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Effects of curcumin on memory, hippocampal acetylcholine level and neuroapoptosis in repeated cerebral ischemia rat model

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Abstract: The aim of this study was to determine the protective effects of curcumin on memory, hippocampal acetylcholine level and apoptosis in a rat model of repeated cerebral ischemia. Male Wistar rats were divided into sham rats that received saline and the other 3 groups underwent 4-vessel occlusion brain ischemia (4VOI), received oral administration of either saline or curcumin at doses rate of 25mg/kg/day and 50mg/kg/day for 7 days. Memory function was evaluated by eight-arm radial maze task and Morris water maze (MWM) test, Acetylcholine release (ACh) in the dorsal hippocampus was evaluated by microdialysis-HPLC) and neuron apoptosis was investigated by terminal deoxynucleotidyltransferase mediated fluorescein-deoxyuridine triphosphate nick-end labeling. 4VOI test reviled impaired memory, reduced dorsal hippocampus Ach level and induced apoptosis in the Repeated Cerebral Ischemia rat model. Curcumin significantly improved the memory deficit ($p<0.001$), increased Ach level ($p<0.001$) and prevented hippocampal neuron apoptosis ($p<0.001$). Curcumin may be suggested as a promising therapy for ischemic cerebrovascular dementia and its beneficial effect is due to its memory preserving, ACh-increasing and neuroprotective effects in the rat.

Keywords: Curcumin, neuroprotective, memory, acetylcholine, apoptosis.

INTRODUCTION

The human brain is an organ with a very high rate of oxygen consumption due to its high metabolism (Watts *et al*., 2018). The brain consumes oxygen nearly 10 times more than the average body oxygen consumption corrected for the organ weight (Furtado *et al*., 2018). This high level of oxygen dependence causes severe brain damage in case of ischemia caused by any pathology (Jurcau and Simion, 2021). There is a complex mechanism to regulate cerebral homeostasis and blood flow to supply enough oxygen for the metabolic needs of the brain, which is called cerebrovascular autoregulation (Nogueira *et al*., 2022). Reduction of cerebral blood flow is one of the most common causes of disruption of cerebrovascular autoregulation, which can lead to serious damage to brain function (Megjhani *et al*., 2021). Cerebral ischemia caused by stroke is the most common clinical presentation of this disorder (Kolouri *et al*., 2016).

Curcuma longa L. (Turmeric) is traditionally used as a medicinal spice in different ancient civilizations like China, Egypt, Persia and India (Prasad and Aggarwal, 2011, Khiljee *et al*., 2015, Mosavat *et al*., 2018). It was used for different ailments including age-related memory loss (Sumathi *et al*., 2017, Ayati *et al*., 2019, Debjit Bhowmik *et al*., 2009). Curcumin is known as the main constituent responsible for turmeric's medicinal effects including anti-inflammatory (Ferguson *et al*., 2021), anti-cancer (Wong *et al*., 2021), antibacterial (Teow and Ali, 2015), antioxidant (Miao *et al*., 2015), anti-atherosclerotic (Singh *et al*., 2021), anti-hyperlipidemic (Singh *et al*., 2021) and anti-diabetic effects (Quispe *et al*., 2022).

Recent evidence has demonstrated the neuroprotective effects of curcumin in various experimental and clinical models of neurologic disorders (Wynn *et al*., 2018, Small *et al*., 2018, Sarraf *et al*., 2019). Curcumin's molecular structure provides it with the potential to cross the blood-brain barrier. Curcumin showed to have promising effects against Alzheimer's disease, memory impairment and cerebral ischemia (Ovbiagele, 2008, Bhat *et al*., 2019). Experimental studies indicate the protective effects of curcumin against ischemic reperfusion injury in a rat model of stroke (Wang and Xu, 2020, Wu *et al*., 2020, Zhou *et al*., 2020). It is also shown that curcumin can affect molecular pathways involved in memory performance including dopamine, serotonin, as well as acetylcholine-esterase activity Amiri *et al*., 2021). In general, these reports show that curcumin has potential effects in reducing neuronal and brain injury in an experimental model of ischemia. However, its therapeutic effect in reducing hippocampal neuronal apoptosis and preserving the acetylcholine level as well as memory is not yet demonstrated in the experimental model of repeated cerebral ischemia. Therefore, the aim of this study was to determine the protective effects of various doses of curcumin on memory, hippocampal acetylcholine level and apoptosis in a rat model of repeated cerebral ischemia.

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MATERIALS AND METHODS

Animals

Forty male Wistar rats aged 8-10 weeks old (weighing 200-250 g) were housed under a temperature of 23±2 °C and humidity of 60±5% with a 12 hours light/dark cycle. Food and water were available ad libitum. Animal housing and experimental procedures were compatible with the Guidelines for Ethical Conduct in the Care and Use of Animals. All procedures and experimental protocols were approved by the local Animal Ethics Committee (Approval number EC-2021-754).

Forty selected rats were divided into four random equal groups (n=10) after training sessions. The three active experimental groups were 4VOI rats that received either distilled water or curcumin (Santa Cruz Biotechnology, CAS 458-37-7, Purity≥95%) 25 or 50 mg/kg/day orally for 7 days. The rats in the sham group underwent vertebral artery cauterization while receiving distilled water. The occluders were inserted into the common carotid arteries in this group without occlusion. No mortality was observed.

Radial 8-arm maze (RAM) performance

The RAM performance was evaluated as previously explained in detail (Shukitt-Hale *et al.,* 2004). Each behavioral testing session was conducted in a standard RAM consisting of a central platform 25 cm in diameter with eight arms of 70 cm (length)×10 cm (width)×15 cm (height) each, radiating equiangular from the central platform that served as a starting base. The white-painted maze was placed at a fixed position to reduce the variability of each test (Nogami-Hara *et al*., 2018).

The rats were pre-trained and trained before the assessment of the effects of 4VOI and interventions on memory as explained previously (Traystman, 2003). Briefly, the animals (in 5 rat groups) were adapted to the apparatus and the food pellets in 3 daily sessions of ten minutes for three days as pre-training. After that, the fourteen days training course was started. In this stage, the rats were permitted to freely move to the arms. The tests were finished on entering all arms eating the baits, or after 10 minutes were passed. The performance of the animals was assessed using the number of correct and error choices in the first eight chosen arms. Only rats that made less than two errors were enrolled with further steps of the experiment. This protocol was selected to evaluate working memory that is mainly affected by the 4VOI.

Morris water maze (MWM) test

MWM was also used to evaluate learning and memory performance seven days after 4VOI as described previously (Barnhart *et al*., 2015). Briefly, a 94 cm round white swimming pool filled with 25±1°C opaque water with 30cm depth was used as a maze. The escape platform (25cm^2) was positioned in the center of one quadrant of the pool and submerged at 1cm beneath the water's surface. Rats were allowed to find the platform (60sec/trial). Escape latencies are defined as the time spent to locate the platform through the test.

Induction of 4VOI

4VOI experimental model was induced as explained previously *(Deng and Xu, 2009)*. 4VOI was performed after the completion of the training. Intraperitoneal sodium pentobarbital with a dose of 50 mg/kg was used for anesthetizing. Stereotaxic apparatus was applied for the immobilization of rats. Electrocauterization of vertebral arteries was done with a bipolar coagulator. Common carotid arteries were exposed. Three days after electrocauterization, both common carotid arteries were occluded using aneurysm clips. The occlusion was performed by awakening rats repeatedly for 10 minutes at 60-minute intervals. The temperature was stabilized at 37°C, using a heating lamp and pad during anesthesia and4VOI procedure. The rats with saved righting reflex after arterial occlusion were excluded from further experiments. Sham rats underwent cauterization of the vertebral arteries and fitting common carotid arteries occluders without occlusion.

Microdialysis procedures

The Acetylcholine (ACh) levels were evaluated in the extracellular space of the dorsal hippocampus by microdialysis in post-4VOI rats before and after the intervention as explained in detail previously *(Bianchi et al., 2003)*. Microdialysis probes (CMA Microdialysis, North Chelmsford, Mass) with the size of 13*0.6*0.5 mm were used. An active U shape dialysis membrane made from cellulose fiber was applied. The probes were inserted through a guide into the dorsal hippocampus penetrating up to 2mm into the brain. Probes were primed with Ringer and then implanted into the dorsal hippocampus. Perfusion fluid was pumped into the probe via a perfusion pump at a rate of 2µl/min. The dialysate was collected into a collection device. It was then investigated by high-performance liquid chromatography (HPLC) (Shimadzu 10ADvp HPLC system) for ACh level.

Histochemical studies

Histochemical analysis was performed as previously explained in detail *(Nogami-Hara et al., 2018).* Fifteen days post-4VOI intervention, the rats were perfused transcardially with heparinized saline followed by 4% paraformaldehyde under sodium pentobarbital anesthesia (50mg/kg Intraperitoneally). The brains were removed and fixed by paraformaldehyde. After lipid clearance and dehydration using an auto-degreasing machine, the brain samples were embedded in paraffin. Five µm thickness coronal sections were mounted on slides. The samples underwent deparaffinization and rehydration and then

they were incubated in proteinase K buffer for 10 minutes. Terminal deoxynucleotidyl transferase UTP nick end labeling (TUNEL) was used for the detection of apoptotic cells of the dorsal hippocampus. The cross-sections were examined for TUNEL-positive cells by fluorescence microscopy. CA1 cells were stained using propidium iodide and cresyl violet staining. NIS-Elements software (Nikon, Tokyo, Japan) was used for quantitative analysis of TUNEL staining results.

STATISTICAL ANALYSIS

Quantitative outcomes including the level of ACh and RAM performance were evaluated by one-way ANOVA after confirmation of homogeneity of variance. Tukey post hoc test was used for multiple comparisons with significant differences among groups in the one-way ANOVA test. $p<0.05$ was considered statistically significant. Data are presented as the mean±standard deviation. IBM SPSS Statistics version 25 was used for the analysis of data. GraphPad Prism 8 was used for graph plotting

RESULTS

Morris water maze test

The MWM test demonstrated that 4VOI significantly increased the escape latency whereas curcumin in both (25mg/kg/day and 50mg/kg/day) doses significantly improved the escape latency ($F=4.992$, $P<0.0001$). The data also demonstrated a significant decrease in the percentage of time spent in the target quadrants with 4VOI which was improved by curcumin ($F=51.20$; $P<0.001$]. No significant difference was observed between different doses of curcumin in MWM test results. (fig. 2)

RAM performance

The RAM performance study demonstrated that 4VOI disturbed this function, showing as decreased corrected choices, whereas curcumin improved memory [$F=14.82$; $P<0.001$]. As shown in fig. 3A, corrected choices were decreased by 31% ($P<0.001$) in 4VOI, while correct choices increased in curcumin (25mg/kg/day and 50mg/kg/day) receiving rats by 23%, ($P<0.028$) and 34% ($P<0.001$), respectively. The data also demonstrated an increase in error choices with 4VOI which was improved by curcumin ($F = 93.64$; $P<0.001$]. fig. 3B demonstrates increased error choices by 359% ($P<0.001$) in 4VOI rats which was decreased by 32% ($P<0.001$) and 47% ($P<0.001$) in curcumin (25mg/kg/day and 50mg/kg/day) groups, respectively.

Acetylcholine level

The basal ACh levels were 89.90±21.25 and 102.10±7.31fmol/100 µL in the sham rats before and after the vehicle, respectively. No significant difference was observed between the vehicle and curcumin groups before the intervention ($P=1.000$, $P=0.953$). There was a significant difference between the ACh levels in different groups [$F=67.36$; $P<0.001$] after the intervention. 4VOI group showed a 51% reduction of ACh to 50.1000± 7.03fmol/100µL and a significant increase ($P<0.001$) by curcumin (25mg/kg/day and 50mg/kg/day) to 73.50± 7.17fmol/100µL ($P<0.001$) and 79.70±6.77fmol/100µL ($P<0.001$) (fig. 4). Hippocampus neuronal apoptosis

The cellular structure of CA1 of the hippocampus of the sham rats is demonstrated in fig. 4A. 4VOI induced neuronal damage in the CA1 (fig. 5B). Sections of rats that received different doses of curcumin (fig. 5C and 5D) showed more preserved cellular architecture. It can be seen in fig. 5B that there are more apoptotic cells (TUNEL-positive) in the CA1 of the 4VOI rats than sham group (fig. 5E). On the other hand, the neuronal features and expression of TUNEL-positive cells of curcumin-treated- 4VOI rats are relatively similar to the sham rats (fig. 5F, 5H). Quantitative analysis of the number of TUNEL-positive cells in each group showed statistically significant less apoptosis in both curcumin groups compared to the sham group ($F =103.40$; $P<0.001$) (fig. 5 graph).

DISCUSSION

Although the memory-improving effect of curcumin has been suggested by previous studies, the associated role of acetylcholine release and apoptosis in the hippocampus is not well investigated. Therefore, in the current study, the effects of curcumin on 4VOI-induced memory impairment with a focus on the ACh level and apoptosis in the CA1 region of the dorsal hippocampus were investigated.

The study demonstrated that 4VOI-induced neuronal apoptosis in the CA1 region of the dorsal hippocampus decreased ACh in this area and provoked memory impairment. Results showed a greater increase in the number of error choices caused by 4VOI compared to the observed decline in the correct choices. This could be explained by more impairment of working memory compared to reference memory in 4VOI(Davis *et al*., 1987). The animal model of ischemia used in this investigation showed previously to induce apoptosis of the pyramidal neurons of the dorsal hippocampusCA1 region (Haraguchi *et al*., 2010). This study confirmed the apoptosis induction in the dorsal hippocampus neurons by 4VOI and also showed the preventive effect of curcumin in 25mg/kg/day and 50mg/kg/day doses against the apoptosis. The experiment also showed a protective effect of curcumin on 4VOI-induced decreased ACh level in the dorsal hippocampus.

Memory-improving effects of curcumin were investigated and supported by multiple studies previously.

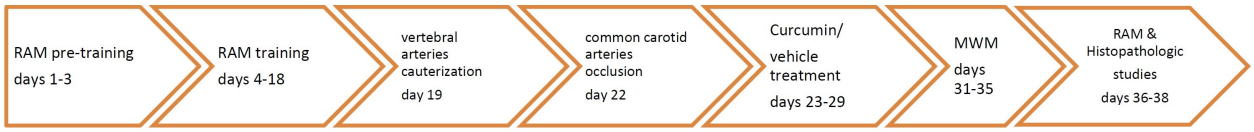

Fig. 1: The experiment timeline

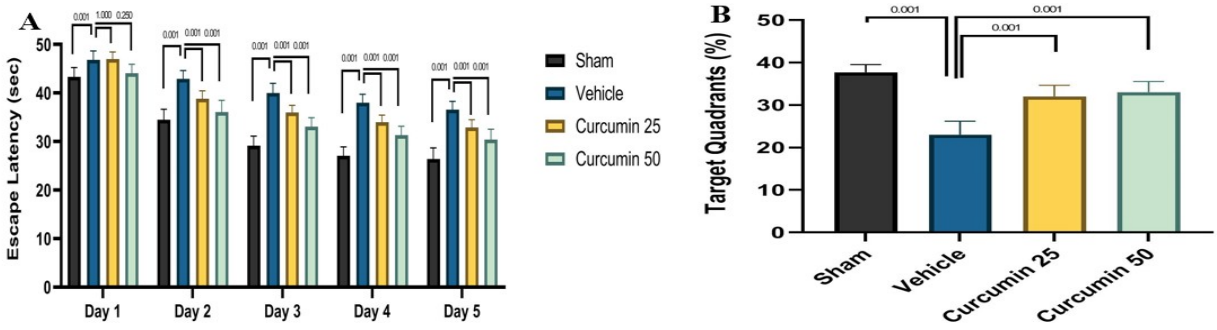

Fig. 2: Escape latency in Morris water maze test from day 1 to day 5 (A). Percentage of time spent in the target quadrants during the Morris water maze test (B). One-way ANOVA is used for statistical comparison among groups.

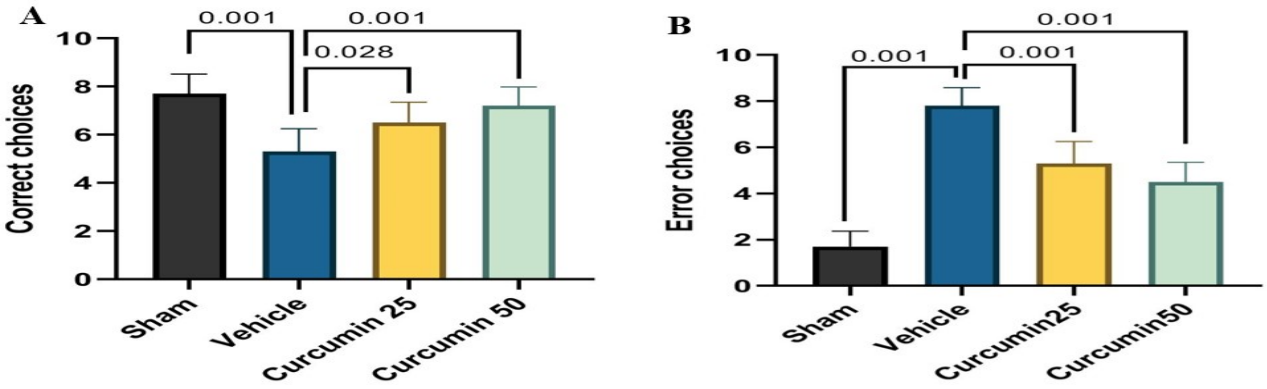

Fig. 3: Correct (A) and error choices (B) in the Radial 8-arm maze performance.
The results show significant decrease in correct choices and significant increase in error choices in 4VOI rats (vehicle group) which was significantly improved by curcumin in 25 and 50mg/kg/day doses. No significant difference was observed between two different doses of curcumin. One-way ANOVA is used for statistical comparison among groups.

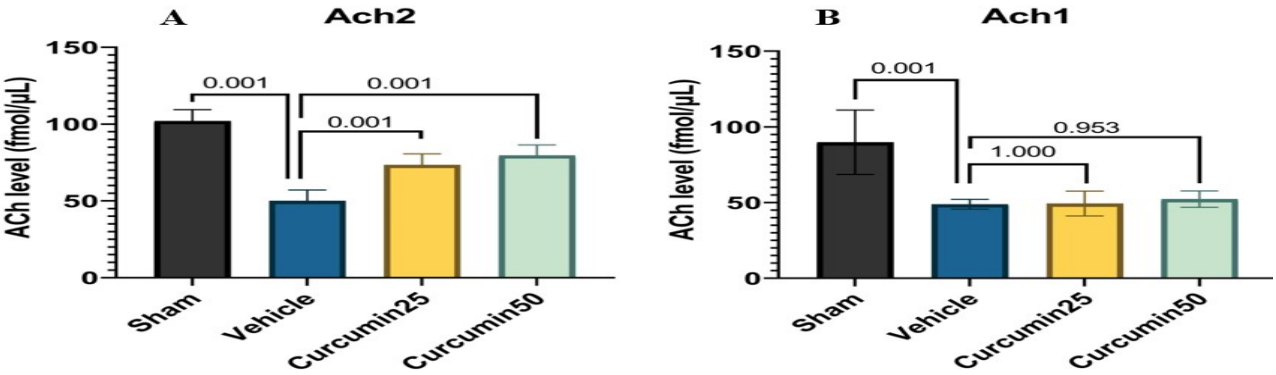

Fig. 4: Effect of curcumin on the ACh level in the dorsal hippocampus before (Ach1) and after (Ach2) final dose of drug administration.
The ACh1 chart shows the ACh level after 4VOI and before the intervention. Significant decrease in ACh level by 4VOI in 3 intervention groups compared to sham group. The ACh2 chart shows ACh level after intervention. Significant increase in Ach level by curcumin in 25 and 50 mg/kg/day doses is observed. No significant difference was observed between two different doses of curcumin. One-way ANOVA is used for statistical comparison among groups.

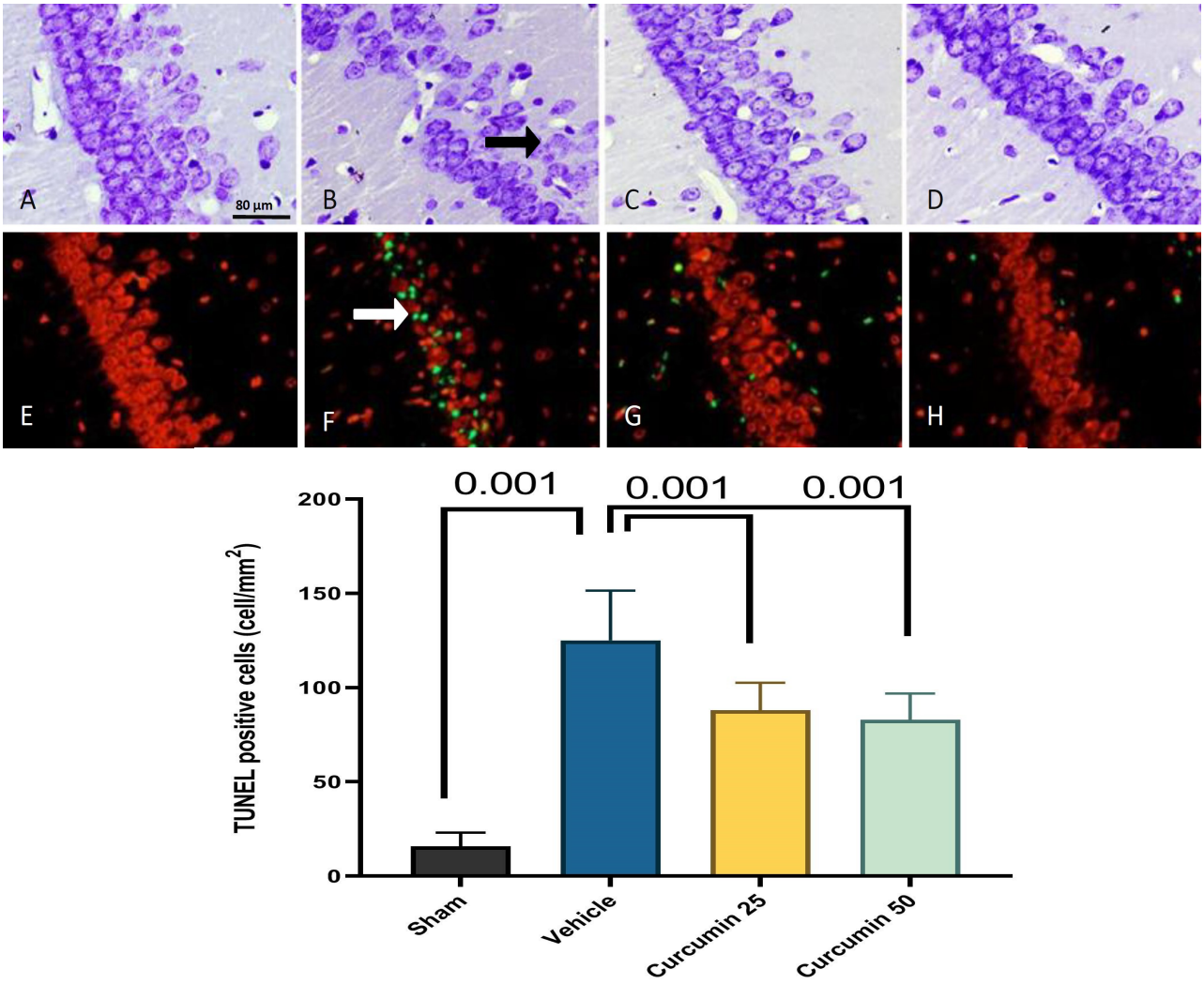

Fig. 5: Effect of oral administration of curcumin on neuronal apoptosis in the CA1 region of the dorsal hippocampus. Cresyl violet staining. A: sham group, B: vehicle group, C: curcumin 25mg/kg, D: curcumin 50mg/kg. TUNEL staining. E: sham group, F: vehicle group, G: curcumin 25mg/kg, H: curcumin 50mg/kg. Apoptotic cells shows green florescence. The black arrow shows the disorganization of neuronal cells in dorsal hippocampus. The green dots indicated by white arrow show the apoptotic cells marked by immune-staining.

The graph shows of quantitative analysis of neuronal apoptosis in the CA1 region of dorsal hippocampus by TUNEL staining by NIS-Elements software in different study groups. There was a significant increase in cell apoptosis by 4VOI in vehicle group compared to sham group which was partially prevented by curcumin. . One-way ANOVA is used for statistical comparison among groups.

Sanei *et al*. systematically reviewed all the evidence and showed that curcumin improved memory in different experimental models such as stress, anticonvulsant, benzodiazepine and age-induced memory impairment (Sanei and Saberi-Demneh, 2019).

A wide range of dosages of curcumin (from 5 to 480 mg/kg/day) and duration of administration (from 1-84 days) were evaluated in these studies. However, no study has evaluated the association of the preventive effect of curcumin on memory impairment with hippocampal acetylcholine level and neuroapoptosis which is well illustrated in our study.

The result of our study was compatible with those of previous reports that showed supplementation with curcumin in an animal model of brain ischemia can reduce neuronal injury (Liu *et al*., 2013). It is shown that this effect can be explained by increased expression in PPARγ by curcumin (Liu *et al*., 2013). The expression of PPARγ was also demonstrated to decrease the inflammatory response mediated by COX-2, TNF-α and IL-1β, which has a critical role in cerebral ischemia(Liu *et al*., 2013). Since curcumin is the main active constituent of turmeric, these findings are also compatible with the reports on the neuroprotective effect of turmeric supplementation in rats (Siahaan *et al*., 2018, Yuliani *et al*., 2017, Yuliani *et al*., 2019).

To the best of our knowledge, this is the first report which demonstrates curcumin is able to increase the ACh level in the dorsal hippocampus of ischemic rats. The ACh level in the brain can be raised through an increase in choline acetyltransferase function or a decrease in acetylcholinesterase activity (Vijayaraghavan *et al*., 2013). Which mechanism corresponds to the observed increase in the ACh level should be evaluated in further studies. Impairment in cholinergic activity is an important pathway in the development of ischemic-associated pathologies in the brain (Wang *et al*., 2009, Martín *et al*., 2018). Pyramidal neuron apoptosis in 4VOI can damage the ACh system, resulting in impairment in memory function (Volpe *et al*., 1992, Bendel *et al*., 2005). Accordingly, it can be suggested that the increase of ACh by curcumin can be responsible for its memory-enhancing activity. However, the increase in Ach level may be only due to the preventive effect on apoptosis. In other words, this study cannot prove that curcumin has a specific effect on the activity of cholinergic neurons in the hippocampus. Further study is needed to evaluate this issue.

It has previously been demonstrated that curcumin can prevent apoptotic cell death in the pyramidal CA3 region induced by methamphetamine neurotoxicity in the rat. It is shown that curcumin can reduce apoptosis in the caspase-3 positive neuron and GFAP positive astrocyte in streptozotocin-induced diabetes rats.

The study faced some important limitations which should be recognized in the interpretation of the results. First, we did not evaluate the long-term preventive effects of curcumin on memory, learning, hippocampal acetylcholine level and neuroapoptosis. The second limitation was that we only evaluate hippocampal acetylcholine level and neuroapoptosis among all the potential underlying cellular mechanisms which are involved in memory impairment and ischemic injury in the brain.

CONCLUSION

The finding of the study suggests that curcumin can increase Ach levels and prevent apoptosis in the dorsal hippocampus in an experimental model of brain ischemia. Curcumin also prevents memory impairment induced by 4VOI. Further investigations are needed to further clarify the pathophysiologic basis of the curcumin effects in neurodegenerative disorders resulting from cerebral ischemia.

REFERENCES

Amiri S, Jafari-Sabet M and Hoormand M (2021). The role of curcumin in ameliorating learning and memory disorders. *Razi J. Med. Sci.,* **28**(9): 118-134.

Ayati Z, Ramezani M, Amiri MS, Moghadam AT, Rahimi H, Abdollahzade A, Sahebkar A and Emami SA (2019). Ethnobotany, phytochemistry and traditional uses of curcuma spp. and pharmacological profile of two important species (*C. longa* and *C. zedoaria*): A review. *Curr. Pharm. Des.,* **25**(8): 871-935.

Barnhart CD, Yang D and Lein PJ (2015). Using the Morris water maze to assess spatial learning and memory in weanling mice. *PlosOne,* **10**(4): 10.

Bendel O, Bueters T, Von Euler M, Ögren SO, Sandin J and Von Euler G (2005). Reappearance of hippocampal CA1 neurons after ischemia is associated with recovery of learning and memory. *J. Cereb. Blood Flow. Metab.,* **25**(12): 1586-1595.

Bhat A, Mahalakshmi AM, Ray B, Tuladhar S, Hediyal TA, Manthiannem E, Padamati J, Chandra R, Chidambaram SB and Sakharkar MK (2019). Benefits of curcumin in brain disorders. *Biofactors,* **45**(5): 666-689.

Bianchi L, Ballini C, Colivicchi M, Della Corte L, Giovannini M and Pepeu G (2003). Investigation on acetylcholine, aspartate, glutamate and GABA extracellular levels from ventral hippocampus during repeated exploratory activity in the rat. *Neurochem. Res.,* **28**(3-4): 565-573.

Davis HP, Baranowski JR, Pulsinelli WA and Volpe BT (1987). Retention of reference memory following ischemic hippocampal damage. *Physiol. Behav.,* **39**(6): 783-786.

Debjit Bhowmik C, Kumar KS, Chandira M and Jayakar B (2009). Turmeric: A herbal and traditional medicine. *Arch. Appl. Sci. Res.,* **1**(2): 86-108.

Deng P and Xu ZC (2009). Four-vessel occlusion model in rats. *In*: Animal models of acute neurological injuries (eds. Chen J, Xu ZC, Xu XM and Zhang JH). Springer Protocols Handbooks, Humana Press.

Ferguson JJ, Abbott KA and Garg ML (2021). Anti-inflammatory effects of oral supplementation with curcumin: A systematic review and meta-analysis of randomized controlled trials. *Nutr. Rev.,* **79**(9): 1043-1066.

Furtado D, Björnmalm M, Ayton S, Bush AI, Kempe K and Caruso F (2018). Overcoming the blood-brain barrier: the role of nanomaterials in treating neurological diseases. *Adv. Mater,* **30**(46): 1801362.

Haraguchi T, Iwasaki K, Takasaki K, Uchida K, Naito T, Nogami A, Kubota K, Shindo T, Uchida N and Katsurabayashi S (2010). Telmisartan, a partial agonist of peroxisome proliferator-activated receptor γ, improves impairment of spatial memory and hippocampal apoptosis in rats treated with repeated cerebral ischemia. *Brain Res,* **1353**(1): 125-132.

Jurcau A and Simion A (2021). Neuroinflammation in cerebral ischemia and ischemia/reperfusion injuries: From pathophysiology to therapeutic strategies. *Int. J. Mol. Sci.,* **23**(1): 14.

Khiljee S, Rehman N, Khiljee T, Loebenberg R and Ahmad RS (2015). Formulation and clinical evaluation of topical dosage forms of Indian Penny Wort, walnut

and turmeric in eczema. *Pak. J. Pharm. Sci.,* **28**(6): 2001-2007.

Kolouri S, Firoozabadi A, Salehi A, Zarshenas MM, Dastgheib SA, Heydari M and Rezaeizadeh H (2016). Nepeta menthoides Boiss. & Buhse freeze-dried aqueous extract versus sertraline in the treatment of major depression: A double blind randomized controlled trial. *Complement. Ther. Med.,* **26**(1): 164-70.

Liu ZJ, Liu W, Liu L, Xiao C, Wang Y and Jiao JS (2013). Curcumin protects neuron against cerebral ischemia-induced inflammation through improving ppar-gamma function. *Evid. Based Complement. Alternat. Med.,* **2013**(1): 470975.

Martín A, Domercq M and Matute C (2018). Inflammation in stroke: The role of cholinergic, purinergic and glutamatergic signaling. *Ther. Adv. Neurol. Disord.,* **11**(1): 1756286418774267.

Megjhani M, Terilli K, Weiss M, Savarraj J, Chen LH, Alkhachroum A, Roh DJ, Agarwal S, Connolly Jr ES and Velazquez A (2021). Dynamic detection of delayed cerebral ischemia: A study in 3 centers. *Stroke,* **52**: 1370-1379.

Miao M, Guo L, Tian S and Wang T (2015). Effects of curcumine on antioxidation in diabetic rats. *Pak. J. Pharm. Sci.,* **28**(1 Suppl): 371-373.

Mosavat SH, Heydari M, Hashempur MH and Dehghani SM (2018). Use of complementary and alternative medicine among paediatric patients with hepatogastrointestinal diseases. *East Mediterr. Health J.,* **24**(10): 1018-1025.

Nogami-Hara A, Nagao M, Takasaki K, Egashira N, Fujikawa R, Kubota K, Watanabe T, Katsurabayashi S, Hatip FB, Hatip-Al-Khatib I and Iwasaki K (2018). The Japanese Angelica acutiloba root and yokukansan increase hippocampal acetylcholine level, prevent apoptosis and improve memory in a rat model of repeated cerebral ischemia. *J. Ethnopharmacol.,* **214**(1): 190-196.

Nogueira RC, Aries M, Minhas JS, H Petersen N, Xiong L, Kainerstorfer JM and Castro P (2022). Review of studies on dynamic cerebral autoregulation in the acute phase of stroke and the relationship with clinical outcome. *J. Cereb. Blood Flow Metab.,* **42**(3): 430-453.

Ovbiagele B (2008). Potential role of curcumin in stroke prevention. *Expert Rev. Neurother.,* **8**(8): 1175-1176.

Prasad S and Aggarwal BB (2011). Turmeric, the golden spice: from traditional medicine to modern medicine. chapter. *In*: Benzie IFF and Wachtel-Galor S (eds.) Herbal Medicine: Biomolecular and Clinical Aspects. Boca Raton FL: © 2011 by Taylor and Francis Group, LLC.

Quispe C, Herrera-Bravo J, Javed Z, Khan K, Raza S, Gulsunoglu-Konuskan Z, Daştan SD, Sytar O, Martorell M and Sharifi-Rad J (2022). Therapeutic applications of curcumin in diabetes: A review and perspective. *Biomed. Res. Int.,* **2022**(1): 1375892.

Sanei M and Saberi-Demneh A (2019). Effect of curcumin on memory impairment: A systematic review. *Phytomedicine,* **52**(1): 98-106.

Sarraf P, Parohan M, Javanbakht MH, Ranji-Burachaloo S and Djalali M (2019). Short-term curcumin supplementation enhances serum brain-derived neurotrophic factor in adult men and women: A systematic review and dose-response meta-analysis of randomized controlled trials. *Nutr. Res.,* **69**(1): 1-8.

Shukitt-Hale B, Mcewen JJ, Szprengiel A and Joseph JA (2004). Effect of age on the radial arm water maze: A test of spatial learning and memory. *Neurobiol. Aging,* **25**(2): 223-229.

Siahaan AMP, Japardi I, Rambe AS, Indharty RS and Ichwan M (2018). Turmeric extract supplementation reduces tau protein level in repetitive traumatic brain injury model. *Maced. J. Med. Sci.,* **6**(11): 1953-1958.

Singh L, Sharma S, Xu S, Tewari D and Fang J (2021). Curcumin as a natural remedy for atherosclerosis: a pharmacological review. *Molecules,* **26**(13): 4036.

Small GW, Siddarth P, Li Z, Miller KJ, Ercoli L, Emerson ND, Martinez J, Wong KP, Liu J, Merrill DA, Chen ST, Henning SM, Satyamurthy N, Huang SC, Heber D and Barrio JR (2018). Memory and brain amyloid and tau effects of a bioavailable form of curcumin in non-demented adults: A double-blind, placebo-controlled 18-month trial. *Am. J. Geriatr. Psychiatry,* **26**(3): 266-277.

Sumathi CS, Rajesh P and Kannan VR (2017). The biological potentialsof indian traditional medicine, curcumin for treating human diseases. *Cardiovasc. Hematol. Agents Med. Chem*. Online ahead of print.

Teow S-Y and Ali SA (2015). Synergistic antibacterial activity of curcumin with antibiotics against *Staphylococcus aureus*. *Pak. J. Pharm. Sci.,* **28**(6): 2109-2114.

Traystman RJ (2003). Animal models of focal and global cerebral ischemia. *ILAR Journal,* **44**(2): 85-95.

Vijayaraghavan S, Karami A, Aeinehband S, Behbahani H, Grandien A, Nilsson B, Ekdahl KN, Lindblom RP, Piehl F and Darreh-Shori T (2013). Regulated extracellular choline acetyltransferase activity-the plausible missing link of the distant action of acetylcholine in the cholinergic anti-inflammatory pathway. *PlosOne,* **8**(6): 8.

Volpe BT, Davis HP, Towle A and Dunlap WP (1992). Loss of hippocampal CA1 pyramidal neurons correlates with memory impairment in rats with ischemic or neurotoxin lesions. *Behav. Neurosci.,* **106**(3): 457.

Wang J, Zhang H-Y and Tang X-C (2009). Cholinergic deficiency involved in vascular dementia: Possible mechanism and strategy of treatment. *Acta Pharmacol. Sin.,* **30**(7): 879-888.

Wang W and Xu J (2020). Curcumin attenuates cerebral ischemia-reperfusion injury through regulating mitophagy and preserving mitochondrial function. *Curr. Neurovasc. Res*., **17**(2): 113-122.

Watts ME, Pocock R and Claudianos C (2018). Brain energy and oxygen metabolism: Emerging role in normal function and disease. *Front. Mol. Neurosci.,* **11**(1): 216.

Wong SC, Kamarudin MNA and Naidu R (2021). Anticancer mechanism of curcumin on human glioblastoma. *Nutrients,* **13**(3): 950.

Wu L, Jiang C, Kang Y, Dai Y, Fang W and Huang P (2020). Curcumin exerts protective effects against hypoxiareoxygenation injury via the enhancement of apurinic/apyrimidinic endonuclease 1 in SHSY5Y cells: Involvement of the PI3K/AKT pathway. *Int. J. Mol. Med.,* **45**(4): 993-1004.

Wynn JK, Green MF, Hellemann G, Karunaratne K, Davis MC and Marder SR (2018). The effects of curcumin on brain-derived neurotrophic factor and cognition in schizophrenia: A randomized controlled study. *Schizophr. Res.,* **195**(1): 572-573.

Yuliani S, Mustofa and Partadiredja G (2019). The neuroprotective effects of an ethanolic turmeric (*Curcuma longa* L.) extract against trimethyltin-induced oxidative stress in rats. *Nutr. Neurosci.,* **22**(11): 797-804.

Yuliani S, Widyarini S, Mustofa and Partadiredja G (2017). Turmeric extract inhibits apoptosis of hippocampal neurons of trimethyltin-exposed rats. *Bratisl. Lek. Listy,* **118**(3): 142-148.

Zhou J, Wu N and Lin L (2020). Curcumin suppresses apoptosis and inflammation in hypoxia/reperfusion-exposed neurons via wnt signaling pathway. *Med. Sci. Monit.,* **26**(1): e920445.