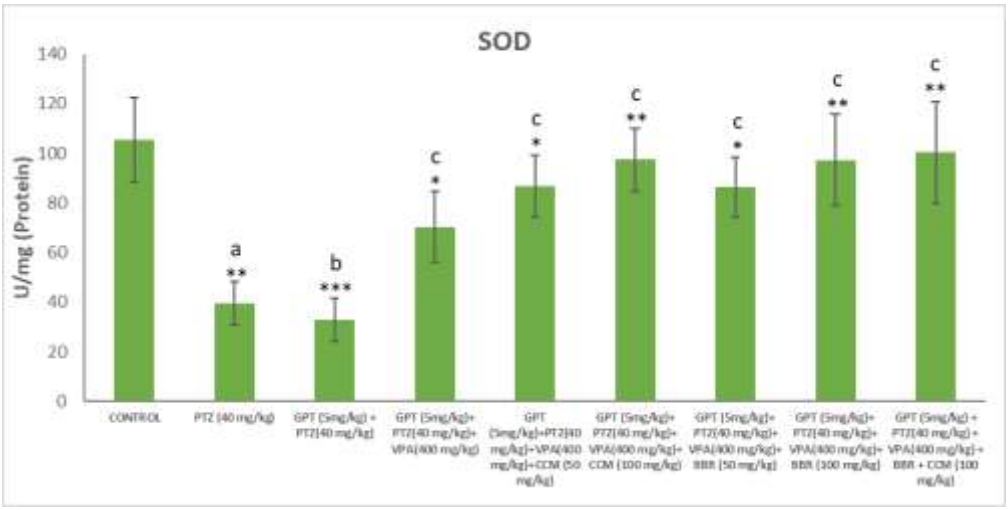


Figure:-10 Effect of Berberine and Curcumin on Superoxide Dismutase (SOD) levels in PTZ+GPT induced experimental animals

Results are expressed as mean ± SEM (n = 6). Statistical analysis was performed using one-way ANOVA followed by Tukey's multiple comparison test [F (8, 45) = 9.932, P<0.05; ^{ab}p<0.05 vs control and ^{c}p<0.05 vs PTZ and PTZ+GPT] was considered statistically significant. SOD(Superoxide Dismutase)**,**VPA(Valproic Acid), PTZ(Pentylene Tetrazole), GPT(Gabapentine), CCM(Curcumin), BBR(Berberine).

3.10. ESTIMATION OF ACETYLCHOLINESTERASE (ACHE)

When compared to the control group, the level of Acetylcholinesterase was significantly increased in the PTZ and GPT+PTZ treated group [F (8, 45) = 7.999, P<0.05; ^{ab}p<0.05 vs control]. When the compared treatment group with PTZ and GPT+PTZ groups significantly decreased the level of Acetylcholinesterase [F (8, 45) = 8.232, P<0.05; ^{c}p<0.05 vs PTZ and GPT+PTZ], the dose dependent effect has been observed.

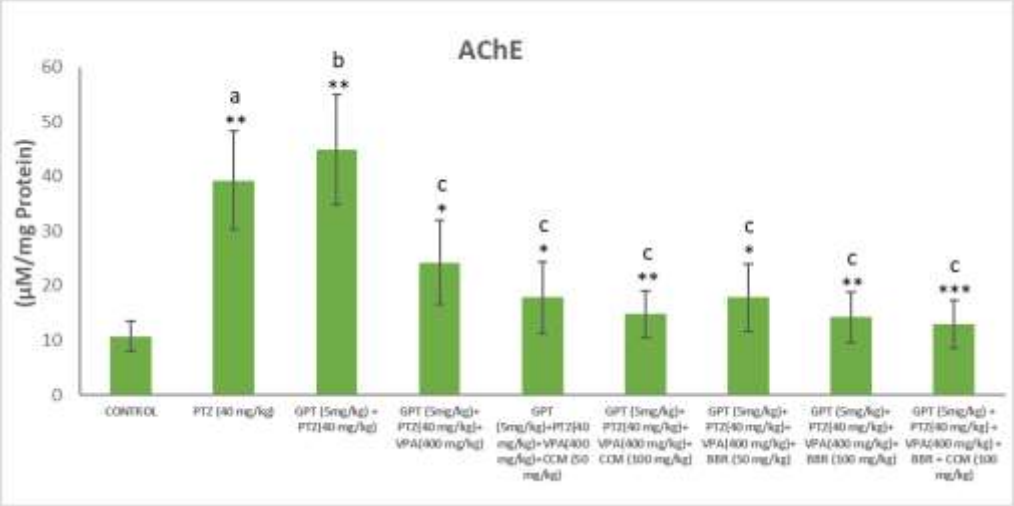
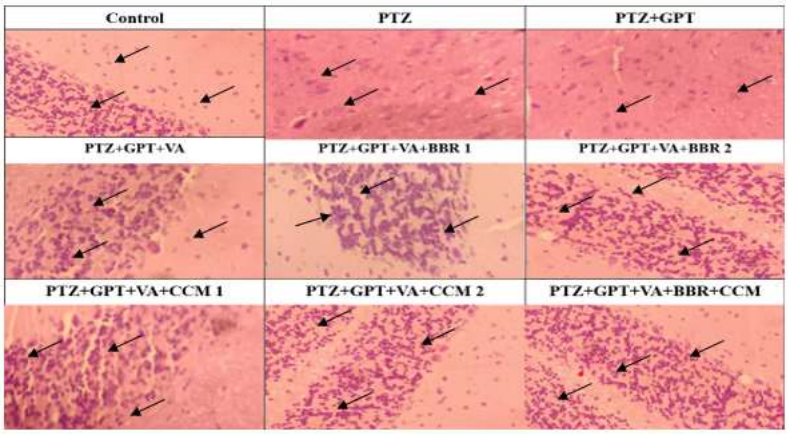


Figure:-11 Effect of Berberine and Curcumin on Acetylcholinesterase (AChE) levels in PTZ+GPT induced experimental animals

Results are expressed as mean ± SEM (n = 6). Statistical analysis was performed using one-way ANOVA followed by Tukey's multiple comparison test [F (8, 45) = 7.999, $P<0.05$; $^{ab}p<0.05$ vs control and $^{c}p<0.05$ vs PTZ and PTZ+GPT] was considered statistically significant. AChE(Acetylcholinesterase), VPA (Valproic Acid), PTZ(Pentylene Tetrazole), GPT(Gabapentine), CCM(Curcumin), BBR(Berberine).

3.11. HISTOPATHOLOGICAL EVALUATION OF BRAIN TISSUE

The H&E stained sections of brain slices showed significant variation between the groups under examination.



Figure;-12 Illustrative pictures of the H&E-stained (Magnification x 400) in the Cortex area.

I. Normal Control Group - Normal histomorphology was observed in the brain sections of group I, consisting of well-ordered neurons, preserved morphology of neurons, normal pyramid cells and absence of degeneration, edema and neuronal inflammatory cell infiltration.

II. PTZ (40 mg/kg) Group - Extreme changes were recorded in brain sections including extensive neuron degeneration, neuronal atrophy, cytoplasmic vacuolation, pyknotic nuclei, perivascular edema, congestion and neuronal inflammatory cells. The architectural arrangement of hippocampus was disturbed showing neuronal damage induced by PTZ.

III. Gabapentin 20 mg/kg + PTZ Group - Gabapentin offered moderate neuroprotective effect from PTZ toxicity.

The brain sections revealed reduced neuronal degeneration and neuronal inflammatory changes as compared to the PTZ treated group, though mild neuron loss and few pyknotic cells were seen.

IV. Gabapentin 20 mg/kg + PTZ + Valproic Acid 400 mg/kg Group - Higher protective potential than Gabapentin alone was obtained by administration of Valproic acid with Gabapentin against PTZ induced neurotoxicity. Brain section showed better preservation of cellular arrangement, reduced degenerating neuron, edema and neuronal inflammatory cells.

V. Gabapentin 20 mg/kg + Valproic Acid 400 mg/kg + Berberine1 50 mg/kg + PTZ Group - Administration of Berberine (50 mg/kg) resulted in much better recovery and improvement in histopathology than Group IV. Brain section revealed mild neuronal degeneration and reduced vacoulation, with well-preserved neurons of the hippocampus in comparison to Group IV.

VI. Gabapentin 20 mg/kg + Valproic Acid 400 mg/kg + Berberine2 100 mg/kg + PTZ Group – Significant improvement and protection against neuronal degeneration was obtained with treatment with Berberine (100

mg/kg). Neuronal architecture was well preserved and minimal neuronal loss, minimal neuronal inflammation and almost normal cellular morphology.

VII. Gabapentin 20 mg/kg + Valproic Acid 400 mg/kg + Curcumin1 50 mg/kg + PTZ Group - Significant neuroprotection was observed in the case of Curcumin (50 mg/kg). Brain sections exhibited reduced neuron degeneration and neuronal inflammatory cells with improved arrangement of neurons although few pyknotic cells were seen.

VIII. Gabapentin 20 mg/kg + Valproic Acid 400 mg/kg + Curcumin2 100 mg/kg + PTZ Group - With higher dose of Curcumin (100 mg/kg) marked improved protection were obtained with good Preservation of Neuronal architecture, minimal neuronal inflammatory cells and minimum neuronal degeneration.

IX. Gabapentin 20 mg/kg + Valproic Acid 400 mg/kg + Curcumin2 100 mg/kg + Berberine2 100 mg/kg + PTZ Group- Almost normal histopathological alterations were seen, as good neuronal architecture and well-preserved hippocampal neurons, and minimal neuronal degeneration and inflammation with nearly normal cellular architecture similar to normal control group was observed.

The histopathological changes in the brain clearly show that PTZ induces extensive neuronal damage, whereas treatment with Gabapentin and Valproic acid provides protection. Further protection is gained with the administration of either Berberine or Curcumin in a dose dependent manner. Maximum neuroprotection is seen when both Curcumin and Berberine are administrated along with Gabapentin and Valproic acid.

4. Discussion

Present investigation successfully induced the development of drug-resistant epilepsy by repeat administration of PTZ as evaluated by enhanced severity of seizures, behavioral deficits, oxidative stress and neuronal damages. [29] Moreover, pre-treatment with gabapentin afforded a protective effect against seizure propagation, while its co-administration with valproic acid resulted in higher anticonvulsant efficacy. Both curcumin and berberine provided dose dependent neuroprotective activities and the highest neuroprotective effects were obtained for the combination of curcumin (100 mg/kg) with berberine (100 mg/kg) as in our previous investigation. [30] We have also noted that treated mice regained motor activity, reduced anxiety related behaviors and improved cognitive and memory function. We also showed a reduction of MDA and NO and a significant restoration of GSH, SOD, CAT, AChE, and total protein levels that confirm attenuation of oxidative stress in brain regions of the treated animals. Histopathological studies provided a structural evidence of preservation of the neuronal architectures and minimization of neuro degeneration in neuronal pathways of treated animals. Thus the results have clearly suggested that curcumin and berberine improved the anticonvulsant and neuroprotective effects of gabapentin and valproic acid by providing protection from oxidative stress. Thus, they represent potential adjunctive treatment options in the therapy of drug resistant epilepsy.
[31,32]

5. Conclusion

In the current investigation, it was concluded that Curcumin and Berberine exhibited substantial dose-dependent neuroprotection against PTZ-induced drug-resistant epilepsy in albino mice. Co-administration of both curcumin (50 and 100 mg/kg) and Berberine (50 and 100 mg/kg) with gabapentin and valproic acid improved seizure control, behavioural responses, antioxidant profiles, and neuronal health of PTZ-induced drug-resistant epilepsy. The beneficial effect was greater at 100 mg/kg dosage for Curcumin and Berberine compared to the 50 mg/kg dosages. In particular, co-administration of Curcumin (100 mg/kg) and Berberine (100 mg/kg) resulted in marked attenuation of seizure occurrence, oxidative stress and histopathological changes in the hippocampus along with improvement of the cognitive as well as locomotor behaviors in albino mice. Therefore, curcumin and berberine could be potential dose-dependent adjunctive therapeutic agents for drug resistant epilepsy. However, detailed molecular and clinical studies are needed for the evaluation of its long-term efficacy and safety in human being.

Authors' Contributions

Each author actively contributed to the idea and design, collection of the data, or the analysis and interpretation of the findings. All authors also made substantial contributions in drafting the article and reviewing it.

Ethics Approval And Consent To Participate

The protocol was approved by Institutional Animals Ethics Committee (IAEC) (1024/PO/Re/S/08/CPCSEA).

Human And Animal Rights

No humans were used in this research. All animal experimentation was done according to IAEC guidelines.

Consent For Publication

Not applicable.

Availability Of Data And Materials

The data and supportive information is available within the article.

Funding

None.

Conflict Of Interest
The authors declare no conflict of interest, financial or otherwise.

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