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CAFFEIC ACID AVERTED INTRACEREBROVENTRICULAR COLCHICINE-INDUCED MEMORY DEFICITS IN RATS

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ABSTRACT

In elderly people chronic oxidative stress initiates a cascade of neurodegenerative events that hasten severe memory decline akin to Alzheimer's disease (AD) and not commensurate to normal age-associated dementia. Caffeic acid (CA) is a natural bioactive polyphenol known to possess robust antioxidant property. The present study investigated repercussions of chronic administration of CA against colchicine (COL) induced oxidative stress and inflammation in brain of rats. Wistar rats (200 g) were subjected to stereotaxic surgery and slow intra cerebro ventricle (ICV) injection of colchicine (15µg/rat) was given to induce AD type dementia. CA (50 and 100 mg/kg, p.o.) was administered to separate groups of rats for 25 successive days. Morris water maze (MWM) and elevated plus maze (EPM) paradigms were utilized to assess the spatial memory of rats. After behavioural studies, rats were sacrificed and whole brain lipid peroxidation (TBARS), reduced glutathione (GSH) and TNF-α levels were measured. CA treatment for 25 days negated the COL triggered cognitive deficits as evident by a significant ($p<0.001$) reduction in mean escape latency during training trials and enhanced ($p<0.001$) percent of time spent in target quadrant [TSTQ (%)] during probe trial in MWM test, and reduction ($p<0.001$) in transfer latency in EPM test. Furthermore, CA (50 and 100 mg/kg) attenuated COL induced rise in brain TBARS as well as TNF-α and simultaneously enhanced the GSH content. CA ameliorates COL induced dementia by attenuating the brain oxidative damage and neuroinflammation in rats.

KEY WORDS

Alzheimer's disease, caffeic acid, colchicine, inflammation, oxidative stress

INTRODUCTION

Alzheimer's disease (AD) is a common type of dementia that progresses in different stages from mild cognitive impairment to severe memory decline along with irreversible neurodegeneration primarily in older people [1]. Albeit impairment of cognitive abilities severely hampers the daily activities of AD affected patients, however, an active lifestyle helps to keep high cognitive reserve [2]. Deposition of intracellular neurofibrillary tangles and extracellular Aβ-plaques in diverse brain regions (e.g. hippocampus and cerebral cortex) accompanied by loss of cholinergic activity in basal forebrain are the neuropathological hallmarks of AD [3]. Prolonged oxidative burden and inflammatory changes in brain are *prima facie* etiologic factors that initiate progressive neurodegenerative changes in brain leading to cognitive dysfunctions [4, 5]. Taupathy is precipitated by free radicals through posttranslational hyperphosphorylation of tau, and the consequent destabilization of microtubule-assembly in hippocampus and cortex leads to neuronal as well as synaptic loss, thereby cause toxic protein aggregation [6]. Free radicals also enhance neurotoxic $A\beta_{42}$ accumulation in senile plaques by increasing amyloidogenic proteolysis of amyloid precursor protein (APP). Advanced end products of oxidative stress

enhance stability of Aβ-plaques and reduce their clearance from brain [7]. These noxious aggregates in brain amplify free radical generation and toxicity.

The therapeutic approaches of AD are still palliative, confined to upregulation of cholinergic transmission (e.g. galantamine, rivastigmine), and reduction of excitoxicity mediated neurodegeneration. However, at present there is dearth of an effective agent which may prove valuable in curative therapy of AD. Polyphenols are ubiquitous natural products, reported to possess neuroprotective property by virtue of their robust antioxidant activity. The literature reports also confirmed the dementia ameliorative potential of polyphenols (e.g. resveratrol, quercetin, curcumin) in animal models of AD and clinical studies [8-10]. Caffeic acid (3,4-dihydroxycinnamic acid) is a shikimic acid pathway origin polyphenol and it is reported to comprise of diverse pharmacological activities such as antioxidant, anti-inflammatory, antidiabetic, anticancer, cardioprotective, antimicrobial and antiparasitic [11,12]. Furthermore, it was observed that caffeic acid (CA) prevented the neurotoxic effects of intracerebroventricular (ICV) administered $A\beta_{25-35}$, aluminium and acrolein with concomitant decline in brain lipid peroxidation, pro-inflammatory cytokine levels, neurodegeneration and memory loss in rodents [8,13,14]. The *in vitro* studies indicated reduction in tau phosphorylation and oxidative stress induced calcium influx by CA [15]. These studies suggest that CA might have potential to prevent or reverse dementia of AD type.

ICV administration of colchicine (COL) in adult rats is one of the leading animal models to indicate dementia in experimental animals [4]. It induces cognitive decline primarily through oxidative stress and neuroinflammation mediated selective neurodegeneration in hippocampus and cerebral cortex [16]. Furthermore, COL enhance accumulation of APP, reduce axoplasmic transport and severely hampers the cholinergic functions of brain by causing neurodegeneration in nucleus basalis of Meynert in substantia innominata of basal forebrain, similar to that observed in autopsy of AD brains [17,18]. In previous studies neuroprotective potential of CA is demonstrated in several animal models of AD. The phenylpropanoid scaffold of CA has been widely used as template to develop CA derivatives (alkyl esters e.g. phenethyl ester) and new chemical entities possessing strong antioxidant property. Human studies revealed that oral dose of CA is well absorbed from small intestine and a significant amount reaches the brain [19]. However, no sufficient reports in the literature are available indicating its putative effect in the management of dementia to the best of our knowledge. Therefore, the present study was undertaken to explore the potential of CA against COL-induced AD type dementia in rats.

MATERIALS AND METHODS

Animals

Adult Wistar rats (either sex, 180-200 g) were procured from Disease Free Small Animal House, Lala Lajpat Rai University of Veterinary and Animal Science, Hisar. The study protocol is duly approved by the institutional animal ethics committee and the animals were maintained under standard laboratory conditions with controlled temperature (23±2°C), humidity (40±10 %) and natural (12 h each) light-dark cycle. The animals were fed with standard rodent pellet diet (Ashirwad Industries, Mohali) and water *ad libitum*. The experiments were carried out between 09:00 and 18:00 h. The care of laboratory animals was done following the guidelines of CPCSEA, Ministry of Forests and Environment, Government of India.

Drugs and chemicals

CA procured from Researcher-Lab Fine Chem Industries, Mumbai was suspended in canola oil. Colchicine (COL), dihydrogen orthophosphate, 5,5'-Dithiobis-(2-nitrobenzoic acid) (DTNB) (Himedia Laboratories, Mumbai); thiobarbituric acid, trichloroacetic acid, sodium dodecyl sulphate (Loba Chemie, Mumbai); TNF-α ELISA kit (Krishgen, Mumbai) were used. Artificial cerebrospinal fluid (aCSF, pH 7.3) was prepared as following: (In mmol/l) 147 mMNaCl, 2.9 mMKCl, 1.6 mM $MgCl_2$, 1.7 mM $CaCl_2$, 2.2 mM dextrose was dissolved in 10 ml of water for injection [16]. All the drug solutions were freshly prepared before injections.

Intracerebroventricular injection of colchicine

The rats were anaesthetized with chloral hydrate (300 mg/kg, *i.p.*). The head was positioned in the frame of stereotaxic apparatus (INCO, Ambala, India), middle sagittal incision was made in the scalp and skull was exposed. Two holes were drilled through the skull for bilateral placement of microinjector into the lateral cerebral ventricles using the following coordinates: 0.8 mm posterior to bregma, 1.5 mm lateral to sagittal suture and 3.6 mm beneath the surface of the brain

[20]. A freshly prepared COL solution (15 µg in 10 µl aCSF) was slowly administered bilaterally through the cerebral ventricles (5 µl in each ventricle) by maintaining rate of injection 1 µl/min [16]. The microsyringe (Hamilton®) was not disturbed from its location for 10 min to avoid the backflow along the injection track and enhance the diffusion of COL solution in CSF. ICV administration was limited to 10 µl. After surgery the skin of all the animals was sutured and antiseptic powder (Cipladine®) was applied.

Experimental protocol

CA was suspended in canola oil and administered orally (dosing volume 10 ml/kg) in two doses (50 and 100 mg/kg) to separate groups of rats for 25 consecutive days [21]. Dementia was induced in rats by administering colchicine (15 µg/rat) through ICV route on day 5. Donepezil is approved by FDA for pharmacotherapy of AD and served as the standard drug in present study [22]. The animals were divided into nine different groups having 7 animals in each group: Normal control group was administered canola oil (10 ml/kg, *p.o.*) for 25 days; CA50 group was given CA (50 mg/kg, *p.o.*) for 25 days; CA100 group was treated with CA (100 mg/kg, *p.o.*) for 25 days; Donepezil group was given donepezil (1 mg/kg, *p.o.*) for 25 days; Sham group rats received sham surgery and administered with canola oil (10 ml/kg, *p.o.*) for 25 days; COL group was injected colchicine (15 µg/rat) through ICV route on day 5 and administered canola oil (10 ml/kg, *p.o.*) for 25 days daily; COL+CA50 group rats received CA (50 mg/kg, *p.o.*) for 25 days and colchicine (15 µg/rat) on day 5; COL+CA100 group was treated with CA (100 mg/kg, *p.o.*) for 25 days and colchicine (15 µg/rat) on day 5; COL+Donepezil group was administered donepezil (1 mg/kg, *p.o.*) for 25 days and colchicine (15 µg/rat) on day 5. Morris water maze (MWM) and elevated plus maze (EPM) tests were employed to measure the spatial memory of rats. MWM test was performed for 5 consecutive days (day 21 to day 25) and was divided in two trials: training trial of 4 days and retrieval trial was performed 24 h after last training trial. Mean escape latency (MEL) is noted during training trials and time spent in the target quadrant (TSTQ) during retrieval trial. The EPM studies were carried out on day 24 and 25 for measurement of transfer latency (TL). The locomotor activity of animals was also measured before surgery and before behavioural studies. After behavioural studies, the animals were sacrificed by decapitation to estimate whole brain TBARS, GSH and TNF-α levels (Figure 1).

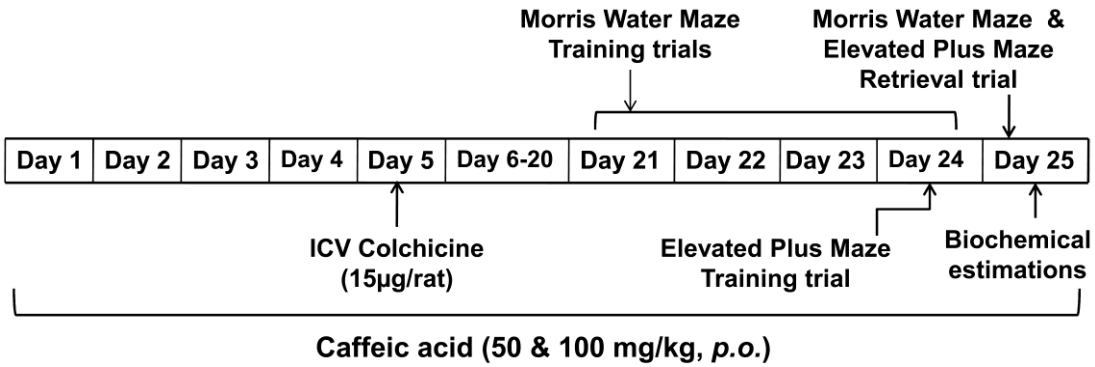

Figure 1: Experimental protocol and drug treatment schedule.

Morris water maze test

MWM is a swimming-based model to assess spatial learning of rodents where the animal learns to escape on to a hidden platform. It consisted of large circular pool (200 cm in diameter, 60 cm in height, filled to a depth of 30 cm with water at 25±1°C). The tank was divided into four equal quadrants with help of two threads, fixed at right angle to each other on the rim of the pool. A submerged platform (11 cm^2) was placed inside the target quadrants of this pool, 1 cm below surface of water. The position of platform was kept unaltered throughout the training session. Each animal was subjected to four consecutive training trials on each day with inter-trial gap of 5 min. The rat was gently placed in the water between quadrants, facing the wall of pool with drop location changing for each trial (Table 1), and allowed 120 s to locate submerged platform. Then, it was allowed to stay on the platform for 20 s. If it failed to find the platform within 120 s, it was guided gently onto platform and allowed to remain there for 20 s. Mean escape latency (MEL) is the average time taken by the rat to locate the hidden platform in water maze.

Day 4 MEL *vs.* day 1 MEL was noted as index of training or learning. On fifth day during the retrieval trial, platform was removed, and each rat was allowed to explore the pool for 120 s. Mean time spent in all four quadrants was noted. The mean time spent by the animal in target quadrant (TSTQ) searching for the hidden platform was noted as index of retrieval or memory and expressed as percentage of TSTQ [TSTQ (%)]. Care was taken that relative location of water maze with respect to other objects in the laboratory serving, as prominent visual clues were not disturbed during the total duration of study [23].

Elevated plus maze test

The EPM apparatus for rats consisted of a central platform (10 cm×10 cm) connected to two open arms (50 cm×10 cm) and two covered arms (50 cm×40 cm×10 cm). The maze was elevated to a height of 50 cm from the floor. Transfer Latency is defined as the time taken by the animal to move from the open arm into one of the covered arms with all of its four paws. In order to record transfer latency (TL), each rat was placed at the end of an open arm facing away from the central platform. TL was recorded on first day for each animal. The rat was allowed to explore the maze for 20 s and then returned to its home cage. The cut off time to reach the closed arm is 90 s. In case the rat does not locate the closed arm in 90 s, it is gently guided to one of the closed arm. Retention of this learned task was examined 24 h after the first day trial [16].

Table 1: Morris water maze training trials chart

Day	Quadrant for rat			
Day 1	Q 1	Q 2	Q 3	Q 4
Day 2	Q 2	Q 3	Q 4	Q 1
Day 3	Q 3	Q 4	Q 1	Q 2
Day 4	Q 4	Q 1	Q 2	Q 3

One-way ANOVA followed by Tukey's *post-hoc* test was applied. Values are expressed as mean±SEM (n=7). Significance at *** p<0.001, ** p<0.01 *versus* normal control; ### p<0.001 COL *versus* sham group and $$$ p<0.*001versus* COL group. [CA50: Caffeic acid 50 mg/kg, CA100: Caffeic acid 100 mg/kg]

Locomotor activity

Each animal was placed in the actophotometer (INCO, Ambala, India) for habituation (5 min), then the animals were observed for 10 min and ambulation was expressed as counts per 10 min [16].

Biochemical studies

A 10% w/v brain homogenate was prepared in 0.1mM phosphate buffer (pH 7.4) using tissue homogenizer (REMI Elektrotechnik Ltd, India). The homogenate was centrifuged (REMI compufuge, India) at 10,000 g for 15 min and supernatant was separated for biochemical estimation.

Measurement of brain TBARS levels

Thiobarbituric acid reactive substances (TBARS) levels were measured following method of Ohkawa *et al.* [24]. The absorbance was noted at 532 nm employing double beam UV-Visible spectrophotometer (Shimadzu UV-1700, Pharmaspec).

Measurement of reduced glutathione

Reduced GSH was measured according to the method of Ellman [25]. Absorbance was noted at 412 nm using double beam UV-Visible spectrophotometer (Shimadzu UV-1700, Pharmaspec).

Determination of TNF-α

TNF-α value was measured as per the instructions given on the immunoassay kit. The absorbance was noted at 450 nm using ELISA reader (BIORAD).

Statistical analysis

All the results are expressed as mean±SEM. Two-way ANOVA followed by Bonferroni *post-hoc* test and one-way ANOVA followed by Tukey's test were used to assess the inter-group variations using software GraphPad InStat (GraphPad Software Inc., USA). A value of p<0.05 was considered to be significant.

RESULTS

The locomotor activity of rats was unaffected by CA and COL treatments. The normal control and drug treated groups showed no significant statistical differences in ambulation as measured in actophotometer.

Caffeic acid enhances learning and memory of rats in Morris water maze test

During the training trials the normal control and sham group rats exhibited reduction ($p<0.001$) in day 4 MEL relative to day 1 MEL thereby displaying spatial learning by virtue of training. Chronic administration of CA (50 and 100 mg/kg) to separate groups showed reduction of day 4 MEL ($p<0.001$) as compared to normal control group rats which indicates enhanced learning. ICV administration of COL enhanced ($p<0.001$) the day 4 MEL relative to sham rats thereby denoting profound learning impairment during training trials. However, oral administration of CA (50 and 100 mg/kg) abridged the day 4 MEL ($p<0.001$) of COL-treated rats as compared to rats of COL group. Donepezil treated groups reflected considerably shortened day 4 MEL ($p<0.001$) relative to normal control as well as COL groups in MWM tests (Figure 2). In retrieval trial, the COL treatment reduced TSTQ (%) of rats significantly as compared to normal control ($p<0.001$) as well as sham ($p<0.01$) rats. Chronic administration of CA (50 and 100 mg/kg) to different groups of COL treated rats resulted in elevation ($p<0.001$) of TSTQ (%) in comparison to rats that received COL alone. CA100 group rats displayed increase in TSTQ (%) value ($p<0.001$) relative to that of normal control rats. Furthermore, the standard drug donepezil was able to comprehensively increase the TSTQ (%) value ($p<0.001$) with respect to COL-treated rats and rats of normal control group (Figure 3). It is pertinent to state that CA has ability to enhance the memory and prevents COL triggered dementia in rats.

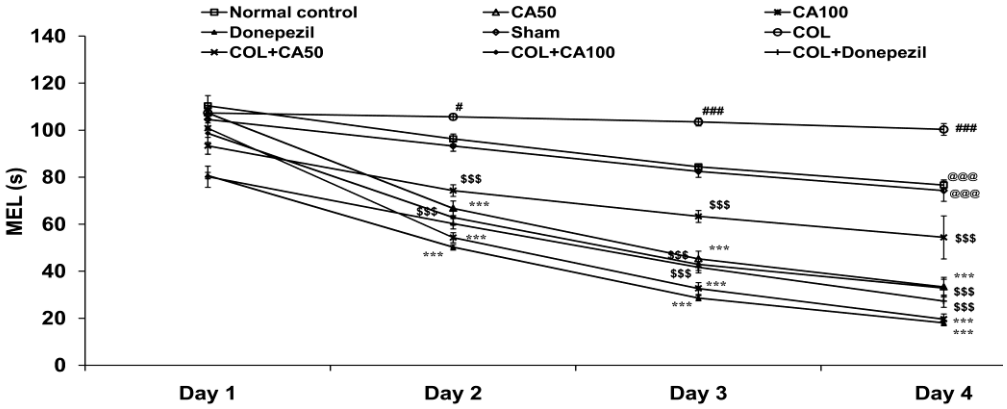

Figure 2: Oral administration of caffeic acid (CA) elevates spatial learning of rats during training trials in Morris water maze task. Mean escape latency (MEL) are compared by applying two-way ANOVA followed by Bonferroni *post-hoc* test. Values are expressed as mean±SEM (n=7). Significance at $^{@@@}$ p<0.001 day 4 MEL *versus* respective day 1 MEL; *** p<0.001 *versus* normal control; $^{###}$ p<0.001, $^{##}$ p<0.01 COL *versus* sham group and $^{\$\$\$}$ p<0.001 *versus* COL group. [CA50: Caffeic acid 50 mg/kg, CA100: Caffeic acid 100 mg/kg]

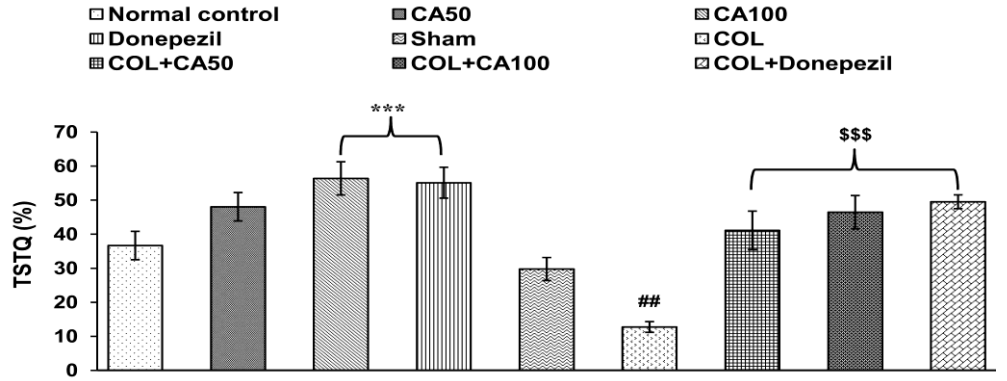

Figure 3: Oral administration of caffeic acid (CA) enhances reference memory of rats during retrieval trial in Morris water maze task. Percentage of time spent in target quadrant [TSTQ (%)] is compared by applying one-way ANOVA followed by Tukey's *post-hoc* test. Values are expressed as mean±SEM (n=7). Significance at *** p<0.001 *versus* normal control; $^{###}$ p<0.001, $^{##}$ p<0.01 COL *versus* sham group and $^{\$\$\$}$ p<0.001 *versus* COL group. [CA50: Caffeic acid 50 mg/kg, CA100: Caffeic acid 100 mg/kg]

Caffeic acid increases memory of rats in elevated plus maze test

The separate groups of rats treated with CA (50 and 100 mg/kg) showed reduction in TL ($p<0.001$) in comparison to rats of normal control group. Centrally administered COL increased ($p<0.001$) the TL of rats relative to sham and normal control group rats. However, oral treatment with CA (50 and 100 mg/kg) abolished the increase in TL ($p<0.001$) of COL-treated rats in comparison to rats of COL group (Figure 4). A decrease in TL ($p<0.001$) of donepezil treated rats relative to rats of normal control and COL groups proved its memory enhancing property.

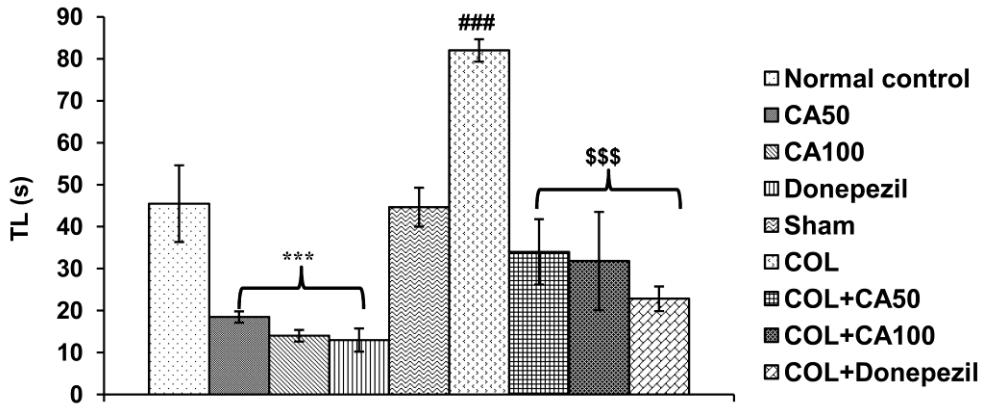

Figure 4: Oral administration of caffeic acid (CA) abrogates mean transfer latency (TL) of rats in elevated plus maze paradigm. One-way ANOVA followed by Tukey's *post-hoc* test was applied. Values are expressed as mean±SEM (n=7). Significance at *** $p<0.001$ *versus* normal control, ### $p<0.001$ COL *versus* sham group and $$$ $p<0.001$ *versus* COL group. [CA50: Caffeic acid 50 mg/kg, CA100: Caffeic acid 100 mg/kg].

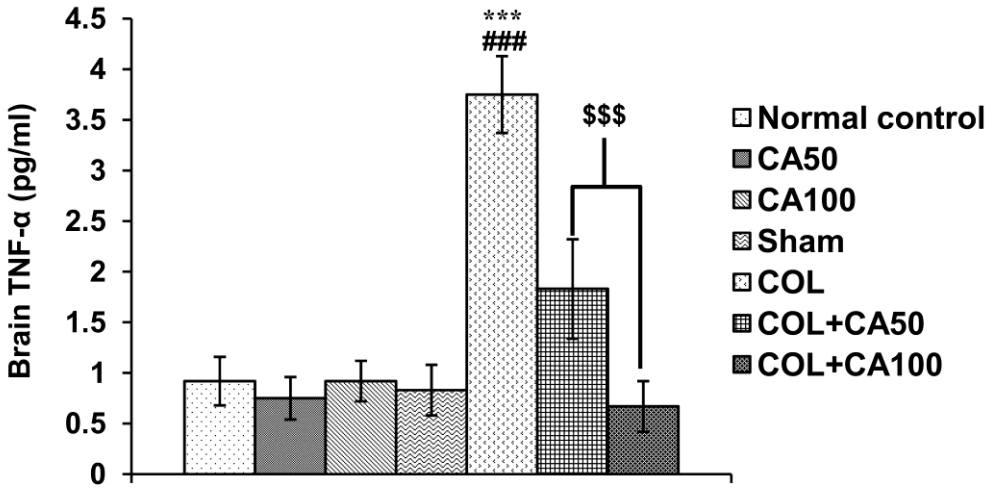

Figure 5: Administration of caffeic acid (CA) for 25 days reduces brain TNF-α level in rats. One-way ANOVA followed by Tukey's *post-hoc* test was applied. Values are expressed as mean±SEM (n=7). Significance at *** $p<0.001$ *versus* normal control, ### $p<0.001$ COL *versus* sham group and $$$ $p<0.001$ *versus* COL group. [CA50: Caffeic acid 50 mg/kg, CA100: Caffeic acid 100 mg/kg]

Caffeic acid attenuates TBARS levels in brain of rats

The CA100 group rats exhibited low whole brain TBARS ($p<0.01$) content relative to normal control group rats. Central administration of COL increased the brain TBARS level ($p<0.001$) in comparison to normal control and sham groups. However, oral administration of CA (50 and 100 mg/kg) for 25 consecutive days to COL-treated rats prevented the increase in brain TBARS level ($p<0.001$) with respect to rats that received COL alone. Donepezil treated rats showed reduced TBARS level ($p<0.001$) relative to rats of normal control and COL administered groups (Table 2). These results showed that CA attenuates oxidative stress and limits ICV

administered COL induced lipid peroxidation in brain of rats.

Table 2: Administration of caffeic acid (CA) for 25 days reduces TBARS and elevates GSH level in the brain of rats

Group	TBARS (µmol/g)	GSH (µmol/g)
Normal control	0.36 ± 0.058	0.57 ± 0.01
CA50	0.31 ± 0.04	0.69 ± 0.06
CA100	0.28 ± 0.04**	0.82 ± 0.05
Donepezil	0.17 ± 0.01***	0.88 ± 0.02***
Sham	0.33 ± 0.02	0.65 ± 0.12
COL	0.55 ± 0.06###	0.28 ± 0.05###
COL+CA50	0.32 ± 0.03$$$	0.61 ± 0.02$$$
COL+CA100	0.22 ± 0.01$$$	0.66 ± 0.05$$$
COL + Donepezil	0.18 ± 0.02$$$	0.85 ± 0.02$$$

Caffeic acid enhances GSH content in brain of rats

COL treatment declined the brain GSH level ($p<0.001$) in comparison to rats of normal control and sham groups. Administration of CA (50 and 100 mg/kg) for 25 days to COL-treated rats prevented ($p<0.001$) the dwindling brain GSH level in comparison to rats of COL group. However, CA50 and CA100 group rats showed no statistical difference in their GSH value relative to normal control rats. Moreover, administration of donepezil enhanced ($p<0.001$) the brain GSH level as compared to COL group and normal control group (Table 2).

Caffeic acid reduces the brain TNF-α content

Both doses of CA (50 and 100 mg/kg) prevented ($p<0.001$) COL induced surge in TNF-α when compared to COL group. However, the higher dose (100 mg/kg) showed more reduction in TNF-α as compared to lower dose. There was no significant difference between the TNF-α level of normal control and CA (50 and 100 mg/kg) treated groups (Figure 5).

DISCUSSION

COL is an ancient natural alkaloid first extracted from *Colchicum autumnale* L. Disruption of microtubular assembly by COL leads to impairment of intracellular trafficking and cytoskeletal framework followed by cell apoptosis and necrosis [26]. Intracerebroventricle administration of COL in rodents is a widely acclaimed model of AD type dementia [4,16]. In the present study, MWM and EPM tests were employed to evaluate spatial learning and memory of the rats. Performance in maze tasks is linked with long-term potentiation and synaptic plasticity in hippocampus and cortex [27]. The rats treated with COL showed decrease of spatial learning during training trials and low retrieval of memory i.e. higher day 4 MEL and lower TSTQ (%) value as compared to sham rats during MWM studies. Furthermore, COL treatment caused increase in TL value of rats relative to sham rats in EPM study. These results are consistent to the reports from other studies which revealed similar findings in response to COL treatment [16]. Long-term treatment with CA reduced day 4 MEL and enhanced TSTQ (%) in rats that were previously administered COL. Moreover, CA treatment to rats that were previously administered COL showed reduction in TL value in EPM test. Furthermore, it was observed that CA enhanced the memory in rats in addition to its prevention of COL-induced dementia. CA50 and CA100 group rats showed significantly lower day 4 MEL, higher TSTQ (%) and reduced TL relative to normal control rats. These findings are comparable to the outcome of behavioural studies from other laboratories which signify the memory improving property of CA [4,23]. The locomotor activity of normal control, COL and CA group rats showed no significant difference. This excludes the possibility that locomotor activity *per se* may have contributed to any changes during behavioural studies.

The TBARS assay correlates with lipid peroxidation product malondialdehyde (MDA) levels. In the present study, COL treatment increased brain TBARS level and reduced the GSH content of rats. These findings are consistent with previous literature reports where COL increased brain MDA level in rodents [4,16,23]. MDA mediates neurotoxicity through formation of bio-molecular adducts (proteins and nucleic acids), advanced lipid-peroxidation end products, advanced

glycation end products (AGEs) and methylglyoxal-acetaldehyde adducts that are extremely immunogenic and cause cell senescence, autophagy, apoptosis and necrosis leading to neurodegeneration [28]. GSH is a major thiol-antioxidant that detoxifies free radicals, peroxidation products and maintains cellular redox status by acting as cysteine reservoir, cofactor for many antioxidant enzymes and directly scavenging free radicals [29]. GSH depletion hastens accumulation of several neurotoxic aggregates due to failure of GSH dependent glyoxalase mediated metabolization of methylglyoxal. Aging and AD both result in lowering of brain GSH/GSSH ratio. Clinical studies demonstrate that the AD patients have high brain as well as plasma MDA level and low GSH content relative to age matched controls [30]. In the present study, oral treatment with CA prevented the rise in brain TBARS and enhanced the GSH level in rat brains that were previously treated with COL. Moreover, CA100 group rats also showed decrease in brain TBARS level. These findings are in confirmation with the review of Magnani *et al.*, which highlighted that CA possesses robust antioxidant property [11]. In the present study COL treated rats showed increased brain TNF-α content relative to the sham rats. Unceasing elevated brain TNF-α is a key feature in AD patients [31] that leads to neurodegeneration through NMDA receptor mediated excitotoxic necrosis, apoptosis and overexpression of amyloid-β in synergism with IL-1 and IL-6 [5]. However, CA treatment for successive 25 days prevented the COL-induced rise in brain TNF-α level.

In addition to investigations pertaining to long-term memory performed in the present study, further evaluation of memory enhancing property of CA is needed by employing different behavioural models. The assessment of mechanism of action of CA requires use of different pharmacological interventions such as wortmannin, LY294002, PD98059 or SB 415286.

CONCLUSION

Thus, it may be concluded that caffeic acid ameliorates COL-induced memory deficits by mitigating oxidative stress and inflammation and simultaneously improved the memory of *per se* group rats. However, further studies are very much required to answer the molecular basis of therapeutic activity of caffeic acid in neurodegenerative disorders such as AD.

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CONFLICTS OF INTEREST

No conflict of interest associated with this work.

CONTRIBUTION OF AUTHORS

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors.

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