

A comparative study showing greater effects of curcumin compared to donepezil on memory function in rats

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Abstract: Curcumin possesses wide spectrum of biological actions, on that account the current study was aimed to investigate the beneficial effectiveness of curcumin on memory and oxidative stress if any, over synthetic drug donepezil approved for the treatment of memory disorders. Eighteen Albino *wistar* (male) rats were divided into 3 groups namely vehicle control which received neutral oil orally and 0.9% saline intraperitoneally, curcumin which received curcumin orally dissolved in neutral oil at the dose of 100mg/ml/kg for seven days, donepezil which received donepezil intraperitoneally at the dose of 1mg/ml/kg for seven days. To assess memory and cognition Elevated Plus Maze and Morris Water Maze tests were performed. Rats were sacrificed after behavioral analysis and their brains were removed for biochemical assays including lipid peroxidation and antioxidant enzymes such as catalase, superoxide dismutase, glutathione peroxidase. Acetylcholine esterase activity and acetylcholine levels were also determined. Our results showed that both curcumin and donepezil improved memory and inhibited acetylcholinesterase, however curcumin inhibited AchE with more potency than donepezil when compared to vehicle control rats. Moreover curcumin exhibited greater antioxidant potential to decrease the load of oxidative stress in brain cells than donepezil as compared to vehicle control rats. In conclusion present study proposed that increased antioxidant potential of curcumin may be responsible for its increased acetylcholine levels and associated enhanced memory performance.

Keywords: Curcumin, donepezil, memory.

INTRODUCTION

Curcuma Longa is a perennial plant, cultivated extensively in Asia, India, China and other countries of the world. It is the part of the Zingiberaceae (ginger family). *Curcuma longa* in dried form is the source of spice turmeric which is widely used in foods for color, flavor and for preserving them and as a yellow dye for textiles. Cytotoxic, tumor-reducing actions of curcumin have also been reported (Hatcher *et al.*, 2008). Curcumin provides protection against reactive oxygen (ROS) and nitrogen species (RNS) (Noorafshan and Ashkani-Esfahani, 2013; Mehla *et al.*, 2010). It is an inhibitor of arachidonic acid metabolism and is a good anti-inflammatory agent as well (Aggarwal and Sung, 2009). Increased expression of Brain Derived Neurotrophic Factor (BDNF) and 5-HT_{1A} receptors by curcumin reverses the hippocampal neurodegeneration in chronically stressed rats (Xu *et al.*, 2007). Neuroprotective effects of curcumin against heavy metals, glutamate toxicity and diseases like epilepsy, Alzheimer's have also been reported (Agarwal *et al.*, 2010; Mehla *et al.*, 2010).

Donepezil is a reversible, non-competitive, second generation acetyl choline esterase inhibitor (AChEI) (Pompeia *et al.*, 2013). It improves cognition in mild and moderate Alzheimer's disease (AD) and also used for the treatment of number of other psychological and

neurological disorders including Parkinson's disease, Down's syndrome and schizophrenia (Pompeia *et al.*, 2013). It is well tolerated and safe, especially for long term use (Kosasa *et al.*, 2000). Anti-inflammatory properties of donepezil have also been reported (Guo *et al.*, 2015). Donepezil is highly specific for brain AChE with longer and most potent duration of action as compare to other second generation AChEI's (Rocca *et al.*, 2002). It has 70h of plasma half-life and 100% bioavailability (Krall *et al.*, 1999). Alzheimer's disease patients treated with donepezil have shown a slower decline in cognition and activities of daily life (Krall *et al.*, 1999). Donepezil has also been found effective in treating depression, panic and irritability like conditions (Girish and Pradhan, 2012). Since a large number of researches have been done on curcumin and revealed its beneficial aspects, the present study was aimed to explore the potential benefits of herbal drug curcumin over commercially available standard drug donepezil specifically on memory. Donepezil is widely used to treat Alzheimer's disease, however role of donepezil in oxidative stress is not much studied, therefore the current study also aimed to compare the role of curcumin and donepezil on oxidative stress.

MATERIALS AND METHODS

Animals

The study was implemented on eighteen male Albino wistar rats weighing 180-200gms. All animals were healthy and purchased from Dow University of Health

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Sciences (OJHA campus, Karachi, Pakistan). All animals were kept individually in their cages under a 12h light-dark cycle and precise temperature ($22\pm 2^{\circ}\text{C}$) with open access to standard rodent diet and tap water for 3-4 days at minimum before experimentation so that animals could acclimate themselves to the novel environment. The experimental procedures were approved by the institutional ethics and animal care committee and executed in strict accordance with National Institute of Health Guide for Care and Use of Laboratory Animals (Publication No. 85-23, revised 1985).

Chemicals

Curcumin and Donepezil hydrochloride were purchased from Sigma Chemical Co. (St. Louis, USA). Thiobarbituric acid (TBA), Hydrogen peroxide (H_2O_2) stock (35%) solution, Dithio-bisnitrobenzoic acid (DTNB), Nitroblue tetrazolium (NBT), and Trichloroacetic acid (TCA) were purchased from British Drug House (BDH, Dorset, UK). Sodium carbonate (Na_2CO_3), EDTA, Hydroxylamine hydrochloride, Dichromatic-acetic reagent, Sodium azide, GSH, Acetylthiocholine (ATC) were also purchased from Sigma Chemical Co. (St. Louis, USA).

Procedure

Animals were randomly divided into three groups: vehicle control (VC), curcumin (CUR) and donepezil (DON). VC animals were orally administered with neutral oil and injected intraperitoneally with saline, Animals in the CUR group were orally administered with curcumin (100mg/ml/kg) and animals in the DON were intraperitoneally injected with donepezil (1mg/ml/kg). All drugs were administered for seven days and then behavioral tests were performed. Curcumin was dissolved in neutral oil while donepezil was dissolved in saline. On the basis of previously reported studies doses of drugs and period of treatment procedure were decided (Girish and Pradhan, 2012; Marighetto *et al.*, 2008).

Behavioral analysis

All the behaviors were analyzed in a quiet and closed room in balance design.

Morris water maze test

Morris Water Maze (MWM) test is used to study the effects on spatial memory. The method is principally the same as defined by Callaway *et al.* (2012) with minor changes. The apparatus comprised of a spherical pool of water with a width of 45cm, length 37cm and depth of water is 12cm. The pool is a metal container painted white on the interior side and the escape platform is also made of metal container with level metallic top having a surface diameter of 8cm and is 2cm under the surface of water during training. The pool was divided into four quadrants with an imaginary line and filled with opaque water maintained at ($23 \pm 2^{\circ}\text{C}$). The test consisted of four

training trials and one probe trial. On the start of each training trial, rat was introduced softly into the pool in north-east quadrant and allowed 90s to locate the submerged platform, located in south-west quadrant. The location of platform was remain constant and the time taken by each rat to reach the platform (escape latency) was noticed. Rats that failed to locate the submerged platform within 90s, were gently directed to the platform and allowed to stay there for 10s. At the completion of each trial, the rats were taken out from the pool, dried and send back to their home cages. Four trials sessions were carried out with an intervening period of 24h. On the fifth day, probe trial was carried out in which animals were placed in the pool for 90s lacking the platform and the period of time spent in target quadrant was noticed.

Elevated plus maze test

EPM is employed to access spatial learning and memory in animals. The apparatus comprised of two open arms (50x10 cm) intersected with two closed arms of the similar dimensions with walls 40cm high. The arms were linked with a central square (5x5 cm) to give the apparatus a plus sign look. The maze was raised 60cm above the floor. Elevation and open arms of the maze serve as aversive stimuli for rodents as they are nocturnal animals and tend to prefer to move to closed arm for safety and protection. The method is same as described by Mutlu *et al.* (2011). The test comprised of two sessions (acquisition and retention). In the acquisition session each rat was positioned at the end of open arm facing away from the central platform and the transfer latency to come into one of the closed arm with all four paws was monitored. Recurrent exposure of the animal in open arms may shorten this time as a result of learning acquisition and memory retention. The rat was further permitted to move openly for 10s in the maze irrespective of open and closed arms when it entered in the closed arm. After 24h retention session was performed and transfer latency was again monitored.

Bio-chemical parameters

After behavioral analysis all animals were sacrificed on the same day. Brains were taken out, washed in saline, and weighed. A 10% (w/v) tissue homogenate was made with 0.1 M phosphate buffer (pH 7.4) which was obtained by centrifugation at $12,000 \times g$ for 20 min at 4°C for the assessment of LPO, CAT, GPx, SOD, and AchE activities. Acetylcholine (Ach) levels were also determined.

Estimation of MDA

Assessment of lipid peroxidation was principally the similar as defined by Chow and Tappel, (1972) with minor changes. In a reaction mixture 100–500 μl homogenate was taken and 2 ml of TCA (15%)–TBA (0.375%) mixture was added. The mixture was boiled for 20 min in water bath, cooled with ice cold water at 4°C and then centrifuged at 3500 rpm for 10 min. Light pink

colored supernatant was collected and absorbance was taken at 532 nm. LPO was expressed as mM of MDA/g of brain tissue.

Estimation of CAT activity

CAT was assessed using a previously described method (Sinha, 1972). The reaction mixture contained 1.0ml of 0.01 M phosphate buffer (pH 7.4), 0.1ml of homogenate, and 0.4 ml of 0.2 M H₂O₂. The tubes were incubated at 37°C for 90s. The reaction was ceased by adding 2.0ml of dichromatic-acetic acid reagent (5%). Samples were then incubated at 100°C for 15min in a boiling water bath. The control was carried out without addition of homogenate, and then amount of H₂O₂ consumed was determined by recording absorbance at 570 nm. The activity of CAT was expressed as mmol of H₂O₂ consumed/min/g of brain tissue.

Estimation of GPx activity

The activity of GPx was determined by the method of Flohe and Gunzler (1984). 1ml of reaction mixture was prepared which comprised of 0.3ml of phosphate buffer (0.1M, pH7.4), 0.2ml of reduced glutathione (2mM), 0.1ml of sodium azide (10mM), 0.1ml of H₂O₂ (1mM), and 0.3ml of brain supernatant. After incubation at 37 °C for 15min, reaction was stopped by the addition of 0.5ml 5% TCA. Tubes were centrifuged at 1500×g for 5min, and supernatant was collected. Phosphate buffer 0.2ml (0.1M, pH7.4) and DTNB 0.7ml (0.4mg/ml) were added to 0.1ml of reaction supernatant. After mixing, absorbance was recorded at 420nm. GPx activity was expressed as μ mol/min/g of brain.

Estimation of SOD activity

The SOD was determined by the procedure of Chidambara *et al.* (2002), based on the reduction of NBT to water insoluble blue formazan. Homogenate (0.5ml) was mixed with 1ml of 50mM sodium carbonate, 0.4ml of 24 μ M NBT, and 0.2ml of 0.1mM EDTA. There action was initiated by adding 0.4ml of 1mM hydroxylamine hydrochloride. Change in absorbance was recorded from zero time followed by 5min at 560nm at 25°C. at the same time control was run without homogenate. Units of SOD activity were expressed as the amount of enzyme required to inhibit the reduction of NBT by 50%. The activity was expressed in terms of μ U/g of brain tissue.

Estimation of AchE activity

AchE activity in homogenate was assessed according to the procedure of Ellman *et al.* (1961) using (acetylthiocholine) ATC as substrate. The reaction mixture consisted of 0.4ml brain homogenate (0.02 g/ml), 2.6ml phosphate buffer (0.1M, pH8.0), 100 μ l DTNB. The reaction mixture was mixed by bubbling air, and placed in the spectrophotometer. Once the reaction content was stable, the absorbance was noted at 412nm for the basal reading followed by addition of 5.2 μ l of ATC to this cuvette. Change in absorbance was recorded from zero

time followed by 10 min at 25°C. AchE activity was expressed as μ mol/min/g of brain tissue.

Estimation of ach levels

Acetyl choline content of tissue was determined by the procedure of Hestrin (1949) as explained by Augustinsson (1957). The sample of tissue was boiled to deactivate the enzyme and release the bound acetylcholine which reacts with ferric chloride and the brown color developed was read at 540nm against the reagent blank. The levels were expressed as μ mol/g of brain tissue.

STATISTICAL ANALYSIS

The statistical assessment was done by one-way ANOVA, two-way ANOVA with repeated measure design and student's *t*-test while post-hoc analysis was done by Tukey's test via SPSS version 20. Results are expressed as the mean $\pm$ SD; *p* value <0.05 was considered significant.

RESULTS

Effect of curcumin and donepezil in MWM

Analysis of data by two-way ANOVA with repeated measure design revealed significant effect of drugs [F (2, 15)=579.5, *p*<0.01], training [F (3, 45)=271.14, *p*<0.01] and interaction [F (6, 45)=20.05, *p*<0.01].

Training session

Post-hoc analysis for the effect of drugs showed significant (*p*<0.01) decline in escape latencies in both CUR and DON rats as compared to VC rats during the four training sessions (fig. 1a).

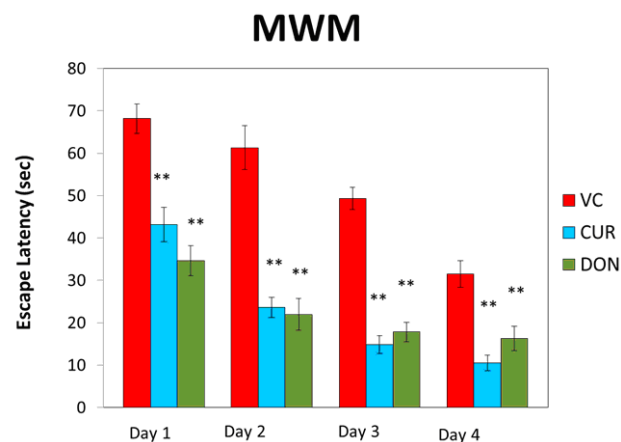


Fig. 1a: Effect of curcumin and donepezil on escape latency in MWM during training session. Data represent mean $\pm$ SD; *n*= 6 rats per group. ***p*<0.01 versus vehicle control group; Tukey's test.

Post-hoc analysis for the training effect showed significant (*p*<0.01) decrease in escape latency of VC rats on 3rd and 4th day of training session as compared to 1st

day of training session. Significant ($p<0.01$) decline in escape latency was detected in CUR rats from day second day to fourth day as compared to day 1 of the training session. However in DON rats decrease in escape latency on 2nd day of training session was only ($p<0.05$) significant while ($p<0.01$) significant on 3rd and 4th day of training session as compared to 1st day (fig 1b).

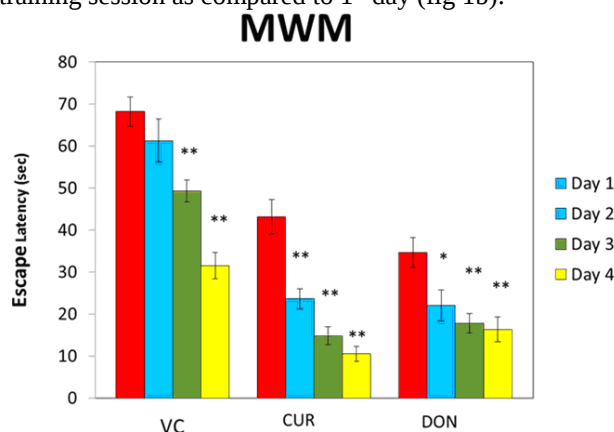


Fig. 1b: Effect of repeated training on escape latency in MWM in VC, CUR and DON groups. Data represent mean $\pm$ SD; $n=6$ rats per group. * $p<0.05$; ** $p<0.01$ versus first training day of respective group; Tukey's test.

Probe trial session

On day 5 after training probe trial session was performed in which % time spent in target quadrant was recorded. Analysis of data by one-way ANOVA revealed a significant [$F(2, 15)=21.50$, $p<0.01$] effect of drugs i.e., curcumin and donepezil. Post hoc analysis showed a significant rise ($p<0.01$) in percent time spent in target quadrant in both CUR and DON treated rats as compared to vehicle controls indicating memory enhancement (fig. 1c).

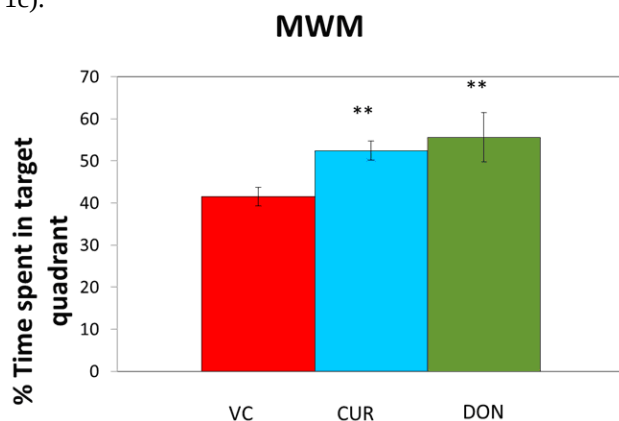


Fig. 1c: Effect of curcumin and donepezil on % time spent in target quadrant on probe trail day. Data represent mean $\pm$ SD; $n=6$ rats per group. ** $p<0.01$ versus vehicle control group; Tukey's test.

Effect of curcumin and donepezil on memory in EPM

Analysis of data by student's t -test revealed significant reduction ($p<0.01$) in transfer latency in retention session

in animals of the CUR group as compare to acquisition session. In the animals of DON group decrease in transfer latency was only ($p<0.05$) significant in retention session as compared to acquisition session. However no significant difference in transfer latencies of learning and acquisition session was observed in vehicle controls (fig. 2).

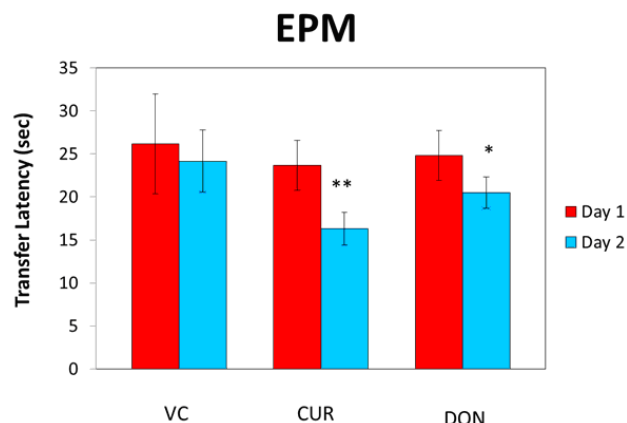


Fig. 2: Effect of curcumin and donepezil on transfer latency in EPM. Data represent mean $\pm$ SD; $n=6$ rats per group. * $p<0.05$; ** $p<0.01$ versus day 1 (acquisition session) of same group; t -test.

Effect of curcumin and donepezil on oxidative stress markers

Analysis of data by one-way ANOVA revealed a significant effect [$F(2, 15) = 8.69$, $p<0.01$] of curcumin and donepezil on CAT activity. Post hoc analysis showed a significant rise ($p<0.01$) in activity of CAT in CUR rats as compared to vehicle controls. However in the animals of DON group increase in CAT activity was only ($p<0.05$) significant as compared to vehicle controls (fig.3a).

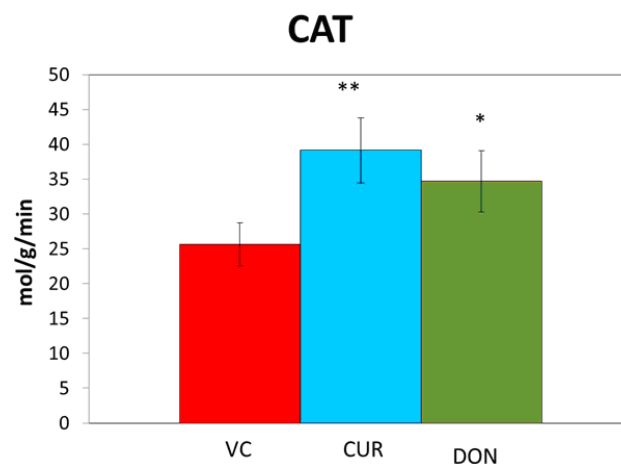


Fig. 3a: Effect of curcumin and donepezil on CAT activity. Data represented as mean $\pm$ SD; $n=6$ rats per group. * $p<0.05$; ** $p<0.01$ versus vehicle control group; Tukey's test.

Analysis of data by one-way ANOVA showed a significant effect of curcumin on SOD activity [$F(2, 15)=14.69$, $p<0.01$]. Post-hoc analysis indicated a significant increase ($p<0.01$) in activity of SOD in CUR rats as compared to VC rats. However no significant effect of donepezil on SOD activity was observed (fig. 3b).

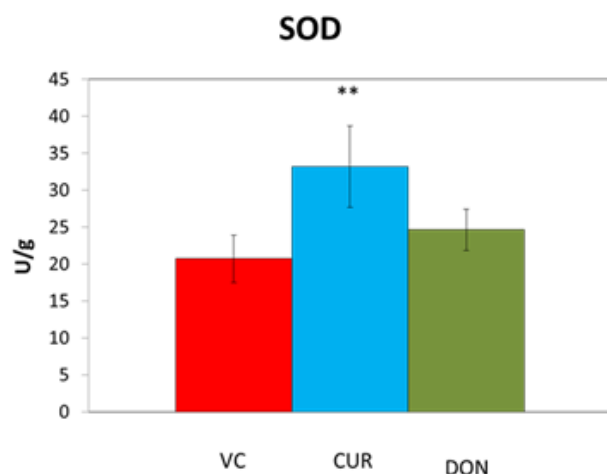


Fig. 3b: Effect of curcumin and donepezil on SOD activity. Data represented as mean $\pm$ SD; $n=6$ rats per group. ** $p<0.01$ versus vehicle control group; Tukey's test.

No significant effects of drugs were observed on GPx activity and MDA levels as shown in fig. 3c and 3d respectively.

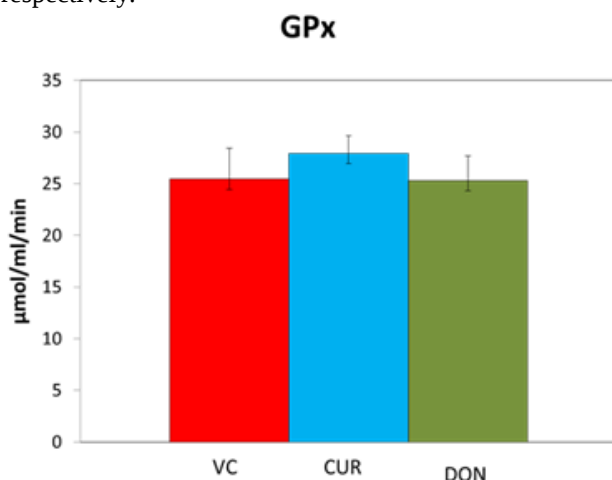


Fig. 3c: Effect of curcumin and donepezil on GPx activity. Data represented as $\pm$ SD; $n=6$ rats per group. Analysis by Tukey's test showed no significant difference

Effect of Curcumin and Donepezil on AchE activity

Analysis of data by one-way ANOVA showed a significant [$F(2, 15)=10.72$, $p<0.01$] effect of curcumin and donepezil on AchE activity. Post-hoc analysis revealed a significant ($p<0.01$) and ($p<0.05$) increase in AchE activity in CUR and DON treated rats respectively as compared to vehicle controls (fig. 4).

LPO

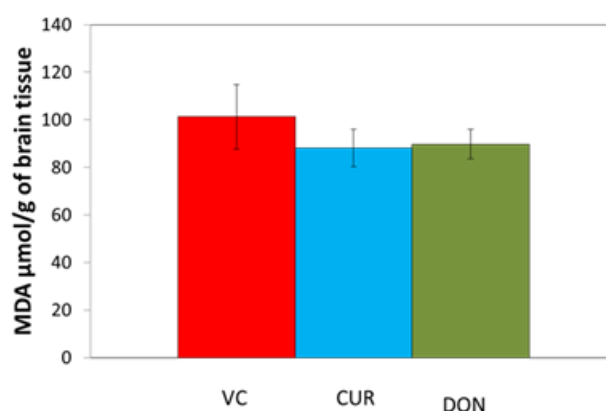


Fig. 3d: Effect of curcumin and donepezil on LPO. Data represented as mean $\pm$ SD; $n=6$ rats per group. Analysis by Tukey's test showed no significant difference.

AchE

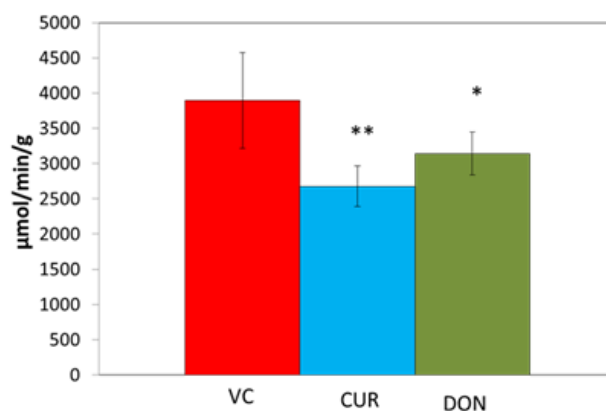


Fig. 4: Effect of curcumin and donepezil on AchE activity. Data represented as mean $\pm$ SD; $n=6$ rats per group. * $p<0.05$; ** $p<0.01$ versus vehicle control group; Tukey's test

Effect of curcumin and donepezil on acetylcholine levels

Analysis of data by one-way ANOVA revealed a significant [$F(2, 15) = 23.16$, $p<0.01$] effect of curcumin and donepezil on acetylcholine levels. Post-hoc analysis indicated a significant increase ($p<0.01$) in CUR rats and DON ($p<0.05$) rats as compared to VC rats in acetylcholine levels (fig. 5).

DISCUSSION

In the present study administration of curcumin for 7 days significantly improved memory in MWM, evident by decreased time spent to locate the hidden platform (escape latency) in all training sessions. Similarly administration of donepezil also improved memory as shown by decreased escape latency which is in accordance with the previous reported findings (Guo *et al.*, 2015). Rats administered with curcumin showed higher tendency of learning than donepezil during training session indicating more memory enhancing effects of curcumin. On probe

trial day both CUR and DON treated rats spent more time in target quadrant as compared to vehicle controls indicating memory enhancing effects of these drugs. Results of EPM also showed improvement in memory as shown by decreased transfer latency in retention session as compared to acquisition session. Previously administration of curcumin attenuated antiepileptic drugs induced memory deficits (Reeta *et al.*, 2009). Improvement in memory in EPM by donepezil was also significant however retention of memory in CUR rats was 31% while in DON group rat's retention of memory was only 17% indicating potential benefit of curcumin over donepezil. Previously it has been reported that curcumin improves learning and memory function and exerts neuropreventive effect (Haider *et al.*, 2015; Noorafshan *et al.*, 2013; Pan *et al.*, 2008).

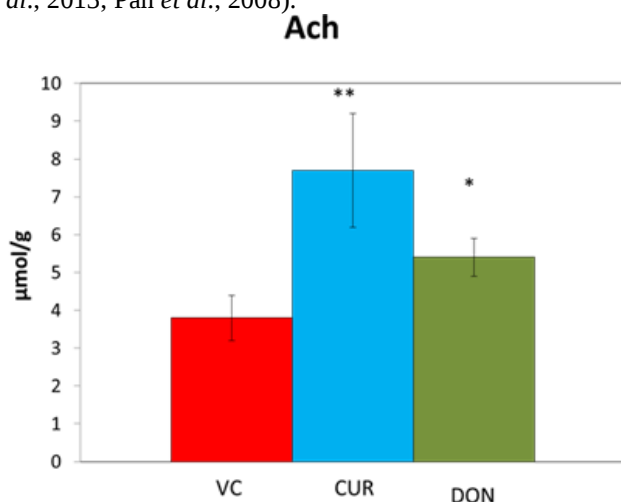


Fig. 5: Effect of curcumin and donepezil on AchE activity. Data represented as mean $\pm$ SD; n= 6 rats per group. *p<0.05; ** p<0.01 versus vehicle control group; Tukey's test.

To further confirm the results of behavioral data of curcumin and donepezil biochemical tests were performed in which AchE activity and acetylcholine levels were determined as estimation of AchE activity is used as an index of cholinergic function. Acetyl choline is a major neurotransmitter involved in learning and memory (Papandreou *et al.*, 2011). It increases long term potentiation (LTP) in many regions of brain including hippocampus (Adams *et al.*, 2004). Role of muscarinic and cholinergic receptors in the encoding of new memories have also been reported (Hasselmo, 2006). It has been reported that cognitive decline observed in AD is due to the loss of cholinergic tone and decline in acetylcholine levels in the brain (Lombardo and Maskos, 2015). Acetyl choline is hydrolyzed by the action of enzyme acetyl cholinesterase the key enzyme in cholinergic nervous system (Auld *et al.*, 2002). Increasing evidence suggested that during the progression of AD the expression of AchE is influenced by various proteins (Garcia-Ayllon *et al.*, 2011). Increased AchE activity in

the plasma of AD patients has also been documented (Garcia-Ayllon *et al.*, 2010). Acetyl cholinesterase inhibitors inhibit this enzyme from degrading Ach, and increase both the level and period of transmission of nerve impulse (Colovic *et al.*, 2013). In the current study significant decrease in the activity of AchE and concomitant increase in the acetyl choline levels in brain following curcumin administration clearly demonstrate the anti-cholinesterase potential of curcumin (Jaques *et al.*, 2012; Ahmed and Gilani, 2009; Kuhad and Chopra, 2007). Donepezil is a known selective, reversible AchEI developed for the management of AD (Guo *et al.*, 2015; Rocca *et al.*, 2002; Colovic *et al.*, 2013). Results of the present study are consistent with the previous findings as a significant decline in AchE activity and associated rise in acetylcholine levels was observed following donepezil treatment.

Oxidative stress is defined as the imbalance between antioxidant defenses which leads to the generation of increased reactive oxygen species or free radicals of oxygen (Haider *et al.*, 2014). Large number of studies have demonstrated the antioxidant effect of curcumin and its potential to reduce the oxidative stress and associated deficits of cognition (Hatcher *et al.*, 2008; Tokac *et al.*, 2013). Several experimental studies have reported that curcumin has a dual role as an antioxidant; it produce its antioxidant effect directly and indirectly by scavenging ROS (Trujillo *et al.*, 2013). Curcumin also improved intracellular concentration of glutathione, thus decreasing lipid per oxidation (Ciftci *et al.*, 2011, 2012). Efficacy of curcumin to prevent the brain from damages induced by free radicals is thought to be many times stronger than that of vitamin E (Martin-Aragon, 1997). Reduction in amyloid pathology and oxidative damage in Alzheimer's disease by curcumin has also been reported (Ono *et al.*, 2004). Results of the current study are consistent with the previous findings as administration of curcumin significantly increased the activity of antioxidant enzymes specifically SOD and CAT, however no significant effect was observed on the activity of GPx and levels of MDA. On the other hand role of donepezil on the antioxidant enzymes activities was not very significant except for the activity of CAT indicating a decreased tendency of donepezil as an antioxidant as compared to curcumin.

CONCLUSION

From the current study it may be established that both curcumin and donepezil improved learning and memory processes, however the potency of curcumin to enhance memory function is far greater than donepezil and this difference may be attributed to more antioxidant property of curcumin as compared to donepezil. The study also highlights the enhanced potential of curcumin as an AchEI. Apart from this a number of side effects including gastrointestinal anomalies-nausea, diarrhea, anorexia,

abdominal pain, bradycardia have been reported previously during donepezil treatment while curcumin does not produce any side effects, highlighting the pharmacological benefit of curcumin over donepezil. Since in the present study curcumin not only improved memory function but also exhibited potent AchEI property therefore it can be suggested as an alternative drug for the long term treatment of Alzheimer's disease.

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