Ellagic Acid Administration Reverses Colchicine-Induced Dementia in Rats

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Abstract

The late-onset sporadic type of Alzheimer's disease is characterised by chronic oxidative stress, neuroinflammation and cognitive dysfunction. Ellagic acid is a naturally occurring polyphenol known to possess robust antioxidant property. In the present study, memory enhancing potential of ellagic acid has been explored against ICV colchicine induced dementia in rats.

Colchicine (15μg/rat) was administered to Wistar rats (200g) through intracerebroventricular (ICV) route by using stereotaxic apparatus. ICV colchicine induces Alzheimer's disease like changes in the brain such as rampant free radical production, neuroinflammation and selective neurodegeneration in hippocampus and cortex by acting as an antitubulin agent (mitotic poison). Ellagic acid (17.5 and 35 mg/kg, *p.o.*) was administered to rats for 25 successive days. Morris water maze and elevated plus maze paradigms were utilized to assess the spatial memory of rats. Oxidative stress biomarkers along with TNF-α were also measured in brain of rats.

Ellagic acid prevented the ICV colchicine triggered cognitive deficits as evident by a significant ($p<0.05$) reduction in mean escape latency during acquisition trial and increased ($p<0.05$) time spent in target quadrant during probe trial in Morris water maze test, and reduction ($p<0.05$) in transfer latency in elevated plus maze test. Furthermore, both the doses of ellagic acid attenuated ICV colchicine induced rise in brain TBARS as well as TNF-α and simultaneously enhanced the GSH content.

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Ellagic acid prevented the brain of rodents from dementing effects of colchicine by attenuating the oxidative damage.

Keywords: Ellagic acid, colchicine, memory, oxidative stress

1. INTRODUCTION

Alzheimer's disease (AD) is a frequently occurring form of dementia, especially in old-aged persons, manifesting progressive neurodegeneration and severe memory loss (31). Atkinson and Shiffrin (1968) organised the brain memory functions into sensory memory, long- and short-term memory characterised as declarative and non-declarative, and working memory respectively. Both long-term and working/short-term memory are adversely affected in AD due to neurodegeneration in their associated structures. Medial temporal lobe memory (MTL) system maintains the declarative memory (explicit) while other cortical, subcortical and cerebellar structures regulate the non-declarative (implicit) memory (37). The consolidation of long-term memory takes place in neocortex whose retrieval after some time of learning becomes independent of MTL system. The hippocampus and frontal cortex integrity is vital for working memory (10). Loss of declarative memory (episodic and semantic both) in early stages and non-declarative memory failure in later stages leads to aphasia, agnosia, apraxia, agraphia, loss of visuospatial ability, reasoning, skills and behaviour changes in AD patients (41). Further, the pioneer studies on working memory by Baddeley and Hitch (1974) demonstrated that the 'central executive component' governs optimum functioning of working memory by regulating phonological loop, episodic buffer and visuospatial sketchpad (3). The end periods of AD portray gross atrophy in hippocampus and cerebral cortex along with enlargement of ventricles (33). The histopathology of AD describe archetypal neurofibrillary tangles and neuritic plaques originating in MTL memory system which spread to neocortex after a few years, duly accompanied by loss of cholinergic function in nucleus basalis (5). NFTs are intracellular deposits of hyperphosphorylated tau protein whereas extracellular plaques contain amyloid-β fibrils.

Eberus Papyrus (1500 BC), De Materia Medica (Pedanius Dioscorides; AD 1) and Therapeutica (Alexander of Tralles; AD 6) have descriptions regarding the use of colchicum (*Colchicum autumnale* L.) rheumatism and gouty arthritis (11, 12). Colchicine is an antitubulin agent which disrupts microtubular assembly thereby causes apoptotic and necrotic neurodegeneration in selective brain regions viz. hippocampus and cortex (25). Previous studies have illustrated that administration of colchicine through intracerebroventricle (ICV) route in adult rats induces AD type dementia by initiating oxidative

stress and inflammation (4, 18, 13, 14, 15, 16). ICV colchicine augments production of chemically reactive molecules containing oxygen also termed as ROS, reactive nitrogen species (RNS), cycloxygenase-2 (COX-2), inducible nitric oxide synthase (iNOS) and enhance glutamatergic neurotoxicity. The cholinergic functions (thoughts, memory, intelligence and behaviour) of brain are severely disrupted by colchicine by causing neurodegeneration in nucleus basalis of Meynert in substantia innominata of basal forebrain (9, 21). The autopsy of AD brains revealed selective neurodegeneration in nucleus basalis in substantia innominata (42, 43).

Ellagic acid is a polyphenolic hydrolytic product of ellagitannins (punicalagin, sanguiin-H6, pedunculagin) abundantly found in pomegranate, berries, nuts, wines and many spirits. It is known to possess several pharmacological properties such as antioxidant, antiinflammatory, antimicrobial, antidiarrheal and immunomodulatory (20). The cardioprotective property of ellagic acid and its sources has been extensively investigated during pre-clinical as well as clinical studies; and is attributed to anti-angiogenic, anti-atherogenic, vasodilatory, hypoglycaemic, hypocholesterolemic, anti-adipogenic, anti-platelet and serum lipid profile improving activities (39). The pharmacokinetic studies revealed good absorption of ellagic acid (but not ellagitannins) from stomach and duodenum attaining C_{max} after 1 h (20). The digestion of ellagic acid begins in small intestine, where variety of enzymes contribute to its metabolism *via* phase I and phase II reactions, *i.e.* hydroxylation and glucuronidation respectively, in liver and plasma, eliminating majority of metabolites (urolithins) through urine (19). Based on the above discussed facts, we take on to analyse the therapeutic effect of ellagic acid in a colchicine-promoted oxidative stress and inflammation in rat brains.

2. MATERIALS AND METHODS

2.1 Animal selection

Adult Wistar rats (either sex), weighing between 180-200 g were obtained from the Disease Free Small Animal House, Lala Lajpat Rai University of Veterinary and Animal Science, Hisar. The animals were acclimatized for seven days to the housing conditions of Central Animal Facility of ASBASJSM College of Pharmacy, Bela prior to experiments. Animals were kept in polypropylene cages, and husk (free from any dust) was used for bedding in the cages. In additions, controlled temperature (23±2°C), humidity (40±10 %) and natural (12 h each) light-dark cycle was maintained for animals. The animal diet was standard rodent pellet diet (Ashirwad Industries, Mohali) and water *ad libitum*. The experiment was carried out between 09:00 and 18:00 h. The

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research protocol of this study has been approved by IAEC and in laboratory, the animals were handled following the guidelines of CPCSEA, Ministry of Forests and Environment and Government of India.
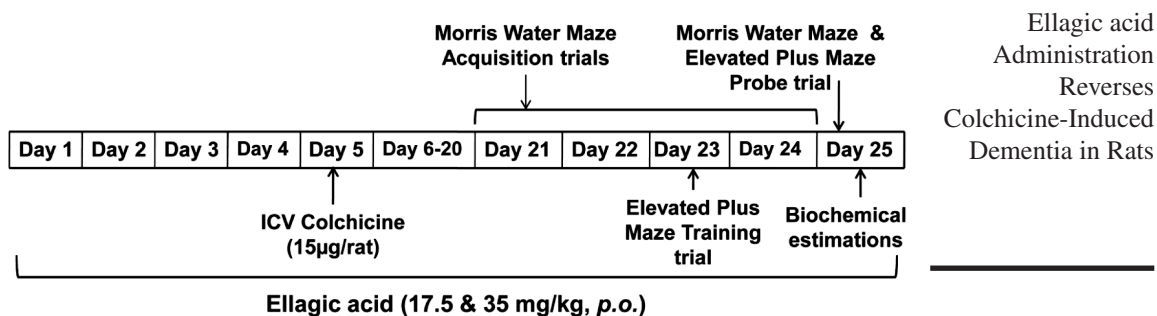
2.2 Drugs and chemicals

Ellagic acid procured from Himedia laboratories, Mumbai was suspended in 0.1% gum acacia maintaining the temperature 40-50°C and pH 5-8. Colchicine, 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) was prepared as following: (In mmol/l) 147 mM NaCl, 2.9 mM KCl, 1.6 mM $MgCl_2$, 1.7 mM $CaCl_2$, 2.2 mM dextrose was dissolved in 10 ml of water for injection (17). The similar electrolytic concentration and osmolality of aCSF and authentic cerebrospinal fluid prompted utilisation of aCSF as solvent for intracerebroventricle (ICV) administration of drugs. All the drug solutions were freshly prepared before injections.

2.3 Intracerebroventricular injection of colchicine (ICV 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), skull was exposed and middle saggital incision was made in the scalp. Two holes were drilled though 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 saggital suture and 3.6 mm beneath the surface of the brain (29). Colchicine (15 µg/rat) was dissolved in freshly prepared aCSF (5 µl) and slowly injected (rate of injection 1 µl/min) though the cerebral ventricle (18, 14). Surgical control animals were given same volume of aCSF only. After surgery the skin of all the animals was sutured and antiseptic powder was applied.

2.4 Experimental protocol

Ellagic acid (17.5 and 35 mg/kg, *p.o.*) was administered once daily to separate groups of rats for 28 consecutive days beginning 4 days prior to colchicine injection (Figure 1) (6). Intracerebroventricle (ICV) injection of colchicine was given to induce memory impairment. The rats were divided into 09 different groups (6-8 rats in each group): Group I, Normal control (0.1% gum acacia for 25 days, *p.o.*); Group II, EGA(17.5) *per se*, ellagic acid (17.5 mg/kg for 25 days, *p.o.*); Group III, EGA(35) *per se*, ellagic acid (35 mg/kg for

| Morris Water Maze Acquisition trials | | | Morris Water Maze & Elevated Plus Maze Probe trial | |

| Day 1 | Day 2 | Day 3 | Day 4 | Day 5 | Day 6-20 | Day 21 | Day 22 | Day 23 | Day 24 | Day 25 |

ICV Colchicine (15µg/rat)

Elevated Plus Maze Training trial

Biochemical estimations

Ellagic acid (17.5 & 35 mg/kg, *p.o.*)

Figure 1: Drugs treatment protocol.

25 days, *p.o.*); Group IV, donepezil *per se* (1 mg/kg for 25 days, *p.o.*); Group V, ICV colchicine (15 µg/5 µl/rat on day 5, ICV); Group VI, sham control (aCSF 5 µl on day 5, ICV); Group VII, colchicine+EGA(17.5), ellagic acid (17.5 mg/kg for 25 days, *p.o.*) + colchicine (15 µg/5 µl/rat on day 5, ICV); Group VIII, colchicine+EGA(35), ellagic acid (35 mg/kg for 25 days, *p.o.*) + colchicine (15 µg/5 µl/rat on day 5, ICV); Group IX, colchicine+donepezil, donepezil (1 mg/kg for 25 days, *p.o.*) + colchicine (15 µg/5 µl/rat on day 5, ICV). Morris water maze (MWM) and elevated plus maze (EPM) tests were employed to measure the spatial memory of rats. On 21st day the rats were exposed to training sessions in MWM after 30 min of administration of ellagic acid and mean escape latency (MEL) is noted for four consecutive days. On 25th day the time spent in the target quadrant (TSTQ) was noted. The EPM studies were carried out on 24th and 25th day for measurement of mean transfer latency (TL). The locomotor activity of animals was also measured before surgery and before behavioural studies. Donepezil (1 mg/kg, *p.o.*) served as the standard drug (36). After behavioural studies, the animals were sacrificed by decapitation to estimate brain TBARS, GSH and TNF-α level.

2.5 Exteroceptive behavioural models

2.5.1 *Morris water maze test*

Morris water maze test was performed to test spatial learning and reference memory of the animals. Spatial learning is measured across repeated training trials (day 1 to 4) and reference memory is gauged during probe trial (on day 5) by preference of rats for the platform area / quadrant when the platform is absent. MWM is a swimming based model, in which the test animal learns to escape on to a hidden platform. The model is composed of a large circular pool

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(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, with the help of two threads, into four equal quadrants fixed at right angle to each other on the rim of pool. A submerged platform (11 cm^2) was placed inside target quadrants of this pool at about 1 cm beneath water surface. The position of platform was not disturbed during whole training session.

Acquisition trial: at inter-trial gap of minutes, each animal was subjected to four successive training trials per day. Each animal (rat) was placed in water pool, between quadrants, facing wall, with change of drop location in each individual trial [Chart 1]. Thereafter, the animal was allowed for 120 s to trace submerged platform, and once identified, it was allowed to be at this for 20 s. In case, animal failed to locate the submerged platform within 120 s, was manually guided towards platform, and allowed to stay there (at platform) for 20 s. Herein, the Escape latency time (ELT) is considered as time period availed by animal to discover hidden submerged platform in water maze. Day 4 ELT vs day 1 ELT was noted as index of acquisition or learning.

Chart 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

Retrieval (Probe) trial: On 5thday of probe trial, submerged platform was removed and each animal was permitted to explore the pool for 120 s. Then, in all four quadrants, the mean time spent was observed. Thereafter, the average time taken by each animal in target quadrant (TSTQ) to locate the submerged platform was considered as index of memory or retrieval. The person performing experiment always attained similar position, and in addition, utmost care was ensured for relative location of water mazein respect to laboratory surroundings, so that the visual indications for animal were constant during whole period of experiment (18, 24).

2.5.2 *Elevated plus maze test*

The EPM apparatus (for rats)is comprised of a main central platform (10×10 cm), which is further linked with two open (50×10 cm) and enclosed arms (50×40×10 cm). The maze was set at a height of 50 cm from the floor. Transfer

latency (TL) is considered as time span taken by rat to get into an enclosed arm, starting from an open arm, with all of its paws inside. For this, each rat was placed at the end of open arm, facing away from central platform. TL was recorded for each animal on their first, and they were permitted to discover the maze for 20 s. Finally, they were returned to their home cage. The cut off time to explore enclosed arm, starting from an open arm, was set to 90 s. If, in case, the rat was unable to discover the enclosed arm within cut off time, it was softly directed to any of enclosed arm. Retention of this learned task was examined 24 h after the first day trial (13).

2.5.3 *Locomotor activity*

The locomotor activity was noted using actophotometer (INCO, Ambala, India) for a period of 5 min. Animal was placed in the actophotometer for habituation (3 min). The animals were then observed for 5 min and expressed as counts per 5 min (17).

2.6 Biochemical studies

2.6.1 *Preparation of brain homogenate*

After behavioural study, the animals were sacrificed by decapitation. The brains were isolated and placed on ice followed by rinsing with ice-cold isotonic saline (0.9% NaCl). A (10% w/v) brain homogenate was prepared in 0.1mM phosphate buffer (pH 7.4), and centrifuged at 10,000 g for 15 min. Thereafter, the supernatant was used for biochemical estimation.

2.6.2 *Measurement of brain TBARS*

Thiobarbituric acid reactive substances (TBARS) assay measures the lipid peroxidation product malondialdehyde (MDA) in tissue sample (26). The absorbance of supernatant was noted at 532 nm employing double beam UV-Visible spectrophotometer (Shimadzu).

2.6.3 *Measurement of reduced glutathione*

Reduced GSH was measured as per literature method (8), and absorbance was noted at 412 nm using double beam UV-Visible spectrophotometer.

2.6.4 *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).

2.7 Statistical analysis

All the results are stated as mean ± SEM, and all group's data was analysed using one-way ANOVA; subsequently, proceeded by Tukey's test with the help

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of software Graph Pad InStat (Graph Pad Software Inc., USA). A value of $p<0.05$ was considered to be significant.

3. RESULTS

Ellagic acid and ICV colchicine treatments did not affected the locomotor activity of rats. There was insignificant statistical differences ($p>0.05$) in locomotion between the normal control and drug treated groups of rats as observed in actophotometer.

3.1 Effect of ellagic acid on learning and memory performance of rats in Morris water maze task

The normal control and sham control group rats displayed spatial learning by virtue of training during acquisition trials as evident by reduction ($p<0.05$) of day 4 MEL relative to day 1 MEL. Ellagic acid *per se* (17.5 and 35 mg/kg, *p.o.*) treated rats showed reduction of day 4 MEL ($p<0.05$) as compared to normal control group rats which indicates enhanced learning. ICV colchicine enhanced ($p<0.05$) day 4 MEL relative to sham control rats denoting profound learning impairment during training trials. However, ellagic acid treatment decreased the day 4 MEL ($p<0.05$) of colchicine treated rats. The standard nootropic drug donepezil considerably shortened ($p<0.05$) the day 4 MEL of rats during acquisition trials [Figure 2].

In probe trial, it was observed that the ICV colchicine treatment reduced the TSTQ ($p<0.05$) of rats significantly as compared to normal control as well as sham control rats. But ICV colchicine induced decline in TSTQ value was arrested by administration of ellagic acid. However, ellagic acid *per se* group rats showed insignificant ($p>0.05$) elevation in TSTQ value in comparison to normal control rats. Furthermore, donepezil comprehensively enhanced the TSTQ value in colchicine treated rats [Figure 2].

### 3.2 Effect of ellagic acid on memory performance of rats in elevated plus maze paradigm

Ellagic acid *per se* group rats displayed reduction in TL ($p<0.05$) relative to rats of normal control group. ICV colchicine treatment increased ($p<0.05$) the TL value of rats relative to sham and normal control group values. However, administration of ellagic acid abolished the increase in TL ($p<0.05$) value of colchicine-treated rats [Figure 3]. A decrease in TL ($p<0.05$) of donepezil treated rats proved its nootropic potential.
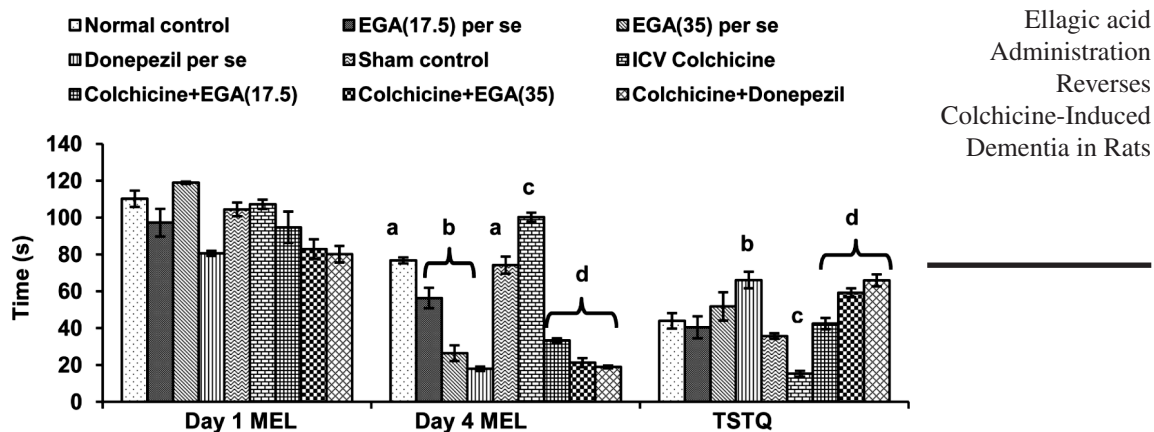
Figure 2: Effect of ellagic acid on spatial learning (day 4 vs. day 1 MEL) and reference memory (TSTQ) of rats during acquisition trials and retrieval trial respectively in Morris water maze task. [Values are expressed as mean ± SEM (n = 6-8), Significance at $p<0.05$, [a]versus respective day 1 MEL, [b]versus normal control, [c]versus sham control group and [d]versus ICV colchicine group].
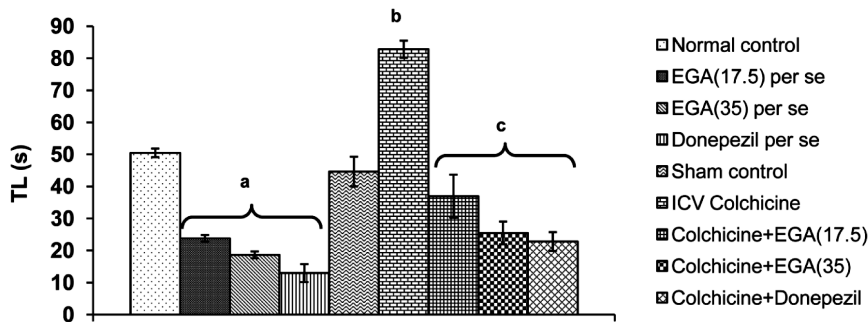
Figure 3: Effect of ellagic acid on mean transfer latency (TL) of rats in elevated plus maze paradigm. [Values are expressed as mean ± SEM (n = 6-8), Significance at $p<0.05$, [a]versus normal control, [b]versus sham control group and [c]versus ICV colchicine group].

3.3 Effect of ellagic acid on brain TBARS level

Administration of plain ellagic acid lowered the brain TBARS ($p<0.05$) content of rats relative to control group rats. ICV colchicine increased the TBARS

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level (p<0.05) in comparison to normal control and sham control. However, administration of ellagic acid to ICV colchicine treated rats do not described increase in brain TBARS level (p<0.05). Donepezil reduced the TBARS level (p<0.05) in different groups of rats [Figure 4].
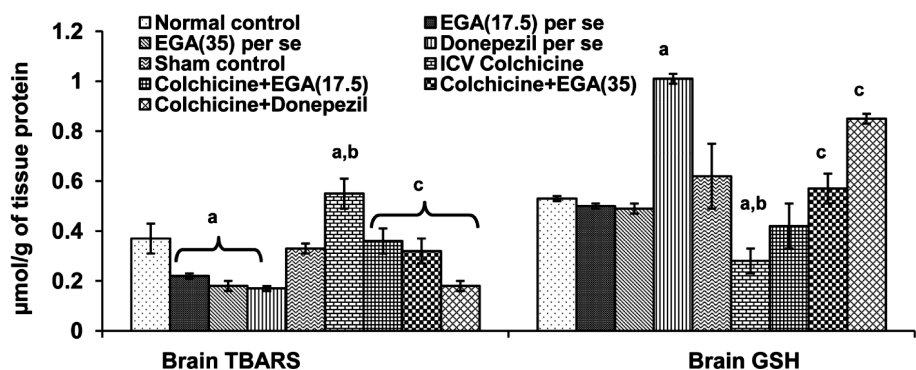
3.4 Effect of ellagic acid on brain GSH content

Administration of ICV colchicine reduced the GSH level (p<0.05) in brain of rats relative to rats of normal control and sham control. Ellagic acid (35 mg/kg) prevented the ICV colchicine triggered diminution of brain GSH level. However, ellagic acid *per se* group rats showed no statistical difference in their GSH value relative to normal control rats. Moreover, donepezil enhanced (p<0.05) the brain GSH level in colchicine-treated groups as well as donepezil *per se* group rats [Figure 4].
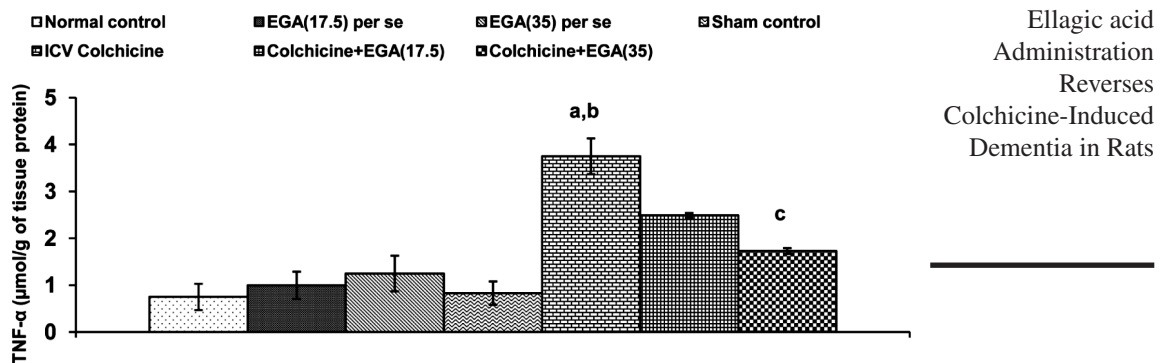
3.5 Effect of ellagic acid on brain TNF-α level

Ellagic acid (35 mg/kg) prevented (p<0.05) colchicine induced surge in TNF-α. There was insignificant difference between TNF-α level of normal control and ellagic acid *per se* treated groups [Figure 5].

4. DISCUSSION

ICV injection of colchicine in rats produces AD like memory deficits (15). In current study, the spatial learning and memory of the rats were evaluated by using MWM and EPM tests. Maze task performance is associated with

Figure 4: Effect of ellagic acid on brain TBARS and GSH level of rats. [Values are expressed as mean ± SEM (n = 6-8), Significance at p<0.05, [a]versus normal control, [b]versus sham control group and [c]versus colchicine group].

Figure 5: Effect of ellagic acid on brain TNF-α level of rats. [Values are expressed as mean $\pm$ SEM (n = 6-8), Significance at $p<0.05$, [a]versus normal control, [b]versus sham control group and [c]versus ICV colchicine group].

long-term potentiation and synaptic plasticity in hippocampus and cortex (40). Ventrolateral frontal cortex maintains the object-information i.e. the hidden platform in MWM task or closed arm in EPM. Dorsolateral frontal cortex maintains the spatial-information i.e. the location of hidden platform or closed arm. Furthermore, the frontal cortex alongwith prefrontal cortex is involved in decision making within the working memory domain (10). The working memory actively maintains limited amount of information sufficient to maintain object-information and spatial-information in maze tasks. The performance of rats in training trials is largely governed by working memory while that in probe trial by long-term memory. Perirhinal cortex, hippocampus and entorhinal cortex determines the acuity of spatial navigation task by rodents in maze paradigms. Perirhinal cortex is involved in visuoperception while hippocampus provides spatial map of surroundings (cognitive mapping) and along with entorhinal cortex it is intricately involved in path integration (27, 30).Colchicine selectively deposits and cause neurodegeneration in hippocampal and cortical regions (34, 13) thereby severely affects the working memory as well as long-term memory of rodents during maze tasks. Acquisition of memory (learning) during training trials is assessed by comparing day 4 MEL vs day 1 MEL and retrieval of memory is assessed by day 5 TSTQ value in MWM task. A lower day 4 MEL indicates normal acquisition of memory and higher TSTQ indicates retrieval of memory. In current study, the rats treated with ICV colchicine showed decreased spatial learning during acquisition trials and low retrieval of memory i.e. higher day 4 MEL and low TSTQ value in contrast to sham control rats. In EPM, the memory of rodents is assessed by time consumed by animal

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to locate enclosed arm starting from open arm, denoted by transfer latency (TL). Colchicine treatment caused increase in TL value of rats relative to sham control rats. Treatment with ellagic acid reduced day 4 MEL and enhanced TSTQ in rats that were previously administered ICV colchicine. Moreover, ellagic acid treatment to rats that were previously administered ICV colchicine showed reduction in TL value. These results comply with literaturereports, which revealed similar findings in response to ICV colchicine treatment (17). Furthermore, it was observed that plain ellagic acid enhanced the memory in rats in addition to its prevention of colchicine induced dementia. Ellagic acid *per se* group rats showed significantly lower day 4 MEL, higher TSTQ and reduced TL relative to normal control rats. These findings are dependable to the outcome of behavioural studies from other laboratories which signify the memory improving property of ellagic acid (18). Hence, ellagic acid ameliorated working memory in frontal cortex regions and hippocampus as well as retrieval of long-term memory in MTL system and neocortex of rats. The locomotor activity of normal control, colchicine group and ellagic acid *per se* rats showed insignificant variation. This occludes the probability that locomotor activity *per se* may have any affect during behavioural studies.

The antitubulin property of colchicine causes derangement of cellular microtubular functions thereby adversely affecting its metabolic machinery that elicits vigorous production of free radicals ultimately leading to apoptotic and necrotic cell death in brain of rodents (25). High oxygen consumption, iron content, low GSH level and utilization of glucose as obligatory energy source by brain further aggravates the frequency and susceptibility toward oxidative stress (7). Free radicals rapidly oxidize cellular polyunsaturated fatty acids that give rise to highly mutagenic and toxic products viz. malondialdehyde (MDA) and 4-hydroxynonenal (4-HNE) (2). MDA forms neurotoxic 'endducts' such as advanced lipid-peroxidation end products (ALEs), advanced glycation end products (AGEs), bio-molecular adducts (proteins and nucleic acids) and methylglyoxal-acetaldehyde adducts that are extremely immunogenic and cause neurodegeneration (2). MDA is a biomarker of lipid peroxidation and is quantified by TBARS assay. In current study ICV colchicine treatment amplified brain TBARS level and reduced the GSH content of rats. These findings are consistent with previous literature reports where ICV colchicine increased brain MDA level in rodents (18). GSH is a key thiol-antioxidant that detoxify free radicals, peroxidation products and preserves cellular redox status by acting as cysteine reservoir, cofactor for many antioxidant enzymes and directly scavenging free radicals (7). Failure of GSH dependent metabolization of methylglyoxal by glyoxalase amplify AGEs accumulation. A sharp decline in GSH/GSSH ratio in brain

directly correlates with aging and AD. AD patients demonstrate high brain as well as plasma MDA level and low GSH content relative to age matched controls (23, 22). MDA and GSH are two key biomarkers of oxidative stress. In current study, ellagic acid prevented ICV colchicine induced rise in brain TBARS and subsequently arrested the decline of GSH level in rodent brains. Moreover, ellagic acid *per se* group rats showed decrease in brain TBARS level which indicates its antioxidant potential. These findings are concordant with results from other researchers that emphasize the antioxidant potential of ellagic acid (39, 19).

ICV colchicine triggers neuro-infammatory changes in brain, enhance COX-2, TNF-α and other pro-inflammatory cytokine production (35). In the present study the brain TNF-α content of colchicine treated rats was greatly enhanced in comparison to the sham control rats. TNF- α plays vital role in adaptive and acquired immunity but microglial overactivation augments the discharge of pro-inflammatory cytokines such as IL-1β, TNF-α, and IL-6 (1). Chronically elevated brain TNF-α in AD patients (38) leads to pro-inflammatory changes (infiltration of leukocytes), excitotoxic neurodegeneration, overproduction of amyloid-β, apoptosis, necrosis, detrimental changes in lipid and glucose metabolism (28, 32). However, treatment of different groups of rats with ellagic acid for successive 25 days prevented the ICV colchicine induced rise in brain TNF-α level.

CONCLUSION

Ellagic acid enhanced the memory of rats against ICV colchicine induced oxidative stress and neuro-inflammation in rats brain. Furthermore, enhanced memory of ellagic acid *per se* group rats indicates the memory boosting potential of ellagic acid.

REFERENCES

[1] Akiyama, H., Barger, S., Barnum, S., Bradt, B., Bauer, J., Cole GM et al. (2000). Inflammation and Alzheimer's disease. Neurobiology of Aging, **21(3),** 383–421. http://dx.doi.org/10.1016/S0197-4580(00)00124-X

[2] Ayala, A., Munoz, F. M., &Arguelles, S. (2014). Lipid peroxidation: production, metabolism, and signalingmechanisms of malondialdehyde and 4-hydroxy-2-nonenal. Oxidative Medicine & Cellular Longevity, 360438. http://dx.doi.org/10.1155/2014/360438

[3] Baddeley, A. D., Bressi, S., Della Sala, S., Logie, R., & Spinnler, H. (1991). The decline of working memory in Alzheimer's disease. A longitudinal study. Brain, 114(Pt6), 2521–2542. http://dx.doi.org/10.1093/brain/114.6.2521

Kaur, J
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[4] Bensimon, G., & Chermat, R. (1991). Microtubule disruption and cognitive defects: effect of colchicine on learning behavior in rats. Pharmacology Biochemistry Behavior, 38(1), 141–145. http://dx.doi.org/10.1016/0091-3057(91)90602-X

[5] Braak, H., & Braak, E. (1995). Staging of Alzheimer's disease related neurofibrillary changes. Neurobiology of Aging, **16(3),** 271–278; discussion 278–284. http://dx.doi.org/10.1016/0197-4580(95)00021-6

[6] Dhingra, D., & Chillar, R. (2012). Antidepressant-like activity of ellagic acid in unstressed and acute immobilization-induced stressed rat. Pharmacological Reports, **64,** 796–807.

[7] Dringen, R. (2000). Metabolism and functions of glutathione in brain. Progress in Neurobiology, **62(6),** 649–671. http://dx.doi.org/10.1016/S0301-0082(99)00060-X

[8] Ellman, G. L. (1959). Tissue sulfhydryl groups. Archives of Biochemistry & Biophysics, **82,** 70–77.

[9] Emerich, D. F., & Walsh, T. J. (1990). Cholinergic cell loss and cognitive impairments following intracerebroventricular or intradentate injection of colchicine. Brain Research, **517,** 157–167. http://dx.doi.org/10.1016/0006-8993(90)91021-8

[10] Fletcher, P. C., & Henson, R. N. (2001). Frontal lobes and human memory: insights from functional neuroimaging. Brain, 124(Pt 5), 849–881. http://dx.doi.org/10.1093/brain/124.5.849

[11] Graham, W., & Roberts, J. B. (1953). Intravenous colchicine in the treatment of gouty arthritis. Annals of the Rheumatic Diseases, **12(1),** 16–19.

[12] Hartung, E. F. (1954). History of the use of colchicum and related medicaments in gout. Annals of the Rheumatic Diseases, **13(3),** 190–200.

[13] Kumar, A., & Dogra, S. (2009). Neuroprotective effect of carvedilol, an adrenergic antagonist against colchicine induced cognitive impairment and oxidative damage in rat. Pharmacology Biochemistry Behavior, **92,** 25-31. http://dx.doi.org/10.1016/j.pbb.2008.10.005

[14] Kumar, A., Dogra, S., & Prakash, A. (2009). Neuroprotective effects of *Centella asiatica* against intracerebroventricular colchicine-induced cognitive impairment and oxidative stress. International Journal of Alzheimer's disease, 972178. http://dx.doi.org/10.4061/2009/972178

[15] Kumar, A., Naidu, P. S., Sehgal, N., & Padi, S. S. V. (2007). Effect of curcumin on intracerebroventricular colchicine-induced cognitive impairment and oxidative stress in rats. Journal of Medicinal Food, **10,** 486–494. http://dx.doi.org/10.1089/jmf.2006.076

[16] Kumar, A., Naidu, P. S., Sehgal, N., Padi, S. S. V., & Goyal, R. (2007). Colchicines induced neurotoxicity as an animal model of sporadic dementia of Alzheimer's type. Pharmacological Reports, **59,** 274–283.

[17] Kumar, A., Dogra, S., & Prakash, A. (2010). Protective effect of naringin, a citrus flavonoid, against colchicine-induced cognitive dysfunction and oxidative damage in rats. Journal of Medicinal Food, **13(4),** 976-984. http://dx.doi.org/10.1089/jmf.2009.1251

[18] Kumar, M. H. V., & Gupta, Y. K. (2002). Intracerebroventricular administration of colchicine produces cognitive impairment associated with oxidative stress in rats. Pharmacology Biochemistry Behavior, **73,** 565–571. http://dx.doi.org/10.1016/S0091-3057(02)00838-9

[19] Landete, J. M. (2011). Ellagitannins, ellagic acid and their derived metabolites: A review about source, metabolism, functions and health. Food Research International, **44(5),** 1150–1160. http://dx.doi.org/10.1016/j.foodres.2011.04.027

[20] Larrosa, M., Garcia-Conesa, M. T., Espin, J. C., &Tomas-Barberan, F. A. (2010). Ellagitannins, ellagic acid and vascular health. Molecular Aspects of Medicine, **31(6),** 513–539. http://dx.doi.org/10.1016/j.mam.2010.09.005

[21] Levey, A. I., Hallanger, A. E., & Wainer, B. H. (1987). Cholinergic nucleus basalis neurons may influence the cortex via the thalamus. Neuroscience Letters, **74(1),** 7–3.

[22] Mandal, P. K., Saharan, S., Tripathi, M., & Murari, G. (2015). Brain glutathione levels-A novel biomarker for mild cognitive impairment and Alzheimer's disease. Biological Psychiatry, **78(10),** 702–710. http://dx.doi.org/10.1016/j.biopsych.2015.04.005

[23] Marcus, D. L., Thomas, C., Rodriguez, C., Simberkoff, K., Tsai, J. S., Strafaci, J. A., & Freedman, M. L. (1998). Increased peroxidation and reduced antioxidant enzyme activity in Alzheimer's disease. Experimental Neurology, **150(1),** 40–44. http://dx.doi.org/10.1006/exnr.1997.6750

[24] Morris, R. (1984). Developments of a water-maze procedure for studying spatial learning in the rat. Journal of Neuroscience Methods, **11(1),** 47–60.

[25] Nakayama, T., &Sawada, T. (2002). Involvement of microtubule integrity in memory impairment caused by colchicine. Pharmacology Biochemistry Behavior, **71(1-2),** 119–38. http://dx.doi.org/10.1016/S0091-3057(01)00634-7

[26] Ohkawa, H., Ohishi, N., & Yagi, K. (1979). Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction.Analytical Biochemistry, **95(2),** 351–358.

[27] O'Keefe, J., & Dostrovsky, J. (1971). The hippocampus as a spatial map. Preliminary evidence from unit activity in the freely-moving rat. Brain Research, **34(1),** 171–175.

[28] Olmos, G., & Llado, J. (2014). Tumor necrosis factor alpha: A link between neuroinflammation and excitotoxicity. Mediators of Inflammation, 861231. http://dx.doi.org/10.1155/2014/861231

[29] Paxinos, G., Watson, C. R., & Emson, P. C. (1980). AChE-stained horizontal sections of the rat brain in stereotaxic coordinates. Journal of Neuroscience Methods, **3,** 129–149.

[30] Pierrot-Deseilligny, C., Muri, R. M., Rivaud-Pechoux, S., Gaymard, B., & Ploner, C. J. (2002). Cortical control of spatial memory in humans: the visuooculomotor model. Annals of Neurology, **52(1),** 10–19. http://dx.doi.org/10.1002/ana.10273

[31] Reitz, C., & Mayeux R. (2014). Alzheimer disease: epidemiology, diagnostic criteria, risk factors and biomarkers. Biochemical Pharmacology, **88(4),** 640–651. http://dx.doi.org/10.1016/j.bcp.2013.12.024

[32] Ringheim, G. E., Szczepanik, A. M., Petko, W., Burgher, K. L., Zhu, S. Z., & Chao, C. C. (1998). Enhancement of beta-amyloid precursor protein transcription and expression by the soluble interleukin-6 receptor/interleukin-6 complex. Molecular Brain Research, **55(1),** 35–44. http://dx.doi.org/10.1016/S0169-328X(97)00356-2

[33] Sabuncu, M. R., Desikan, R. S., Sepulcre, J., Yeo, B. T., Liu, H., Schmansky, N. J., et al. (2011). The dynamics of cortical and hippocampal atrophy in Alzheimer disease. Archives of Neurology, **68(8),** 1040–1048. http://dx.doi.org/10.1001/archneurol.2011.167

[34] Shigematsu, K., & McGeer, P. L. (1992). Accumulation of amyloid precursor protein in neurons after intraventricular injection of colchicine. The American Journal of Pathology, **140(4),** 787–794.

[35] Sil, S., & Ghosh, T. (2016). Role of cox-2 mediated neuroinflammation on the neurodegeneration and cognitive impairments in colchicine induced rat model of Alzheimer's Disease. Journal of Neuroimmunology, **291,** 115–124. http://dx.doi.org/10.1016/j.jneuroim.2015.12.003

Kaur, J
Kumar, M
Bansal, N

[36] Sonkusare, S., Srinivasan, K., Kaul, C., & Ramarao, P. (2005). Effect of donepezil and lercanidipine on memory impairment induced by intracerebroventricular streptozotocin in rats.Life Sciences, **77,** 1–14. http://dx.doi.org/10.1016/j.lfs.2004.10.036

[37] Squire, L. R., & Zola, S. M. (1996). Structure and function of declarative and nondeclarative memory systems. Proceedings of the National Academy of Sciences USA, **93(24),** 13515–13522.

[38] Tan, Z. S., Beiser, A. S., Vasan, R. S., Roubenoff, R., Dinarello, C. A., Harris, T. B., et al. (2007). Inflammatory markers and the risk of Alzheimer disease: the Framingham Study. Neurology, **68(22),** 1902–1908.

[39] Usta, C., Ozdemir, S., Schiariti, M., & Puddu, P. E. (2013). The pharmacological use of ellagic acid-rich pomegranate fruit. International Journal of Food Science & Nutrition, **64(7),** 907–913. http://dx.doi.org/10.3109/09637486.2013.798268

[40] Vorhees, C. V., & Williams, M. T. (2006). Morris water maze: procedures for assessing spatial and related forms of learning and memory. Nature Protocols, **1(2),** 848–858. http://dx.doi.org/10.1038/nprot.2006.116

[41] Walsh, D. M., & Selkoe, D. J. (2004). Deciphering the molecular basis of memory failure in Alzheimer's disease. Neuron, **44(1),** 181–193. http://dx.doi.org/10.1016/j.neuron.2004.09.010

[42] Whitehouse, P. J., Price, D. L., Clark, A. W., Coyle, J. T., & DeLong, M. R. (1981). Alzheimer disease: evidence for selective loss of cholinergic neurons in the nucleus basalis. Annals of Neurology, **10(2),** 122–126.

[43] Whitehouse, P. J., Price, D. L., Struble, R. G., Clark, A. W., Coyle, J. T., Delon, M. R. (1982). Alzheimer's disease and senile dementia: loss of neurons in the basal forebrain. Science, **215(4537),** 1237–1239.