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SUMMARY AND CONCLUSIONS

The present study was carried out to investigate the neuroprotective potential of curcumin in ameliorating neurobehavioral, biochemical and histological changes observed in PTZ induced chronic epilepsy. Keeping in view the above objective, male Wistar rats weighing about 200-250 g were randomly segregated into four groups: Control group [normal saline, intraperitoneally], PTZ treated group [PTZ 40 mg/kg body weight, intraperitoneally for 30 days, (every alternate day)], PTZ + Curcumin treated group [curcumin 100 mg/kg body weight daily for 40 days, 30 min PTZ injection, Curcumin treated group [curcumin 100 mg/kg body weight daily, orally for 40 days]. The effect of curcumin on neurobehavioral deficits, oxidative stress, mitochondrial dysfunctions, neuroinflammation and blood brain barrier impairment associated with PTZ induced chronic epilepsy was studied in cortex and hippocampus. The important findings of the present study are summarized as follows:

1. The effect of curcumin supplementation on the seizure score was studied. Repetitive administration of sub-convulsive dose of PTZ for 30 days resulted in severe generalized clonic-tonic seizures in animals, whereas curcumin supplementation to PTZ treated animals showed no anti-epileptic effect.

2. PTZ treated animals showed no change in the body weight, whereas control animals and PTZ treated animals supplemented with curcumin had a significant gain in body weight.

3. Rotarod test was used to evaluate the motor coordination of PTZ treated animals. No impairment in the motor co-ordination of animals was observed. Curcumin supplementation also had no effect on motor coordination.

4. To examine the effect of PTZ administration on the locomotor activities, actophotometer was used. A significant increase in the number of photo beam counts were observed in PTZ animals compared to controls, suggesting an increase in the locomotor activity of PTZ animals. The locomotor activities remained unaltered in PTZ treated animals supplemented with curcumin.

5. Elevated plus maze was used to access the effect of PTZ induced seizures on anxiety-like behavior of animals. PTZ treated animal showed an increase in the number of entries and time spent in the closed arm suggesting an increase in anxiety. Curcumin supplementation
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to PTZ animals showed increase in the number of entries and time spent in the open arm suggesting an anxiolytic potential of curcumin.

6. The spatial learning and memory of PTZ treated animals was assessed in terms of transfer latency on elevated plus maze. The percentage retention in PTZ treated animal was found to be significantly decreased; suggesting deficits in learning and memory of PTZ treated animals. On the other hand, curcumin supplementation to PTZ treated animals resulted in increased transfer latency suggesting improvement in memory functions in PTZ treated animals.

7. Active and passive avoidance task was performed to assess the effect of PTZ induced seizures on short and long term memory in animals. It showed significant impairment in both consolidation as well as long term memory in PTZ animals (decreased entrance latency dark compartment) after 30 days. However, curcumin supplementation to PTZ treated animals (avoided entering to the dark compartment) showed better consolidated as well as long term memory suggesting curcumin improves memory functions in animals with chronic epilepsy.

8. The exploratory behavior of animals was assessed using Y maze test, PTZ treated animals showed decrease in number of novel arm entries suggesting decreased exploration for the novel environment. On the other hand, curcumin supplementation to PTZ treated animals increased the number of entries to novel arm, hence suggesting increase in the exploratory behavior towards novel environment.

9. Memory impairment was assessed using Morris water maze task, wherein the time taken by PTZ treated animals to reach the hidden platform in maze was more as compared to controls (increased escape latency) suggesting decline in memory functions. Curcumin supplemented to PTZ treated animals decreased the escape latency to the hidden platform, thus improving memory functions in animals with chronic epilepsy.

10. Acetylcholinesterase (AChE) plays a major role in synaptic plasticity, specifically in learning and memory. The AChE activity significantly decreased in hippocampus whereas, an increase was observed in cortex of PTZ treated. Curcumin supplementation to the PTZ animals ameliorated AChE activity in both brain regions.

11. Oxidative stress has been recognized as a contributing factor in pathophysiology of epilepsy. Therefore, the levels of lipid peroxidation in terms of MDA were assessed in
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animals. PTZ administered rats exhibited marked increase in the lipid peroxidation in hippocampus and cortex. On the other hand, curcumin supplementation to PTZ treated animals resulted in reduction of lipid peroxidation.

12. The activity of SOD, catalase and GST was measured in both the hippocampus and cortex to access the antioxidant status in animals following seizures. PTZ treated animals had reduced SOD, catalase and GST activities. PTZ treated animals supplemented with curcumin exhibited increased activities of these enzymes suggesting an improvement in antioxidant defence system.

13. The levels of total thiols including protein thiols and non-protein thiols (GSH) were found to decrease in PTZ treated animals. Curcumin administration to PTZ animals restored thiol levels in hippocampus and cortex, suggesting beneficial effect of curcumin in protecting thiols.

14. Increased in oxidative stress could lead to increased mitochondrial dysfunctions. Therefore, the mitochondrial oxidative stress and respiratory chain functions were measured in PTZ treated animals. A significant increase in mitochondrial ROS and protein carbonyl levels were observed in PTZ treated animals along with decreased activities of NADH dehydrogenase and cytochrome oxidase enzyme in hippocampus and cortex suggesting the involvement of mitochondrial oxidative stress in pathophysiology of epilepsy. The mitochondrial oxidative stress was found to be decreased significantly accompanied by improvement in respiratory chain functions in PTZ treated animals supplemented with curcumin.

15. Mitochondrial structural changes in terms of mitochondrial swelling and ultrastructural alterations were studied using TEM. Mitochondrial swelling was observed in PTZ treated animals, along with it animals exhibited severe damage to mitochondrial structure characterized by disruption of mitochondrial membrane integrity, distorted cristae with clearing of matrix density. These results suggest that abnormalities in the brain mitochondrial activity associated with seizure can induce changes in mitochondrial ultrastructure. Curcumin supplementation decreased mitochondrial swelling and protected the brain mitochondria from seizure induced structural alterations.

16. Generation of free radicals or increased mitochondrial oxidative stress could play a role in CNS inflammation. Thus, neuroinflammation in chronic epilepsy was studied in terms of
activation of astrocytes (GFAP) and microglial (Iba-1) cells. The mRNA and protein expression of GFAP and Iba-1 was found to be increased in PTZ treated animals as evaluated using real time PCR and western blotting respectively suggesting the activation of glial cells following seizures. However, curcumin supplementation to PTZ animals decreased the expression of GFAP and Iba-1 in both the regions of brain.

17. In addition, reactive astrogliosis and microgliosis in chronic epilepsy was examined using immunohistochemistry, which revealed an increase in immunoreactivity for GFAP and Iba-1 in PTZ treated animals suggesting glial cells activation in epilepsy and could play a role in PTZ induced seizures. Curcumin supplementation to PTZ animals resulted in decreased expression of GFAP and Iba-1 in both the regions.

18. Activated microglia cells can release inflammatory mediators such as cytokines and chemokines which could initiate a pro-inflammatory signaling cascade. The release of pro-inflammatory cytokines (TNF-α, IL-1β, IL-6) and chemokines (MCP-1) were assessed in the brain. An increased mRNA and protein expression of TNF-α, IL-1β, IL-6 and MCP-1 were observed in hippocampus and cortex of PTZ treated animals suggesting hyperinflammatory state in chronic epilepsy. Curcumin supplementation to PTZ treated animals decreased the mRNA and protein levels of these pro-inflammatory cytokines and chemokines.

19. Activated glia cells can contribute in leakage across the blood-brain barrier (BBB), causing cerebral edema. Increased uptake of sodium fluorescein and Evans blue dye along with increased water content in hippocampus and cortex suggested increased permeability of BBB and edema in PTZ treated animals. Curcumin supplementation significantly decreased Evans blue and sodium fluorescein extravasation into the brain regions accompanied by decreased edema.

20. Electron micrographs of blood vessels in hippocampus and cortex of PTZ treated animals also showed increased swelling in endothelial and sub-endothelial zone (edema) with disrupted mitochondrial structures. Curcumin supplementation protected the endothelial cells from damage by reducing edema in the brain regions.

21. A significant increase in MMP-9 activity along with its increased mRNA expression was observed in hippocampus with no change in cortex of PTZ treated animals when compared to controls. Curcumin supplementation to PTZ treated animals significantly
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attenuated MMP-9 activity and mRNA levels in hippocampus when compared to PTZ treated animals.

22. Seizure associated oxidative stress and inflammatory changes such as astrogliosis and presence of pro-inflammatory cytokines can contribute to brain damage in epilepsy. Neuronal cell loss was assessed in the brain tissue using cresyl violet and H & E staining. PTZ treated animals showed shrunken nucleus (pyknotic) and cytoplasm in hippocampus and cortex when compared to control animals. Curcumin supplemented to PTZ animals exhibited normal morphology of cells in hippocampus and cortex similar to that of control animals.

23. To examine the degenerating neurons in PTZ treated animals, Fluoro Jade B staining was performed. A few Fluoro Jade B positive cells were observed in hippocampus and cortex following PTZ treatment. Curcumin supplementation to PTZ animals showed no change in Fluoro Jade B staining.

24. TUNEL staining was performed in brain sections of PTZ animals. A very few TUNEL positive cells were observed in hippocampus and cortex of PTZ treated animals when compared to respective controls and the results were consistent with fluoro jade staining. Curcumin supplementation also showed no change in the TUNEL staining among different groups.

25. NF-κB transcription factors was measured using electrophoretic mobility shift assay. No significant induction of NF-κB was observed in PTZ treated animals, neither curcumin supplementation had any effect on the NF-kB binding activity.

In conclusion, these findings clearly demonstrate that PTZ induced seizures increased oxidative stress, resulted in mitochondrial dysfunctions, activated astrocyte and microglial cells. These activated glial cells in turn increased the concentration of pro-inflammatory cytokines and chemokines in the hippocampus and cortex of PTZ animals resulting in increased BBB permeability and edema. Increase in oxidative stress and inflammation in PTZ treated animals contributed to increased neuronal damage. Altered acetylcholinesterase activity and change in antioxidant status in PTZ animals possibly impaired neurobehavioral functions including learning and memory. The results further revealed that curcumin supplementation attenuated glial cells mediated inflammation and BBB damage in chronic epilepsy possibly via inhibiting oxidative stress and improving mitochondrial functions.
Curcumin supplementation also reduced neuronal cell loss and effectively improved learning and memory in chronic epilepsy. Based on these findings, it is suggested that curcumin supplementation has a beneficial role in chronic epilepsy and may be used as a potential therapy to reduce deficits in chronic epilepsy. However, further studies are required at molecular level to elucidate the precise mechanism of action underlying the neuroprotective potential of curcumin.