RSC Advances

ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/advances

Experimental evidence for the potential of Lycopene in the management of Scopolamine induced Amnesia

Rajni Bala, and Deepa Khanna,*

[1]*Cardiovascular Pharmacology Division, Department of Pharmacology,*
Rajendra Institute of Technology and Sciences, Sirsa 125 055, Haryana, India

Running Title: Lycopene prevents Scopolamine induced Amnesia

Word count-

***Correspondence**

Deepa Khanna, Ph.D
Head of Department ofPharmacology
Institute of Pharmacy
Rajendra Institute of Technology and Sciences
Sirsa-125 055
India.
Email: 7drdeepa@gmail.com
Phone: 0091-9416850005

Abstract

Dietary intake of tomatoes and tomato products containing lycopene has been shown to be associated with a decreased risk of chronic diseases. Lycopene is a potent hypocholesterolemic agent, anti-atherosclerotic and anti-cancerous. The present study was conducted to evaluate the effect of lycopene as cognitive enhancer in scopolamine induced amnesic mice. A total of 108 mice divided into 18 groups were employed in the present study. Lycopene was administered orally in two different doses (5 and 10mg/kg) for 15 successive days. Pre-treatment with the tested dose of lycopene (5 and 10 mg/kg) significantly improved the learning ability and retention of learned memory in Elevated plus maze and Hebb Williams maze, taken as exteroceptive behavioral models. Furthermore, scopolamine induced increase in brain acetylcholinesterase and oxidative stress, responsible for amnesia, was significantly reduced by both the doses of lycopene. Serum cholesterol and C-reactive protein (CRP) levels were successfully reduced by lycopene. Moreover the anti-amnesic effect of lycopene were well supported by photomicrographs of hippocampus and cerebral cortex part of brain, where as severity of cell damage, number of pyknotic black neurons, formation of edema and number of neuronal cell death were less comparative to scopolamine group. The observed effects of lycopene claim that it appears to be a promising cognitive enhancer and it would be worthwhile to explore the potential of lycopene in the management of Alzheimer patients.

Key words: Lycopene, Amnesia, Scopolamine

1. Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative brain disorder that is slow in onset but leads to dementia, unusual behavior, personality changes, and ultimately death[1]. AD is characterized by amnesia, agnosia and aphasia[2]. AD caused by degeneration of the brain neurons affects the quality of life of elder people[3]. Amnesia is a loss of memory caused by brain damage, disease, or psychological trauma[4]. The memory can be either, wholly or partially lost due to the extent of damage that was caused[5]. Factors, which play an etiological role in amnesia, are decrease in cholinergic release in brain, increase in free radicals formation leading to oxidative stress, inflammation and Aβ plaques formation[6, 7]. The acetylcholinesterase (AchE) activity has been shown to be increased within and around amnesic brain and associated with increased oxidative stress. Abnormal accumulation of cholesterol levels increase Aβ plaques in cellular and most animal models of AD; and the drugs that inhibit cholesterol synthesis lower Aβ deposits in these models[7]. Essentially amnesia is loss of memory, which can also be caused temporarily by the use of various sedatives and hypnotic drugs[8], while well established memory enhancers like Piracetam, and cholinesterase inhibitors like Donepezil are mainly used to improve memory[9, 10].

Scopolamine-induced cognitive dysfunction is extensively used to probe potential therapeutic agents attenuating cognitive deficits[11, 12, 13]. Intraperitoneal injection of Scopolamine, block the cholinergic neurotransmission, leading to cholinergic dysfunction and impaired cognition in rats, which is relevant to decreased glucose oxidation and increase in brain oxidative stress[14]. Scopolamine induced amnesia leads to increased calcium influx followed by oxidative stress, which in turn increases activity of acetylcholinesterase[15]. One of the mechanisms by which oxidative stress may mediate neurodegeneration is by triggering an inflammatory response. Inflammation is a defense reaction against diverse insults, intended to remove damaging agents and to inhibit their detrimental effects. Scopolamine might increase the expression of pro inflammatory markers Tumor necrosis factor (TNF-α) in the hippocampus[16], which could probably increase C-reactive protein (CRP) levels. Several studies are pouring in showing a strong connection between high cholesterol and high incidence of AD[3, 7]. Scopolamine administration also results in increased serum cholesterol levels as shown in studies carried by Kulkarni et al., 2010[17].

Natural products containing beneficial phytoconstituents can exert numerous favorable effects on brawn and brain and can modify brain aging[18]. Lycopene is a carotenoid that is present in tomatoes, processed tomato products and other fruits. It is one of the most potent quencher of singlet oxygen among dietary carotenoids[19]. Dietary intake of tomatoes and tomato products containing lycopene has been shown to be associated with a decreased risk of chronic diseases [20]. Lycopene is a potent hypocholesterolemic agent[21], anti-atherosclerotic [22], and anti-cancerous [23] It has proven effects in the treatment of osteoporosis[24], dermatology[25] and dental hygiene[26]. Lycopene also exhibits anti-inflammatory[27, 28] and anti-coagulant activity[29]. Recent studies purposed that the anti-oxidative action of lycopene may contribute to the protection against Aβ-induced neurotoxicity[30]. Lycopene attenuates biochemical and cellular alteration in 3-nitropropionic acid (NP)-induced Huntigton's disease (HD) like symptoms in rats[31]. Likewise, lycopene was found to be effective in preventing mitochondrial oxidative stress and dysfunctions in 3-NP-induced HD[32]. Study conducted by Lin et al., described the anti neuro-inflammatory effects of lycopene via activation of adenosine monophosphate-activated protein kinase-α1/heme oxygenase-1 pathways[27]. The above studies certainly suggest neuroprotective potential of lycopene however, the effect of lycopene as cognitive enhancer is not yet known. Therefore, we were motivated to study the potential of lycopene in the management of cognitive dysfunctions and to establish its effects on central nervous system.

2. Materials and methods

2.1. Animals

A total of 108 mice were employed in the present study. All the experiments were carried out with young 3 months old Swiss Albino mice of 22–28 g weight. Animals were procured from disease free small animal house of Lala Lajpat Rai University of Veterinary & Animal Sciences, Hisar, Haryana, India. Animals had free access to food and water, and they were housed in a natural (12h) each light–dark cycle. Animals were fed with mice chow (obtained from Ashirwad Industries, Mohali, India) and water ad libitum. Experiments were carried out between 0900 and 1800 h. The animals were acclimatized for at least 5 days to the laboratory conditions. The experimental protocol; was approved by the Institutional Animal Ethical Committee (IAEC) and the care of laboratory animals was taken as per the guidance of Committee for the purpose of

control and supervision of experiments on animals (CPCSEA), Ministry of Forests and Environment, Government of India (registration number 888/ac/05/CPCSEA 29-04-2005).

2.2. Drugs and Chemicals

Drugs and Chemicals used in this study were obtained from following drug houses. Scopolamine hydrobromide & 5, 5′-dithiobis 2-nitro benzoic acid (DTNB) (Sigma-Aldrich St, Louis, MO, USA), Lycopene obtained as exgratia sample (BAPEX Pvt. Ltd. India), Trichloro-acetic acid (RANKEM, New Delhi, India), Thiobarbituric acid & Acetylcholine iodide (Otto Chemika-Biochemica, Mumbai, India), Reduced glutathione (SD Fine, Mumbai, India). Other chemicals used were of analytical grade.

2.3. Preparation of doses

5 mg/kg & 10 mg/kg dose of lycopene was suspended in 0.5% Sodium carboxy methyl cellulose (CMC) and administered orally in mice. Piracetam, scopolamine and donepezil were dissolved separately in normal saline and injected intraperitonealy. Volume of oral administration and *i.p* injection was 1ml/100g of mouse.

2.4. Experimental design

In the present investigation, the mice were randomly divided into 18 groups. Each group comprised of 6 mice. lycopene (5 mg/kg and 10 mg/kg) was administered orally along with diet for 15 successive days. After 90 min of administration of the last dose (on 15th day), mice were exposed to Elevated plus maze and Hebb William's maze for recording transfer latency (TL) and time taken to reach reward chamber (TRC). Amnesia was induced in separate groups (interoceptive models) of mice by scopolamine hydrobromide (0.4mg/kg; *i.p.*) after 90 min of the last dose of lycopene (5 mg/kg and 10 mg/kg) administration on fifteenth day. The animals were exposed to the training session (learning) using Elevated plus maze and Hebb William's maze (on 15th day) after 45 min of scopolamine injection. Retention (memory) was assessed after 24 hours i.e. on 16th day. Piracetam (400 mg/kg; *i.p.*) was used as an established nootropic agent and was injected for 15 days. Donepezil (1 mg/kg; *i.p.*) was injected 60 min before dissecting brain served as positive control for the comparison of brain acetylcholinesterase activity.

Atorvastatin (10mg/kg; p.o.) for 15 successive days served as positive control for comparing total serum cholesterol levels.

2.5. Elevated plus maze

Elevated plus maze served as the exteroceptive behavioral model to evaluate memory in mice. The procedure, technique and end point for testing memory were followed using the parameters described by Vasudevan and Parle, 2006[3]. The elevated plus maze for mice consisted of two open arms (16 cm×5 cm) and two covered arms (16 cm×5 cm×12 cm) extended from a central platform (5 cm×5 cm), and the maze was elevated to a height of 25 cm from the floor. On the first day, each mouse was placed at the end of an open arm, facing away from the central platform. Transfer latency was defined as the time (in seconds) taken by the animal to move from the open arm into one of the covered arms with all its four legs and was recorded on the first day (training session) for each animal. The mouse was allowed to explore the maze for another 2 minutes and then returned to its home cage. Retention of this learned-task (memory) was examined 24 h after the first day trail. Significant reduction in TL value of retention indicates improvement in memory.

2.6. Hebb-Williams maze

It is an incentive based exteroceptive behavioral model useful for measuring spatial and working memory of mice[33]. It consists of mainly three components. Animal chamber (Start box), which is attached to the middle chamber (exploratory area) and a reward chamber at the other end of the maze in which the reward (Food) is kept. All the three components were provided with guillotine removable doors. 12 h fasted mice were employed in the study. Each mouse was placed in animal chamber (Start box) and door was opened to facilitate the entry of animal into the next chamber. The door of start box was closed immediately after the animal moved in to the next chamber so as to prevent its back entry. Time taken in seconds by the animal to reach reward chamber (TRC) from the start box was noted for each animal. Each animal was allowed to explore the maze for additional 20 seconds, with all its doors opened before returning to its home cage. A fall in TRC on subsequent maze exposures was taken as an index of successful retention.

2.8. Biochemical estimations

2.8.1. Collection of blood and brain samples

The animals were sacrificed by cervical decapitation under light anesthesia on the fifteenth day 90 min after last dose of drug administration. Immediately after decapitation, the trunk blood was collected then whole brain was carefully removed from the skull. The collected brain was washed with ice cold isotonic saline and weighed. The fresh whole brain was transferred to a glass homogenizer and homogenized in an ice bath after adding 10 volumes of 0.9% NaCl solution. The homogenate was centrifuged at 3000 rpm for 10 minutes and the resultant cloudy supernatant liquid was used for estimation of AchE, TBARS and GSH. The collected blood was centrifuged at 3000 rpm for 15 min so as to separate serum. The serum was used for estimation of cholesterol and C-reactive protein (CRP) levels

2.8.2. Estimation of serum total cholesterol

The serum total cholesterol concentration was estimated by cholesterol oxidase-peroxidase (CHOD-PAP) method using commercial available kit (Transasia Biomedical Ltd., Solan, India). The working reagent 1000 µL was added to 20 mL of distilled water, 20 µL standard cholesterol solutions (200 mg/dL) and 20 µL serum samples to prepare blank (B), standard (S) and test (T), respectively, with thorough mixing. All the glass tubes were incubated at 37 ° C for 10 minutes. At the end the absorbance of S and T was measured against B spectrophotometrically at 505 nm.

$$\text{Cholestrol (mg/dL)} = \frac{\text{Absorbance of Test (T)}}{\text{Absorbance of standard (S)}} \times 200$$

2.8.3. Estimation of serum C-reactive protein (CRP)

The serum CRP, a marker of inflammation, was measured using an immunoassay kit (Immunospec Corporation, CA, and USA). This estimation was done from Nalwa laboratories Pvt. Ltd., Hisar, India. Qualitative measurement of CRP was done in rat serum by turbidimetric immunoassay method. In this method, CRP in the sample binds to specific anti-CRP antibodies, which have been absorbed to latex particles and agglutinates. The agglutinate was proportional to

the quantity of CRP in the sample. The actual concentration was then determines by interpolation from a calibration curve prepared from calibrators of known concentrations.

The CRP concentration was calculated by using formula:

$$\text{CRP concentration in mg/L} = \frac{(A2 - A1)\ \text{sample}}{(A2 - A1)\ \text{calibrator}} \times \text{calibrator concentration}$$

Calibrator concentration = 150 mg/L, A1 = Initial concentration, A2 = Final concentration

2.8.4. Estimation of brain acetylcholinesterase (AchE)

Acetylcholinesterase activity was measured by the method of Ellman et al. 1961 [34] with a slight modification[35]. The cloudy supernatant liquid (0.5 ml) was pipette out into 25 ml volumetric flask and dilution 1was made with a freshly prepared DTNB (5, 5-dithiobis-2-nitrobenzoic acid) solution (10 mg DTNB in 100 ml of Sorenson phosphate buffer, pH 8.0). From the volumetric flask, two 4 ml portions were pipetted out into two test tubes. Into one of the test tubes, 2 drops of eserine solution was added. 1 ml of substrate solution (75 mg of acetylcholine iodide per 50 ml of distilled water) was pipetted out into both tubes and incubated for 10 minutes at 30 °C. The solution in the tube containing eserine was used for zeroing the spectrophotometer. The resulting yellow color due to reduction of DTNB by certain substances in the brain homogenate and due to non-enzymatic hydrolysis of substrate. After having calibrated the instrument, change in absorbance per minute of the sample was read at 420 nm.

2.8.5. Assessment of brain oxidative stress:

2.8.5.1. Estimation of thiobarbituric acid reactive substances (TBARS)

Brain TBARS, thiobarbituric acid reactive substances were estimated as per method given by Ohkawa et al., 1979[36]. The reaction mixture was prepared by mixing 0.2 mL brain homogenate, 0.2 mL of 8.1% sodium dodecyl sulphate, 1.5 mL of 20% acetic acid solution (adjusted to pH 3.5 with NaOH), and 1.5 mL of 0.8% aqueous solution of thiobarbituric acid. The reaction mixture was made up to 4.0 mL with distilled water, and incubated at 95 ºC for 60 minutes. Upon cooling in tap water, 1.0 mL distilled water and 5.0 mL of the mixture of n-butanol and

pyridine (15:1 v/v) were added to reaction mixture and shaken using vortex shaker. The test tubes were centrifuged at 4000 rpm for 10 minutes. The absorbance of the developed pink colour was measured spectrophotometrically at 532 nm. The standard curve using 1, 1, 3, 3-tertramethoxyopropane was plotted to calculate the concentration of brain TBARS. The brain TBARS concentration was expressed as nanomol/g.wt of brain.

2.8.5.2. Estimation of reduced glutathione (GSH)

The GSH, reduced glutathione level was estimated using the methods described by Ellman (1959)[37] and Boyne and Ellman (1972)[38]. The brain homogenate was mixed with 10% w/v trichloroacetic acid in 1:1 ratio and centrifuged at 4ºC for 10 minutes at 5000 rpm. The supernatant (0.5 mL) was mixed with 2 mL of 0.3 M disodium hydrogen phosphate buffer (pH 8.4) and 0.4 mL of distilled water. Then, 0.25 mL of 0.001M freshly prepared DTNB [dissolved in 1% w/v sodium citrate] was added to the reaction mixture, and incubated for 10 minutes. The absorbance of the yellow coloured complex was noted spectrophotometrically at 412 nm. A standard curve using the reduced form of glutathione was plotted to calculate the concentration of brain GSH. The brain GSH concentration was expressed as µM/g wet weight of brain.

2.9. Histopathological study

Whole brain of mice which was carefully removed from the skull was kept in 10% formalin solution. The brain was stained with haematoxylin-eosin stain. Brain hippocampus and cortex region were studied under microscope (Olympus India CH 20i). The samples were examined at Nalwa laboratories, Hisar, Haryana, India.

2.10. Statistical analysis

All value were expressed as mean ± S.D. Data obtained from various groups were statistically analyzed using one way ANOVA, followed by Dunnett's t-test. A 'p' value of less than 0.01 was considered statistically significant.

3. Results

3.1. Effect of Lycopene on memory deficits

3.1.1. Effect of Lycopene on transfer latency (TL) using Elevated plus maze

TL of the second day/ 16th day of drug treatment reflected retention of information or memory. Administration of lycopene (5 mg/kg, 10 mg/kg) for 15 successive days significantly reduced ($p< 0.01$) TL of 16th day in mice (6.5±1.87, 8.0±2.28), when compared to normal control (15.6±3.01) group (Fig.1.). This significant reduction in TL indicates improvement in memory of mice. Administration of scopolamine (0.4 mg/kg; *i.p.*) on 15th day showed significant enhancement ($p< 0.01$) in TL value (22.0±3.57) indicating induction of amnesia in mice. However, 5 mg/kg and 10 mg/kg lycopene pretreatment successfully reversed ($p< 0.01$) (15.8±3.18, 15.8±1.70) memory deficits induced by Scopolamine (Fig.2). Mice treated with piracetam, positive control (400mg/kg; *i.p.*) for 15 successive days showed significant improvement (9.0±2.36) in the memory of mice and reversed amnesia induced by scopolamine as expected (Fig.2).

3.1.2. Effect of Lycopene on time taken to reach reward chamber (TRC) using Hebb-Williams maze

TRC is defined as time taken in seconds by the animal to reach reward chamber from the start box. Significant reductions in TRC value indicate retention of learned task and thus improvement of memory. Administration of lycopene (5 mg/kg, 10 mg/kg) for 15 successive days showed significant reduction ($p< 0.01$) in TRC of mice (15.3±2.16, 18.3±1.63), when compared to normal control (24.8±2.6) group indicating significant improvement in memory (Fig.3). Scopolamine (0.4 mg/kg; *i.p.*) significantly increased ($p< 0.01$) TRC (62.5±3.14) indicating impairment in memory. Lycopene pretreatment at 5mg/kg, 10 mg/kg dose successfully reversed ($p< 0.01$) (23.5±1.51, 28.6±1.21) memory deficits induced by scopolamine (Fig.4). Piracetam used as positive control (400mg/kg; *i.p.*) for 15 successive days showed significant improvement (29.1±2.04) in the memory of mice and reversed amnesia induced by scopolamine (Fig.4).

3.2. Effect of Lycopene on serum cholesterol

Administration of lycopene (5 mg/kg, 10 mg/kg) for 15 successive days showed significant reduction ($p< 0.01$) in serum cholesterol levels of mice (79.84±1.15, 83.61±1.17), when

compared to normal control (98.97±1.93) group. Scopolamine administration significantly increased serum cholesterol levels (p< 0.01) (163.51±3.35), when compared to normal control. Lycopene pretreatment at 5mg/kg, 10 mg/kg dose successfully reduce (p< 0.01) (86.76±0.91, 96.61±1.11) serum cholesterol levels elevated by scopolamine (Fig.5). Atorvastatin used as positive control (10 mg/kg; *p.o.*) for 15 successive days showed significant reduction (61.68±1.02) in serum cholesterol levels of mice when compared to normal control. Atorvastatin showed significant reduction of serum cholesterol levels by 37.68% (p< 0.01) in mice when compared to normal control. In lycopene pretreated groups total percentage reduction in serum cholesterol was 24.50% (p< 0.01) at 5 mg/kg dose and 19.33% (p< 0.01) at 10 mg/kg dose.

3.3. Effect of Lycopene on serum C-reactive protein (CRP)

Mice received lycopene (5mg/kg and 10 mg/kg) treatment for 15 successive days showed significant reduction in serum CRP levels. Scopolamine administration significantly increased serum CRP levels (p< 0.01) (0.74±0.01), when compared to normal control (0.57±0.01) group. Lycopene pretreatment at 5mg/kg, 10 mg/kg dose successfully reversed (p< 0.01) (0.62±0.01, 0.58±0.02) serum CRP levels elevated by scopolamine (Fig.6).

3.4. Effect of Lycopene on brain acetyl cholinesterase (AchE)

Administration of lycopene (5 mg/kg, 10 mg/kg) for 15 successive days showed significant reduction (p< 0.01) in brain AchE levels of mice (0.082±0.002, 0.089±0.001), when compared to normal control (0.107±0.001)group. Scopolamine administration significantly increased AchE levels (p< 0.01) (0.251±0.023), when compared to normal control. Lycopene pretreatment at 5mg/kg, 10 mg/kg dose successfully reverse (p< 0.01) (0.109±0.012, 0.127±0.008) AchE levels elevated by scopolamine (Fig.7). Donepezil used as positive control (1mg/kg; *i.p.*) for 15 successive days showed significant reduction (0.077±0.001) in AchE levels of mice when compared to normal control group.

3.5. Effect of Lycopene on lipid peroxidation

Administration of lycopene (5 mg/kg, 10 mg/kg) for 15 successive days showed significant reduction (p< 0.01) in brain TBARS levels of mice (6.71±0.45, 7.34±0.61), when compared to normal control (9.49±0.20) group. Scopolamine administration significantly increased TBARS

levels ($p< 0.01$) (16.08±0.98), when compared to normal control. Lycopene pretreatment at 5mg/kg, 10 mg/kg dose successfully reverse ($p< 0.01$) (10.50±0.33, 11.41±0.68) TBARS levels elevated by scopolamine (Fig.8). Piracetam used as positive control (400mg/kg; *i.p.*) for 15 successive days showed significant improvement (5.16±0.16) in TBARS levels of mice when compared to normal control group.

3.6. Effect of Lycopene on reduced glutathione (GSH)

Administration of lycopene (5 mg/kg, 10 mg/kg) for 15 successive days showed significant increase ($p< 0.01$) in brain GSH levels of mice (0.95±0.03, 0.92±0.04), when compared to normal control (0.74±0.04) group. Scopolamine administration significantly reduced GSH levels ($p< 0.01$) (0.36±0.02), when compared to normal control group. Lycopene pretreatment at 5mg/kg, 10 mg/kg dose successfully reverse ($p< 0.01$) (0.84±0.04, 0.83±0.04) decreased GSH levels reduced by scopolamine (Fig.9). Piracetam used as positive control (400mg/kg; *i.p.*) for 15 successive days showed significant improvement (1.09±0.02) in GSH levels of mice when compared to normal control group.

3.7. Effect of Lycopene on brain histology:

Examination of brain sections of normal control mice showed the normal structure of the cerebral cortex and hippocampus indicated by presence of viable cells in the structure (Fig. 10A, 11A). On the other hand cerebral cortex of scopolamine induced amnesic mice revealed severe congestion in the blood capillaries with perivascular edema and damage consisted of focal gliosis (Fig 10 B1 & 10 B2) and the hippocampus showed neuronal cell degeneration, pyknotic black neurons with condensed nucleus and edema (Fig. 11B). Brain sections of mice pretreated with lycopene (5mg/kg) revealed moderate focal gliosis and moderate shrinkage of blood capillaries in the cerebral cortex (Fig10 C1 & 10 C2), when compared to scopolamine group while, the hippocampus showed less number of degenerated cells (Fig. 11C) indicating moderate protection against scopolamine induced injury whereas, brain sections of mice pretreated with lycopene (10mg/kg) revealed normal cerebral cortex and hippocampus, when compared to scopolamine group (Fig. 10D & 11D) indicating marked protection against scopolamine-induced brain injury.

4. Discussion

Alzheimer's disease is characterized by progressive memory loss, cognitive impairment and personality defects accompanied by diffuse structural abnormalities in the brain. Amnesia is a clinical syndrome characterized by the development of multiple cognitive defects due to synaptic failure, neuronal injury and neuronal death. This sequence involved in brain dysfunctioning is the net result of altered neurotransmitters, inflammation, oxidative stress, cholesterol accumulation[39]. The aim of the study is to evaluate lycopene as cognitive enhancer in scopolamine induced amnesia in mice. Scopolamine-induced cognitive dysfunction is extensively used to probe potential therapeutic agents attenuating cognitive deficits[12]. Systemic administration of scopolamine has been reported to be more effective in disrupting learning and acquisition, and short-term retention of spatial memory [40]. Indeed in the present study, single *i.p.* injection of scopolamine at the dose of 0.4mg/kg causes loss of memory as accessed on exteroceptive behavior models i.e. Elevated plus maze and Hebb's Williams maze. Lycopene (5 mg/kg, 10 mg/kg *p.o.*) treatment for 15 successive days improved the memory of mice as reflected by diminished transfer latency (TL) as well as time taken to reach reward chamber (TRC) values, when compared to their respective scopolamine treated groups. Lycopene (5mg/kg, 10mg/kg *p.o.*) per se and piracetam (400mg/kg *i.p.*) produced memory enhancing effect in mice, when compared to normal control thus, suggesting the reversal of amnesia. Piracetam, the established nootropic agent was used for the comparison as it improves the memory by exerting effect on AchE neurotransmitter and NMDA glutamate receptors[41, 42]. Piracetam, protects neurons from hypoxia, and stimulates growth of acetylcholine receptors[3].

Lycopene was not previously investigated in Scopolamine-induced amnesia model. However, in a study performed by Hsiao et al., (2004), lycopene affords neuroprotection against microglia activation and focal cerebral ischemia in rats[43]. Lycopene was suggested as one of the carotenoid capable of enhancing cognition in healthy elderly population[44]. Huntington's disease is a neurodegenerative disorder characterized by degeneration of the striatal neurons. Lycopene was found to be effective in preventing mitochondrial oxidative stress and dysfunctions in 3-NP-induced Huntington's disease[32]. Lycopene attenuates biochemical and cellular alterations in 3-nitropropionic acid-induced Huntington's disease like symptoms in rats[31]. Likewise neuroprotective effect of lycopene against Polychlorinated biphenyl (PCBs) induced nitrosative stress in cerebral cortex was related to its RNS quenching property[45]. Further lycopene has been

shown as a neuroprotective agent against Aβ-induced neurotoxicity in primary cultured rat and it was suggested as a promising candidate for Alzheimer Disease treatment [30].

The main histological features of AD include deposition of inter neuronal Aβ plaques and intraneuronal neurofibrillary tangles. Abnormal accumulation of cholesterol levels increase Aβ plaques in cellular and most animal models of AD; and the drugs that inhibit cholesterol synthesis lower Aβ deposits in these models[7]. Several studies link high levels of cholesterol and progression of Alzheimer's disease. Lycopene dose-dependently reduced intracellular total cholesterol levels by reducing of 3-hydroxy-3-methylglutaryl coenzyme A reductase expression, which is responsible for the synthesis of cholesterol[21]. Meta-analysis study by Ried and Fakler 2010 suggested protective effects of lycopene in reducing LDL cholesterol as comparable to the effect of low doses of statins in patient with slightly elevated cholesterol levels [46]. Scopolamine administration (0.4mg/kg *i.p.*) results in increased serum cholesterol levels as showed in study carried by Kulkarni and coworkers, 2010[17]. Similarly in the present study scopolamine treated mice showed significant increase in cholesterol levels, when compared to normal control animals. Hypercholesterolemic effects in scopolamine group was successfully reversed by lycopene pretreatment thus suggesting that lycopene at the dose of 5mg/kg and 10mg/kg could be preventing the accumulation of β- amyloid plaques and intraneuronal neurofibrillary tangles by lowering cholesterol levels. Moreover, hypocholesterolemic effects of atorvastatin (10mg/kg *p.o.* standard) and lycopene 5mg/kg, 10mg/kg *per se* groups were significant, when compared to normal control groups.

Published studies tended to show that increase in pro-inflammatory markers TNF-α, IL-6, IL-1 and inflammatory marker, CRP led to progression of cognitive decline [16,47,48]. C-reactive protein, an acute phase protein, is a sensitive marker of inflammation. It has been believed that the synthesis of CRP is induced by pro inflammatory markers [49]. Muscarinic receptors in the central nervous system inhibit systemic inflammation in animals and activation of muscarinic cholinergic transmission in the central nervous system lowers serum TNF levels [50]. Since Scopolamine is a non-selective muscarinic receptor antagonist, blockage of muscarinic receptor by Scopolamine might increase the expression of pro inflammatory markers TNF-α in the hippocampus[16], which could probably increase the CRP levels. Lee et al., (2012), suggested that lycopene promotes barrier integrity, inhibits monocyte adhesion, block activation of pro

inflammatory cytokines, inhibit expression of high-mobility group protein B1 (HMGB1)-mediated tumor necrosis factor and HMGB1- mediated pro-inflammatory signaling responses in endothelial cells, showing its usefulness as a therapy for vascular inflammatory diseases[51]. Lin et al., 2013 suggested that lycopene can be a useful therapeutic agent for the treatment of neuroinflammation-associated disorders as it inhibits lipopolysaccharide-induced COX-2 expression[27]. Lycopene effectively reduced inflammation by inhibiting the release of TNF-α and stimulating IL-10 production[28]. In the present study, scopolamine treated animals showed marked increase in CRP levels, when compared to normal control groups indicating involvement of inflammation as one of the parameter leading to amnesia. Lycopene treatment (5mg/kg and 10mg/kg p.o.) attenuates scopolamine induced increase in C-reactive protein levels thus proving its anti-inflammatory potential.

Scopolamine interferes with central cholinergic functions and memory circuits, leading to decreased acetylcholine release and subsequent amnesia[51]. Acetylcholine is considered to be the most important neurotransmitter responsible for consolidating and maintaining long term memory[52]. Selective loss of cholinergic neurons and increased acetyl cholinesterase (enzyme responsible for degradation of Ach) activity was also reported to be a characteristic feature of Alzheimer's disease. The use of acetylcholinesterase (AchE) inhibitors reversed the amnesia produced by disruption of cholinergic system[53]. To improve cholinergic transmission, different strategies are adopted, including increase of Ach synthesis, the augmentation of presynaptic Ach release, and the stimulation of cholinergic post synaptic muscarinic and nicotinic receptors and the inhibition of Ach synaptic degradation by employing cholinesterase inhibitors[54]. The acetylcholinesterase activity has been shown to be increased within and around amnesic brain. Further AchE activity has been shown to be associated with increased oxidative stress. It has been also observed that acetylcholine plays a neuroprotective role by scavenging superoxide anions, thereby reducing the lipid peroxidation level[55]. Scopolamine induced amnesia leads to increased calcium influx followed by oxidative stress which in turn increases activity of acetylcholinesterase [15]. Lycopene improves the cholinergic transmission in Neurohepatic toxicity induced by manganese chloride exposure by inhibiting AchE activity[56] or by preventing oxidative stress[57]. In the present study acetylcholinesterase activity in the brain homogenate of mice treated with scopolamine was also found to be increased, when compared with the normal control group. Scopolamine induced increase in acetylcholinesterase activity was successfully

attenuated by lycopene 5mg/kg, 10mg/kg dose, suggesting that lycopene pretreatment improve cholinergic transmission by inhibiting acetylcholinesterase enzyme. Donepezil used as standard drug is a highly selective acetylcholinesterase inhibitor and one of the only four drugs currently approved for treatment of Alzheimer's type dementia[58]. Donepezil as standard and lycopene 5mg/kg, 10mg/kg per se as test dose act as acetylcholinestrase inhibitor, when compared to normal control group.

Scopolamine-induced amnesia is relevant to decreased glucose oxidation, increase in brain oxidative stress[14,59] showed in their study that in scopolamine-treated group brain malondialdehyde (MDA) levels was increased and an antioxidant status was deteriorated as observed by changes in superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) activity [59]. In the present study mice after scopolamine treatment showed a significant increase in the brain TBARS levels, which is the measure of lipid peroxidation and free radical generation. Further, scopolamine reduces the level of antioxidant GSH. Qu et al., 2011 purposed that the anti-oxidative action of lycopene may contribute to the protection against Aβ-induced neurotoxicity[30]. The accumulated experimental evidence suggests that lycopene can quench singlet oxygen showing its ability to scavenge free radical of (NOO-, RS- and RSOO-) in free radical models, and also protect cell components against oxidative damage in cell culture models, or in animal models [60, 61]. Lycopene attenuated oxidative stress by altering, glutathione peroxidase and catalase enzyme activities in cadmium-treated rats[62]. Lycopene obtained from cooked tomatoes significantly restored the antioxidant enzymes SOD, GSH-Px, GSH, glutathione reductase (GR), and decreases, the levels of the lipid peroxide MDA in patients suffering from coronary heart disease[63]. Likewise, in the present study lycopene proved its antioxidant potential by reducing elevated TBARS and reduced GSH levels. Increasing scopolamine induced oxidative stress was successfully attenuated by pretreating with lycopene 5mg/kg, 10mg/kg per se as test dose act as antioxidant, when compared to normal control thus confirming the fact that lycopene may be looked upon as a champion of all the antioxidants [24] known till date by virtue of which possibly susceptible brain cells get exposed to less oxidative stress, resulting in reduced brain damage and improved neuronal function.

Histological examination of stained brain sections revealed serious damaging effects of scopolamine on brain tissue, when compared to normal brain sections of normal control group.

Examination of brain sections of normal control mice showed the normal structure of the cerebral cortex and hippocampus while brain sections of scopolamine induced amnesic mice revealed severe congestion in the blood capillaries with perivascular edema and damage consisted of focal gliosis in the cerebral cortex likewise, the hippocampus showed neuronal cell degeneration, pkynotic black neurons with condensed nucleus and edema. Gliosis results from activation of glial cells following induction of inflammation associated brain damage[64].

Pretreatment of lycopene 5mg/kg dose provided partial protection against Scopolamine-induced brain injury by revealing moderate focal gliosis and moderate shrinkage in blood capillaries in the cerebral cortex, when compared to scopolamine group while, the hippocampus showed less number of degenerated cells. Brain sections of mice pretreated with lycopene 10 mg/kg dose provided marked protection against scopolamine-induced brain injury, which was revealed by near restoration of the normal structure of cerebral cortex and hippocampus. Therefore, lycopene prevented the formation/ extent of neuron degeneration by reducing in the severity of cell damage and decrease in the count of dead cells and also reduction in the formation of pyknotic black neurons comparative to scopolamine hippocampus, where as all these parameters were increased, these results are consistent with previous findings [15, 64] thereby, lycopene further supports its role as a promising neuroprotective agent.

5. Conclusion

In the present study, based on behavioral and biochemical parameter assessments we observed that both doses of lycopene successfully reversed the scopolamine induced amnesia. Lycopene pretreatment attenuated (i) elevated TL and TRC values (ii) elevated serum cholesterol levels (iii) elevated CRP levels (iv) increased AchE activity (v) increased oxidative stress as suggested by increased TBARS and reduced GSH levels. Further, scopolamine induced histological changes in cortex and hippocampus of amnesic mice brain was moderately protected by lycopene pretreatment. In light of above, it may be worthwhile to explore the potential of lycopene clinically for the management of amnesia.

Conflict of interest

The authors declare that there is no conflict of interest.

Acknowledgements

The authors are thankful to the chairman, Dr. Rajendra Singh Sra and secretary Dr. Om Parkash and Principal Mr. Sanjeev Kalra, Rajendra Institute of Technology and Sciences, Sirsa, for providing the necessary facilities to carry out the experimental study.

References

References

1. R.D. Jewart, J. Green, C.J. Lu, J. Cellar, L.E. Tune, Cognitive, behavioral, and physiological changes in Alzheimer disease patients as a function of incontinence medications. *Am J Geriatr Psychiatry.,* 2005, **13,** 324-328.

2. D. Dhingra, M. Parle, S.K. Kulkarni, Genetic basis of Alzheimer's disease. Indian J Pharm Sci., 2005, **67,** 409–413.

3. M. Vasudevan, M. Parle, Pharmacological actions of Thespesia populnea relevant to Alzheimer's disease. *Phytomedicine.,* 2006, **3,** 677–687.

4. S.L. Mullally, E.A. Maguire, Learning to remember: The early ontogeny of episodic memory. *Dev Cogn Neurosci.,* 2014, **9C,** 12-29.

5. C. Pittenger, Disorders of memory and plasticity in psychiatric disease. *Dialogues Clin Neurosci*., 2013, **15,** 455-463.

6. X. Zhu, B. Su, X. Wang, M.A. Smith, G. Perry, Causes of oxidative stress in Alzheimer disease. *Cell Mol Life Sci*., 2007, **64,** 2202-2210.

7. L. Puglielli, R.E. Tanzi, D.M. Kovacs, Alzheimer's disease: the cholesterol connection. *Nat Neurosci.,* 2003, **6,** 345–351.

8. C. Jones, R.D. Griffiths, G. Humphris, Disturbed memory and amnesia related to intensive care. *Memory*., 2000, **8,** 79-94.

9. S. Shinagawa, M. Shigeta, Acetylcholinesterase inhibitors for treatment of Alzheimer's disease. *Brain Nerve*., 2014, **66,** 507-516.

10. V.V. Iasnetsov, I.N. Krylova, The antiamnestic effect of nootropic substances in rats. *Eksp Klin Farmakol.,*2013, **76,** 3-6.

11. J. Chen, Y. Long, M. Han, T. Wang, Q. Chen, R. Wang, Water-soluble derivative of propolis mitigates Scopolamine-induced learning and memory impairment in mice. *Pharmacol Biochem Behav*., 2008, **90,** 441-446.

12. P. Mohapel, G. Leanza, M. Kokaia, O. Lindvall, Forebrain acetylcholine regulates adult hippocampal neurogenesis and learning. *Neurobiol Aging.,* 2005, **26,** 939-946.

13. S.E. Molchan, R.A. Martinez, J.L. Hill, H.J. Weingartner, K. Thompson, B. Vitiello, T. Sunderland, Increased cognitive sensitivity to Scopolamine with age and a perspective on the Scopolamine model. *Brain Res Brain Res Rev.,* 1992, **17,** 215-226.

14. P. Goverdhan, A. Sravanthi, T. Mamatha, Neuroprotective effects of meloxicam and selegiline in Scopolamine-induced cognitive impairment and oxidative stress. *Int J Alzheimers Dis*., 2012, **2012**, e974013.

15. S.M. Biradar, H. Joshi, T.K. Chheda, Neuropharmacological effect of Mangiferin on brain cholinesterase and brain biogenic amines in the management of Alzheimer's disease. *Eur J Pharmacol.,* 2012, **683,** 140-147.

16. Y.J. Jang, J. Kim, J. Shim, C.Y. Kim, J.H. Jang, K.W. Lee, H.J. Lee, Decaffeinated coffee prevents Scopolamine-induced memory impairment in rats. *Behav Brain Res.,* 2013, **245,** 113-119.

17. K.S. Kulkarni, S.B. Kasture, S.A. Mengi, Efficacy study of Prunus amygdalus (almond) nuts in Scopolamine-induced amnesia in rats. *Indian J Pharmacol.,* 2010, **42,** 168–173

18. H. Joshi, M. Parle, Brahmi rasayana improves learning and memory in mice. *Evid Based Complement Alternat Med.,* 2006, **3,** 79-85.

19. I. Karahan, A. Ateşşahin, S. Yilmaz, A.O. Ceribaşi, F. Sakin, Protective effect of lycopene on gentamicin-induced oxidative stress and nephrotoxicity in rats. *Toxicology.,* 2005, **215,** 198-204.

20. T. Rissanen, S. Voutilainen, K. Nyyssönen, R. Salonen, J.T. Salonen, Low plasma lycopene concentration is associated with increased intima-media thickness of the carotid artery wall. *Arterioscler Thromb Vasc Biol.,* 2000, **20,** 2677-2681.

21. P. Palozza, N. Parrone, R.E. Simone, A. Catalano, Lycopene in atherosclerosis prevention: An integrated scheme of the potential mechanisms of action from cell culture studies. *Arch Biochem Biophys.,* 2010, **504,** 26–33.

22. L. Badiman, G. Vilahur, T. Padro, Nutraceuticals and atherosclerosis: human trials. *Cardiovasc Ther.,* 2010, **5,** 627-35.

23. F.Y. Tang, H.J. Cho, M.H. Pai, Y.H. Chen, Concomitant supplementation of lycopene and eicosapentaenoic acid inhibits the proliferation of human colon cancer cells. *J Nutr Biochem.,* 2009, **20,** 426-434.

24. L. Kim, A.V. Rao, L.G. Rao, Lycopene II—effect on osteoblasts: the carotenoid lycopene stimulates cell proliferation and alkaline phosphatase activity of SaOS-2 cells. *J Med Food .,* 2003, **6,** 79-86.

25. M. Darvin, A. Patzelt, S. Gehse, S. Schanzer, C. Benderoth, W. Sterry, Lademann., Cutaneous concentration of lycopene correlates significantly with the roughness of the skin. *J .Eur J Pharm Biopharm.,* 2008, **6,** 943-947

26. R.V. Chandra, M.L. Prabhuji, D.A. Roopa, S. Ravirajan, H.C. Kishore, Efficacy of lycopene in the treatment of gingivitis: a randomised, placebo-controlled clinical trial. *Oral Health Prev Dent*., 2007, **5,** 327-336.

27. H.Y. Lin, B.R. Huan,, W.L. Yeh, C.H. Lee, S.S. Huang, C.H. Lai, H. Lin, D.Y. Lu, Antineuroinflammatory effects of lycopene via activation of adenosine monophosphate-activated protein kinase-α1/heme oxygenase-1 pathways. *Neurobiol Aging.*, 2014, **35,** 191-202.

28. M. Hazewindus, G.R. Haenen, A.R. Weseler, A. Bast, The anti-inflammatory effect of lycopene complements the antioxidant action of ascorbic acid and a-tocopherol. *J Food Chem.,* 2012, **132** 954–958.

29. G. Hsiao, Y. Wang, N.H. Tzu, T.H. Fong, M.Y. Shen, K.H. Lin, D.S. Chou, J.R. Sheu, Inhibitory effects of lycopene on in vitro platelet activation and in vivo prevention of thrombus formation. *J Lab Clin Med.,* 2005, **146,** 216-226.

30. M. Qu, L. Li, C. Chen, M. Li, L. Pei, F. Chu, J. Yang, Z. Yu, D. Wang, Z. Zhou, Protective effects of lycopene against amyloid β-induced neurotoxicity in cultured rat cortical neurons. *Neurosci Lett.*, 2011, **505,** 286-290.

31. P. Kumar, H. Kalonia, A. Kumar, Lycopene modulates nitric oxide pathways against 3-nitropropionic acid-induced neurotoxicity. *J Life Sci.,* 2009, **85,** 711–718.

32. R. Sandhir, A. Mehrotra, S.S. Kamboj, Lycopene prevents 3-nitropropionic acid-induced mitochondrial oxidative stress and dysfunctions in nervous system. *J Neurochem Inter.,* 2010, **57,** 579–587.

33. M. Parle, D. Khanna, Procholinergic, hypocholesterolemic and memory improving effect of clove. *IRJP.,* 2011, **2,** 119-126.

34. G.L. Ellman, K.D. Courtney, V. Andres, R.M. Feather-stone, A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochem Pharmacol.,* 1961, **7,** 88-95.

35. G. Voss, K. Sachsse, Red cell and plasma cholinesterase activities in microsamples of human and animal blood determined simultaneously by a modified acetylthiocholine-DTNB procedure. *Toxicol Appl Pharmacol*., 1970, **16,** 764-772.

36. H. Ohkawa, N. Ohishi, K. Yagi, Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal Biochem,.*1979, **95,** 351-358.

37. GL. Ellman, Tissue sulfhydryl groups. *Arch Biochem Biophys.* (1959) **82** 70-77.

38. A.F. Boyne, G.L. Ellman, A methodology for analysis of tissue sulfhydryl components. *Anal Biochem.,* 1972, **46**,639–653.

39. E. Mohandas, V. Rajmohan, B. Raghunath, Neurobiology of Alzheimer's disease. *Indian J Psychiatry*., 2009, **51,** 55-61.

40. I. Klinkenberg, A. Blokland, The validity of scopolamine as a pharmacological model for cognitive impairment: a review of animal behavioral studies. *Neurosci Biobehav Rev*. 2010, **34,** 1307-1350.

41. K. Winnicka, M. Tomasiak, A. Bielawska, Piracetam--an old drug with novel properties? *Acta Pol Pharm.,* 2005, **62,** 405-409.

42. WE. Müller, GP, Eckert, A. Eckert, Piracetam: novelty in a unique mode of action. *Pharmacopsychiatry*., 1999, **32,** 2-9.

43. G. Hsiao, TH. Fong, NH. Tzu, KH. Lin, DS. Chou, JR. Sheu, A potent antioxidant, lycopene, affords neuroprotection against microglia activation and focal cerebral ischemia in rats. *In Vivo.* , 2004, **18,** 351–356.

44. N.T. Akbaraly, H. Faure, V. Gourlet, A. Favier, C. Berr, Plasma carotenoid levels and cognitive performance in an elderly population: results of the EVA study. *J Gerontol.,* 2007, **62,** 308–316.

45. M.M.B.S. Janani, K. Selvakumar,, S. Suganya, A.B.F. Yasmine, G. Krishnamoorthy, J. Arunakaran, Protective role of lycopene against PCBs-induced nitrosative stress in cerebral cortex of adult male rats. *Biomed Aging Pathol.,* 2012, **2,** 151–156.

46. K. Ried, P. Fakler, Protective effect of lycopene on serum cholesterol and blood pressure: Meta-analyses of intervention trials. *Maturitas.* 2010, **68,** 299–310.

47. S. Viau, M.A. Maire, B. Pasquis, S. Grégoire, C. Fourgeux, N. Acar, L. Bretillon, et al. Time course of ocular surface and lacrimal gland changes in a new Scopolamine-induced dry eye model. *Graefes Arch Clin Exp Ophthalmol.,* 2008, **246,** 857-867.

48. J.J. Locascio, H. Fukumoto, L. Yap, T. Bottiglieri, J.H. Growdon, B.T. Hyman, M.C. Irizarry, Plasma amyloid beta-protein and C-reactive protein in relation to the rate of progression of Alzheimer disease. *Arch Neurol.,* 2008, **65,** 776–785.

49. C. Eklund, F. Jahan, T. Pessi, T. Lehtimäki, M. Hurme, Interleukin 1B gene polymorphism is associated with baseline C-reactive protein levels in healthy individuals. *Eur Cytokine Netw.,* 2003, **14,** 168–171.

50. V.A. Pavlov, M. Ochani, , M.G. Puerta, K. Ochani, J.M. Huston, C.J. Czura, Central muscarinic cholinergic regulation of the systemic inflammatory response during endotoxemia. *Proc Natl Acad Sci USA.,* 2006, **103,** 5219–5223.

51. B. Lee, B. Sur, I. Shim, H. Lee, D.H. Hahm, Phellodendron amurense and its major alkaloid compound, Berberine ameliorates Scopolamine-Induced neuronal impairment and memory dysfunction in rats. *Korean J Physiol Pharmacol.,* 2012, **16,** 79-89.

52. A. Blokland, Acetylcholine: a neurotransmitter for learning and memory? *Brain Res Brain Res Rev.,* 1995, **21,** 285-300.

53. M. Yáñez, D. Viña, Dual inhibitors of monoamine oxidase and cholinesterase for the treatment of Alzheimer disease. *Curr Top Med Chem.,* 2013, **13,** 1692-1706.

54. J.L. Cummings, Treatment of Alzheimer's disease: current and future therapeutic approaches. *Rev Neurol Dis.,* 2004, **1,** 60-69.

55. D. Milatovic, R.C. Gupta, M. Aschner, Anticholinesterase Toxicity and Oxidative Stress. *J Sci World.,* 2006, **6,** 295–310.

56. M.A. Lebda, M.S. El-Neweshy, Y.S. El-Sayed, Neurohepatic toxicity of subacute manganese chloride exposure and potential chemoprotective effects of lycopene. *J NeuroToxic.,* 2012, **33,** 98–104.

57. H. Kaur, S. Chauhan, R. Sandhir, Protective effect of lycopene on oxidative stress and cognitive decline in rotenone induced model of Parkinson's disease. *Neurochem Res.,* 2011, **36,** 1435–1443.

58. D. Prvulovic, B. Schneider, Pharmacokinetic and pharmacodynamic evaluation of donepezil for the treatment of Alzheimer's disease. *Expert Opin Drug Metab Toxicol.,* 2014, **10,** 1039-1050.

59. Y. Fan, J. Hu, J. Li, Z. Yang, X. Xin, J. Wang, J. Ding, M. Geng, Effect of acidic oligosaccharide sugar chain on Scopolamine-induced memory impairment in rats and its related mechanisms. *Neurosci Lett.,* 2005, **374,** 222-226.

60. P. DiMascio, S. Kaiser H. Sies, Lycopene as the most efficient biological carotenoid singlet oxygen quencher. *Arch Biochem Biophys.,* 1989, **274,** 532-538.

61. A. Mortensen, LH. Skibsted, Relative stability of carotenoid radical cations and homologue tocopheroxyl radicals. A real time kinetic study of antioxidant hierarchy. *FEBS Lett*., 1997, **417,** 261-266.

62. N. Rencuzogullari, S. Erdogan, Oral administration of lycopene reverses cadmium-suppressed body weight loss and lipid peroxidation in rats. *Biol Trace Elem Res.,* 2007, **118,** 175-183.

63. KS. Bose BK. Agrawal, Effect of lycopene from cooked tomatoes on serum antioxidant enzymes, lipid peroxidation rate and lipid profile in coronary heart disease. *Singapore Med J.,* 2007, **48** 415-20.

64. H.F. Zaki, A. May, E.I. Fattah, A.S. Attia, Naringenin protects against Scopolamine-induced dementia in rats. *Bulletin Faculty Pharmacy Cairo Univ.,* 2014, **52,** 15-25.

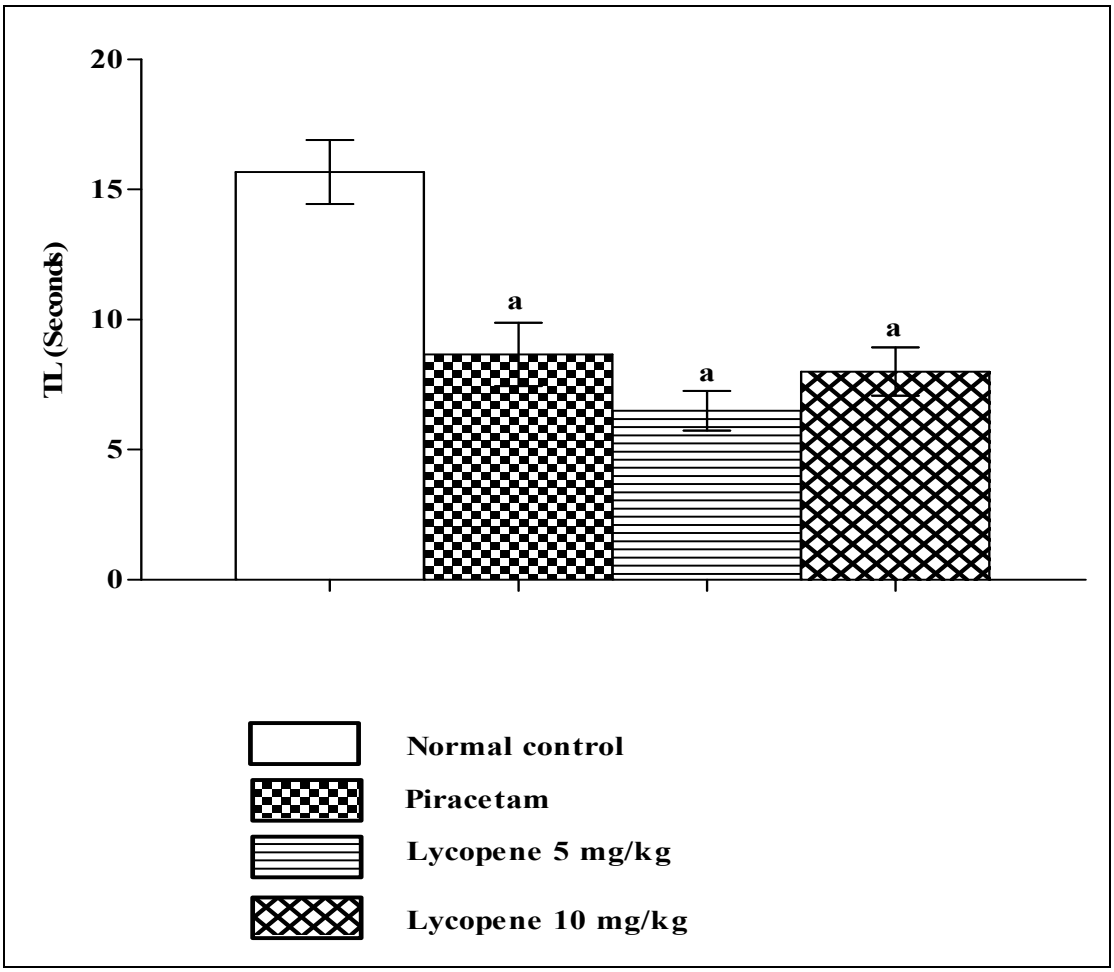


Figure 1: Effect of Lycopene on transfer latency (TL) using Elevated plus maze

Values were expressed as mean ± SD. a, p< 0.01 versus Normal control.

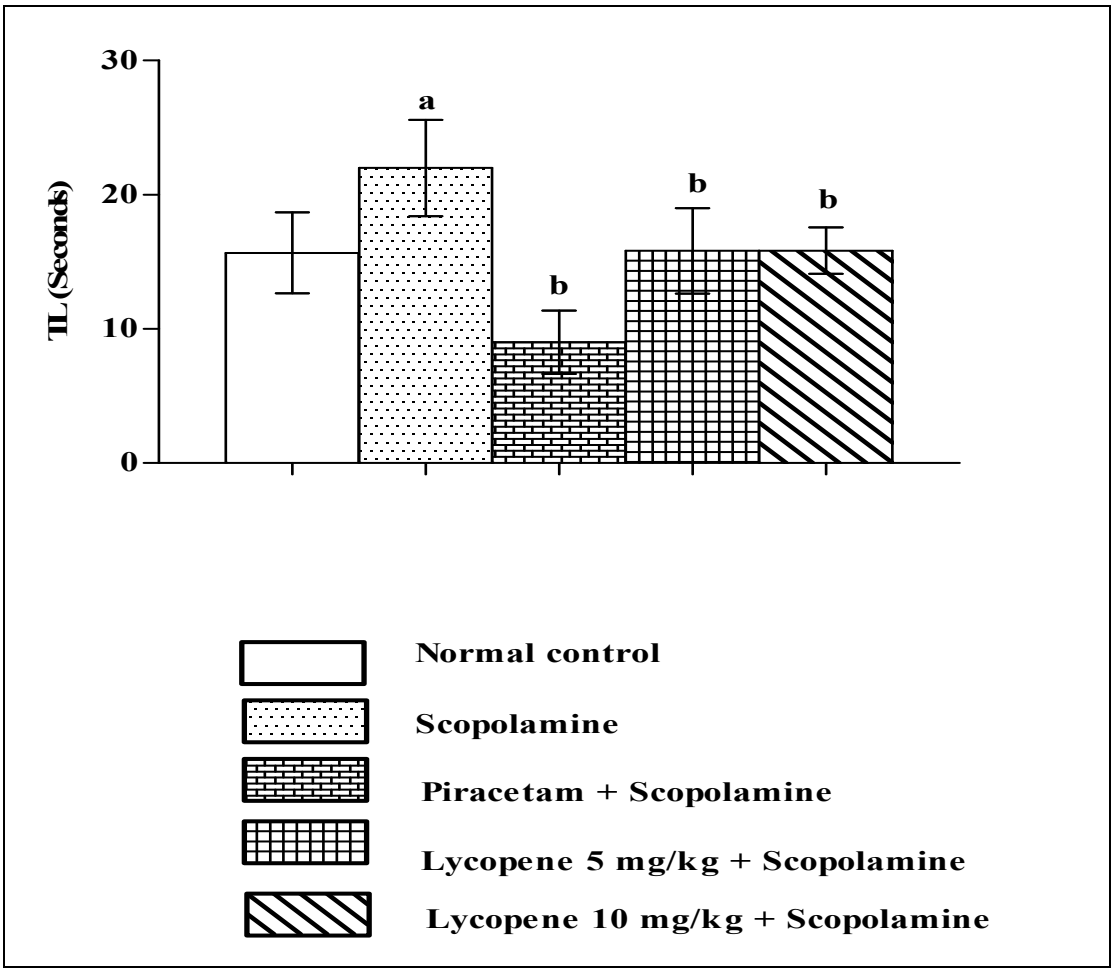


Figure 2: Effect of Lycopene on transfer latency (TL) in Scopolamine induced amnesia using Elevated plus maze

Values were expressed as mean ± SD. a, p< 0.01 versus Normal control; b, p< 0.01 versus Scopolamine.

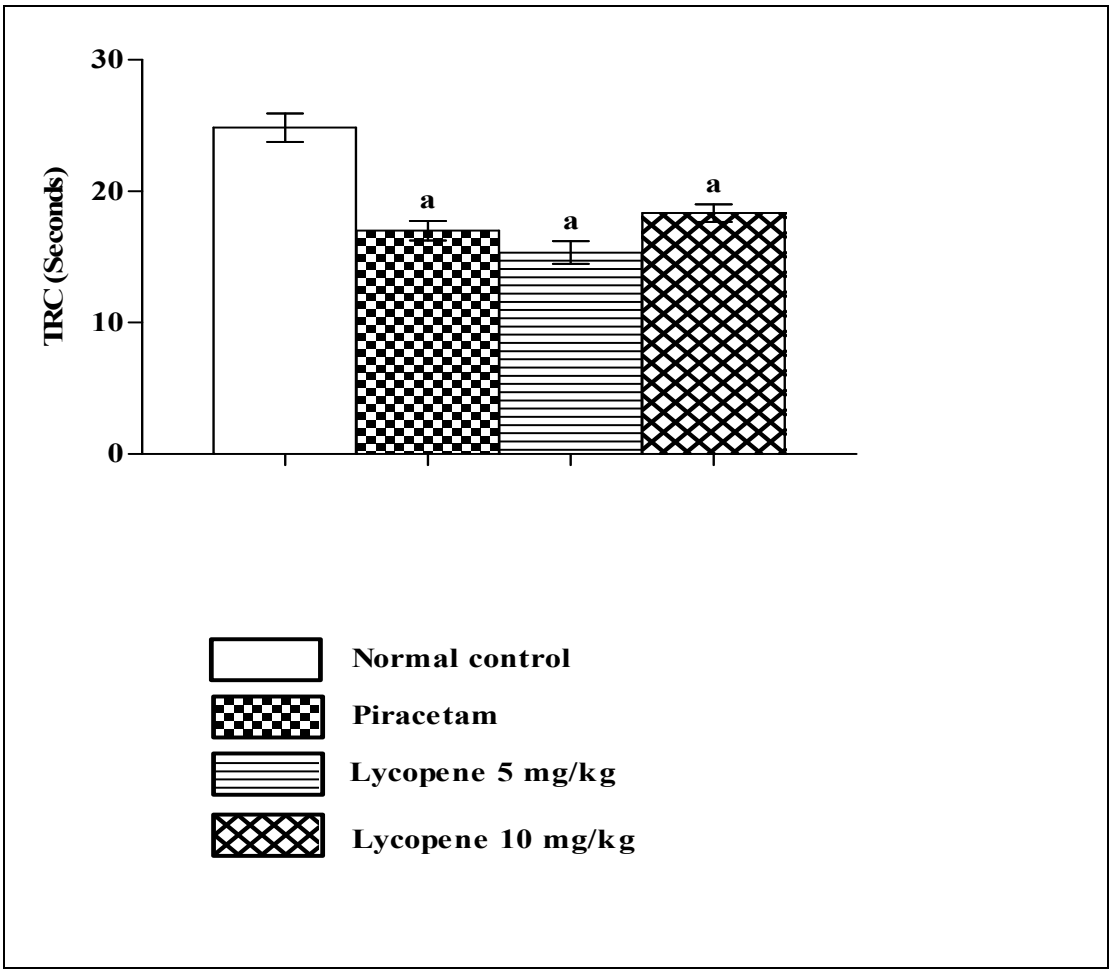


Figure 3: Effect of Lycopene on time taken to reach reward chamber (TRC) using Hebb-Williams maze

Values were expressed as mean ± SD. a, p< 0.01 versus Normal control.

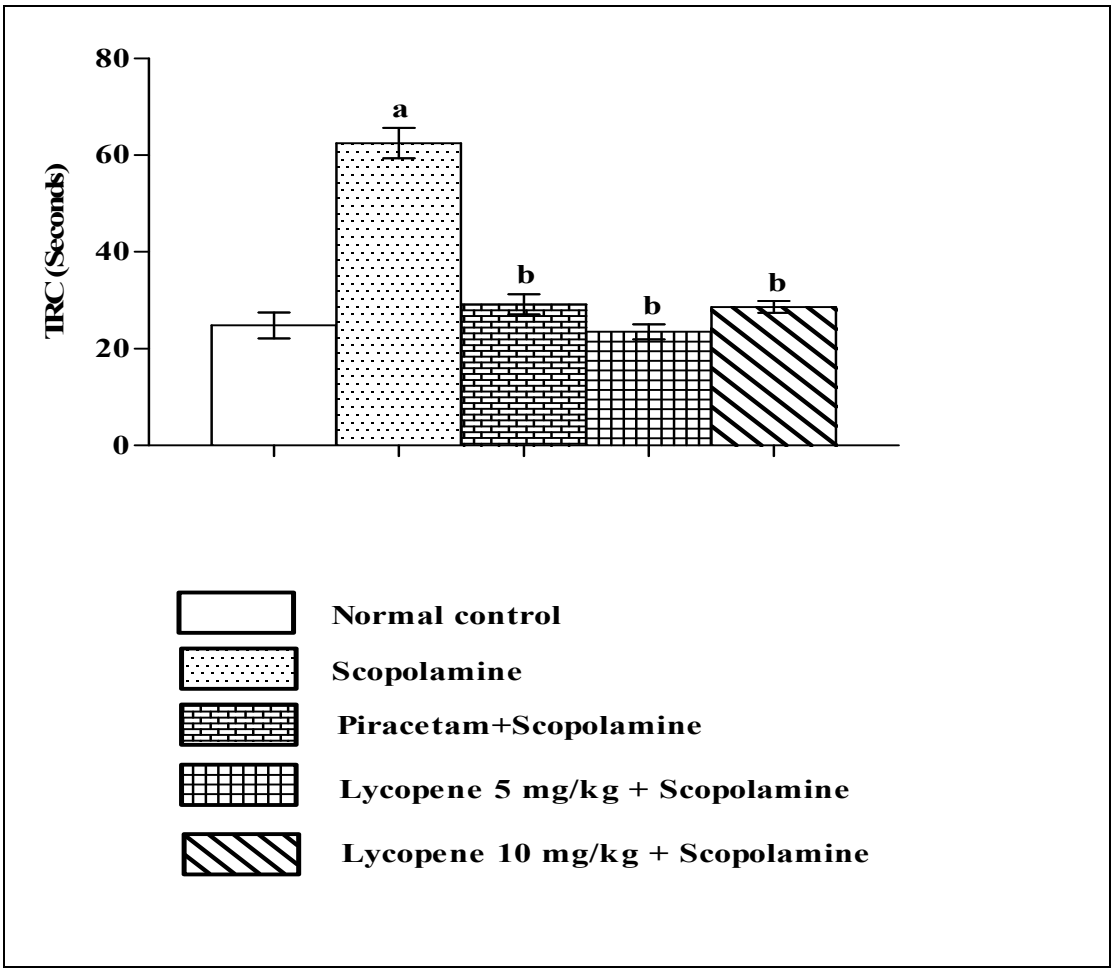


Figure 4: Effect of Lycopene on Scopolamine induced amnesia using Hebb-Williams maze

Values were expressed as mean ± SD. a, p< 0.01 versus Normal control; b, p< 0.01 versus Scopolamine

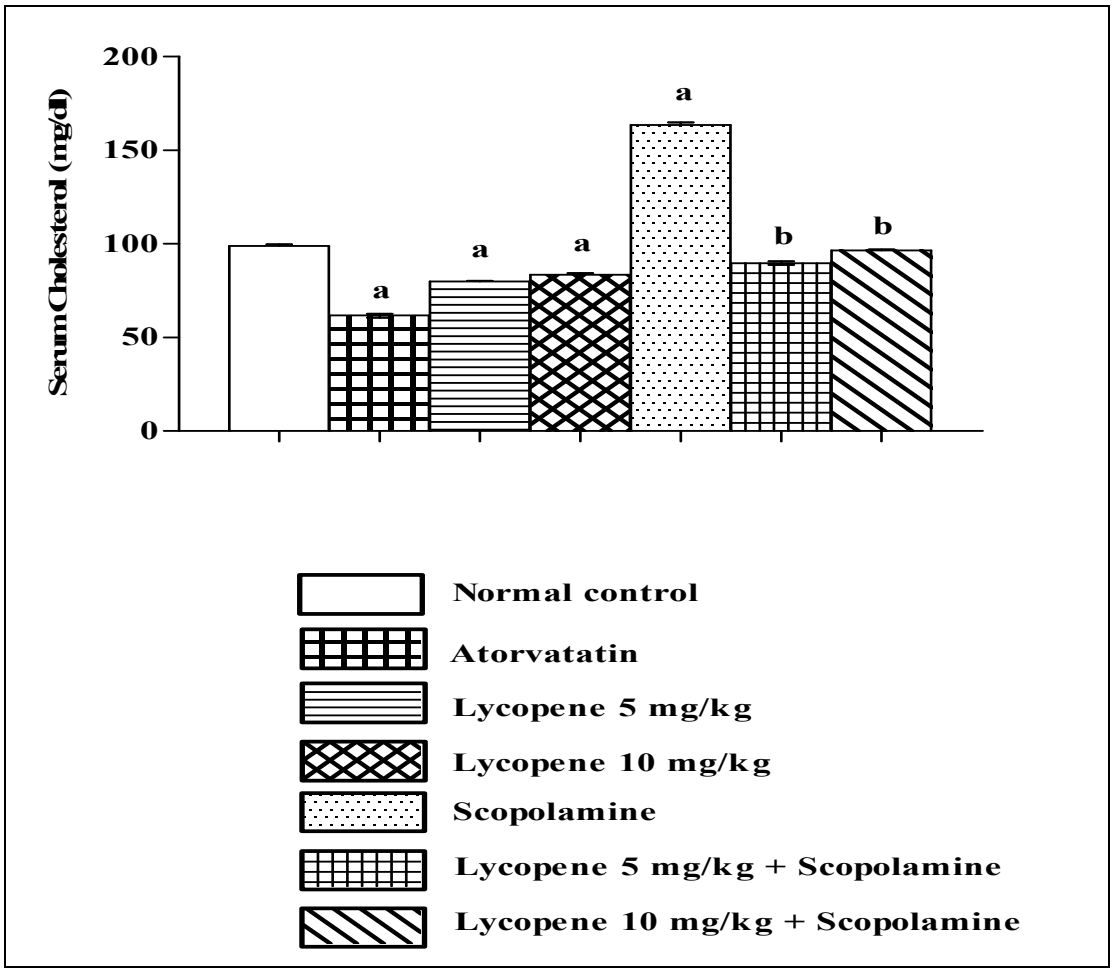


Figure 5: Effect of Lycopene on serum cholesterol

Values were expressed as mean ± SD. a, p< 0.01 versus Normal control; b, p< 0.01 versus Scopolamine.

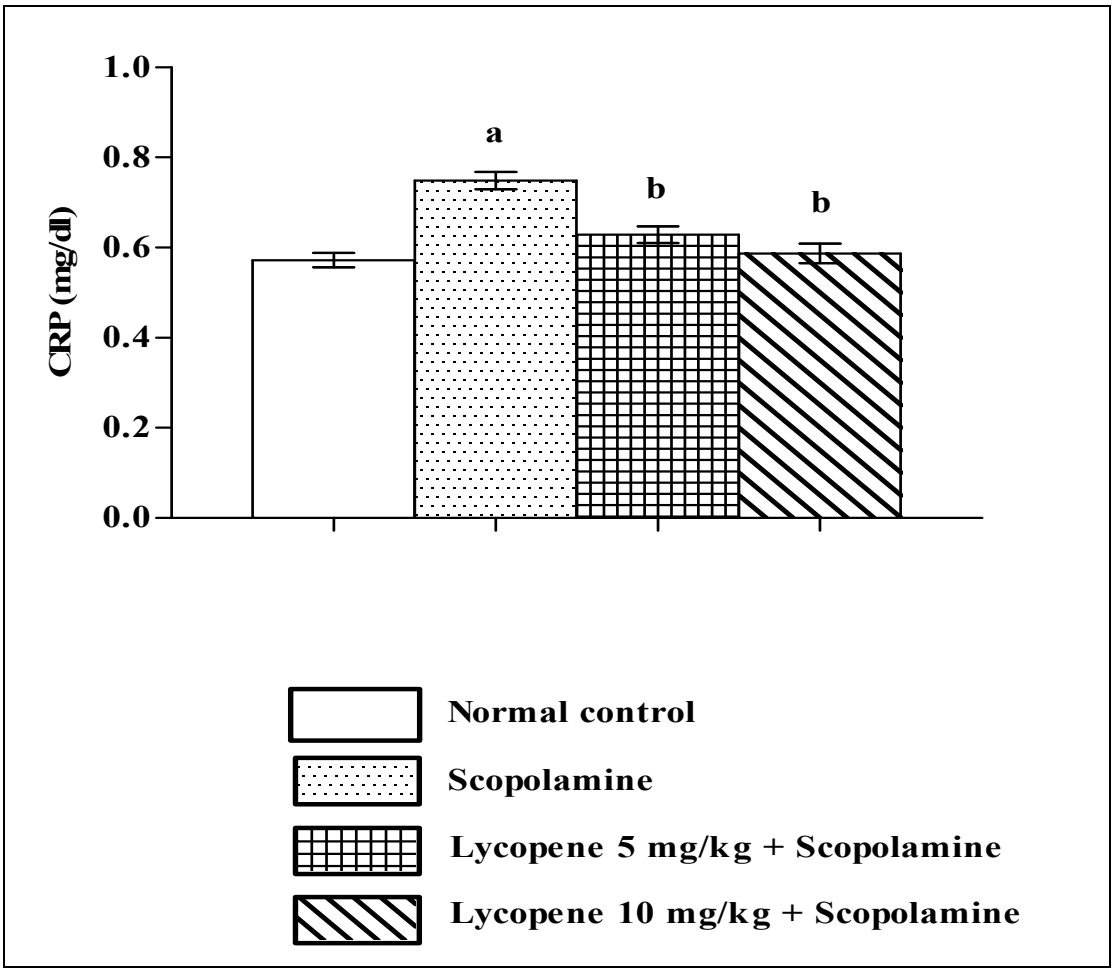


Figure 6: Effect of Lycopene on serum C-reactive protein (CRP)

Values were expressed as mean ± SD. a, p< 0.01 versus Normal control; b, p< 0.01 versus Scopolamine.

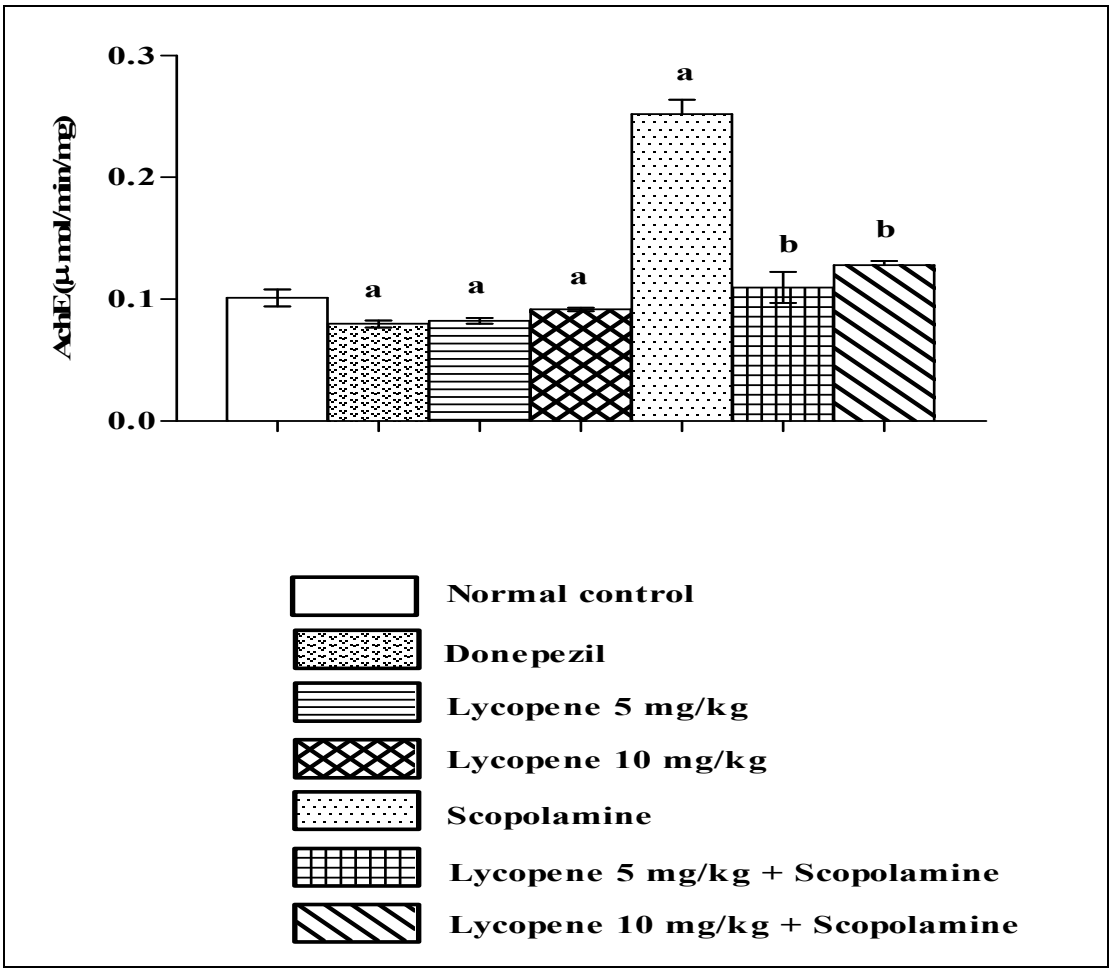


Figure 7: Effect of Lycopene on brain acetyl cholinesterase (AchE)

Values were expressed as mean ± SD. a, p< 0.01 versus Normal control; b, p< 0.01 versus Scopolamine.

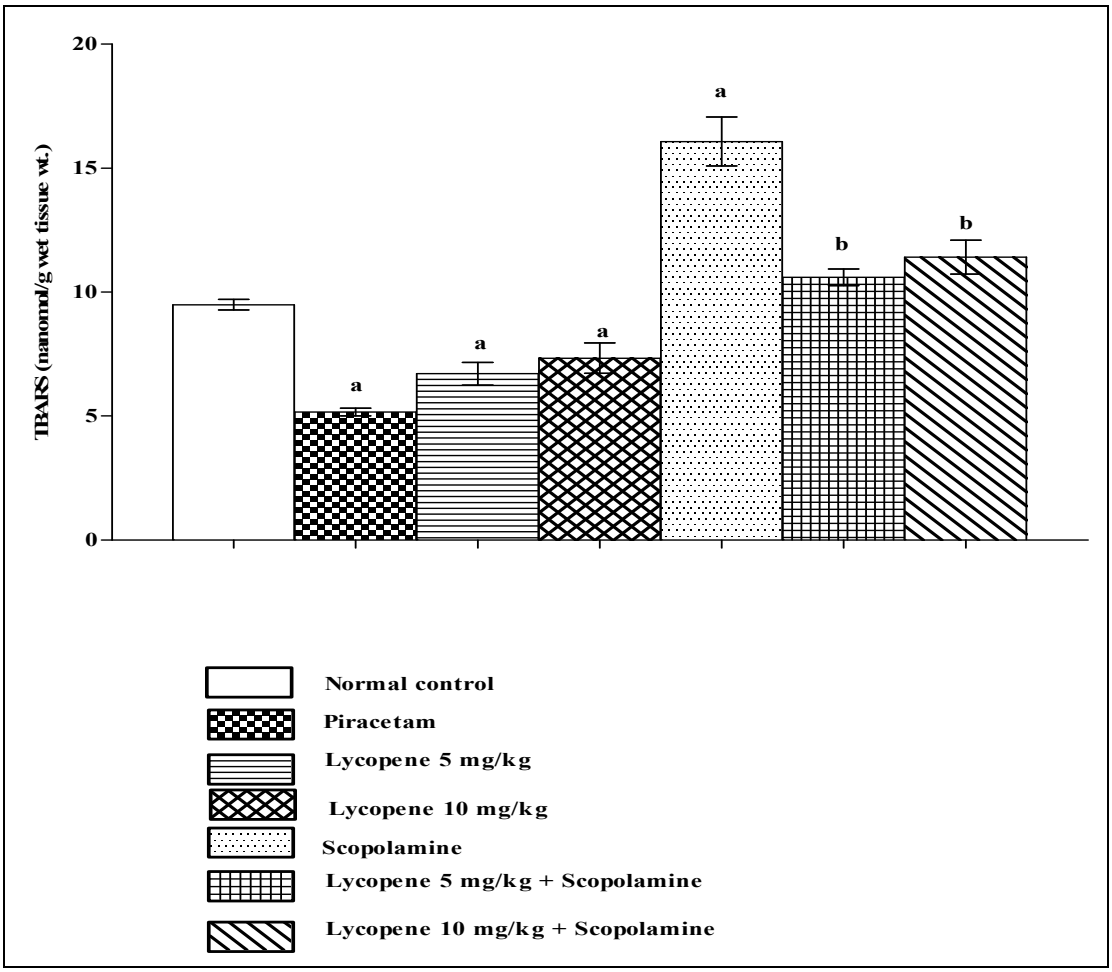


Figure 8: Effect of Lycopene on thiobarburtic acid reactive substance (TABRS)

Values were expressed as mean ± SD. a, p< 0.01 versus Normal control; b, p< 0.01 versus Scopolamine.

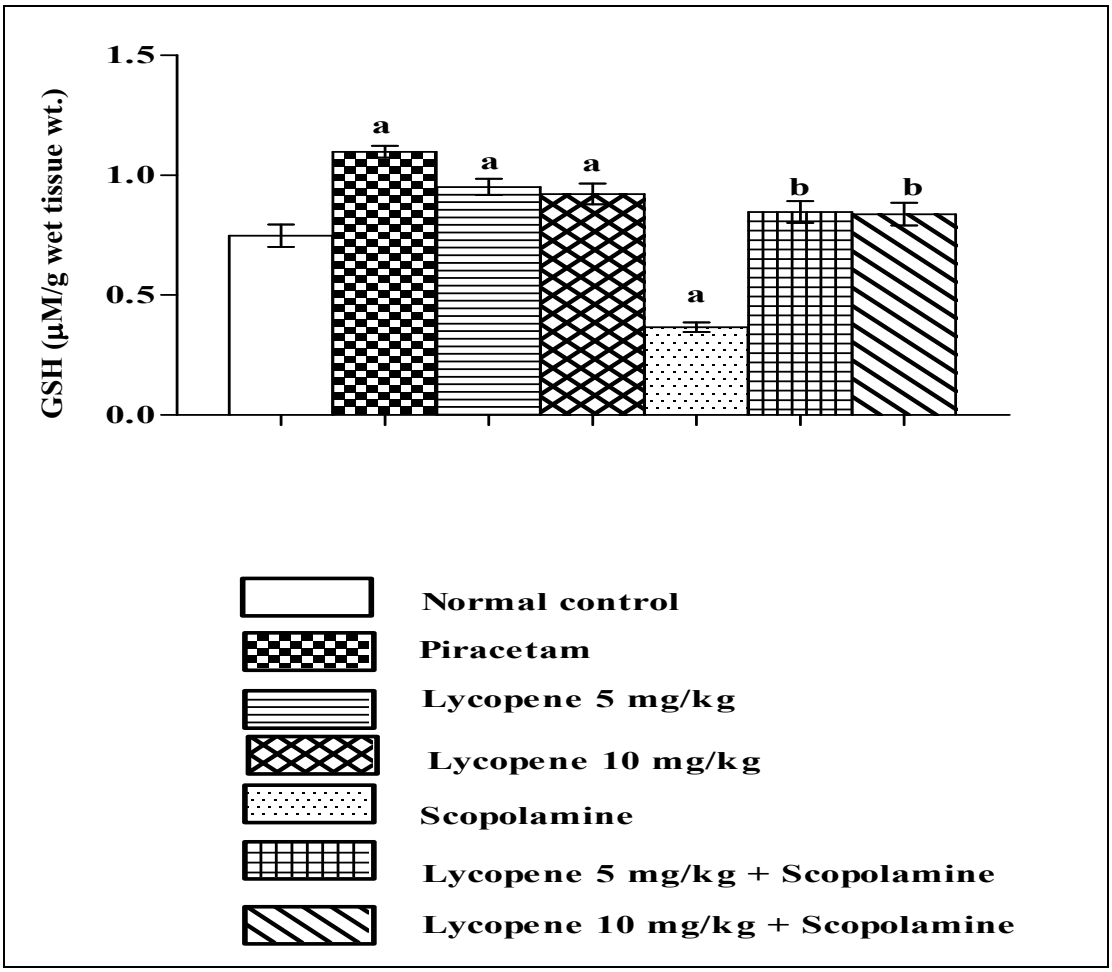
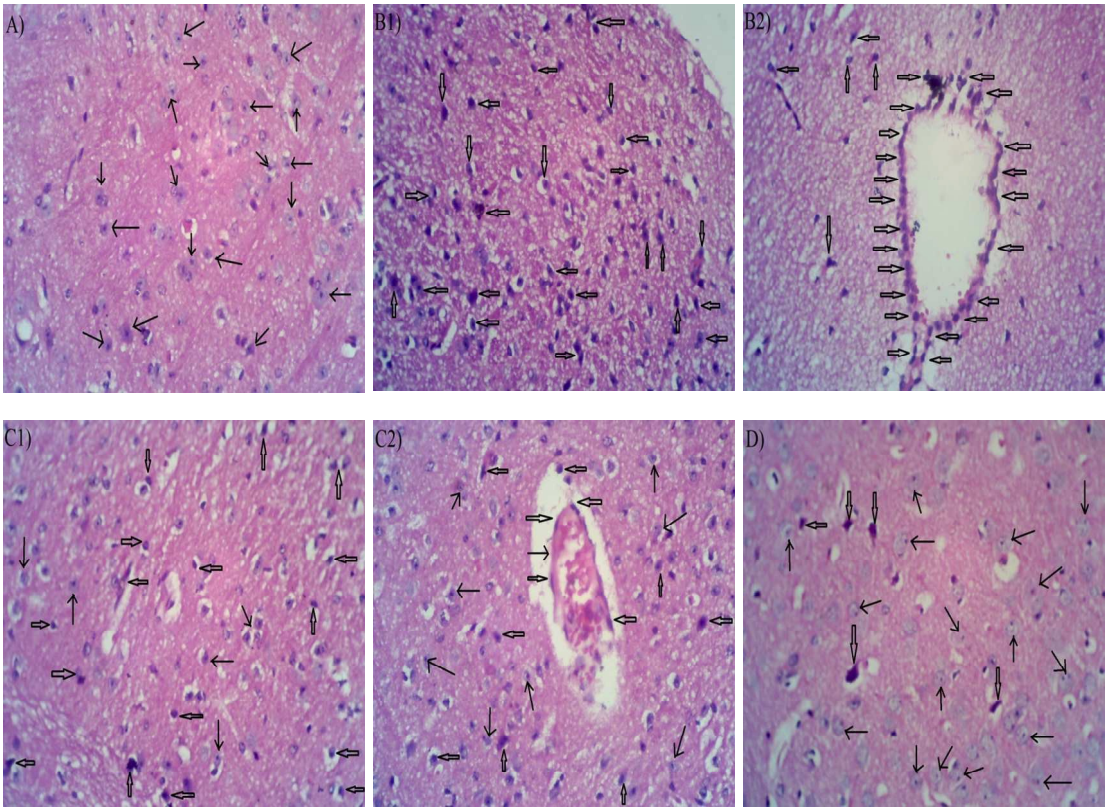


Figure 9: Effect of Lycopene on reduced glutathione (GSH)

Values were expressed as mean ± SD. a, p< 0.01 versus Normal control; b, p< 0.01 versus Scopolamine.



s

Figure 10: Effect of Lycopene on cerebral cortex of brain:

Photomicrograph of the cerebral cortex of brain of (A) Normal control group, (B1) Scopolamine group (B2) Scopolamine cortex representing dilated blood vessel (C1) Lycopene 5 mg/kg + Scopolamine group (C2) Lycopene 5 mg/kg + Scopolamine cortex representing blood vessel (D) Lycopene 10 mg/kg + Scopolamine group. Arrow (→) indicates the presence of viable neuron cells, whereas thick (⇒) arrow indicates the population of degenerated neurons, the detail quantification of nerve cells and severe congestion in the blood capillaries.

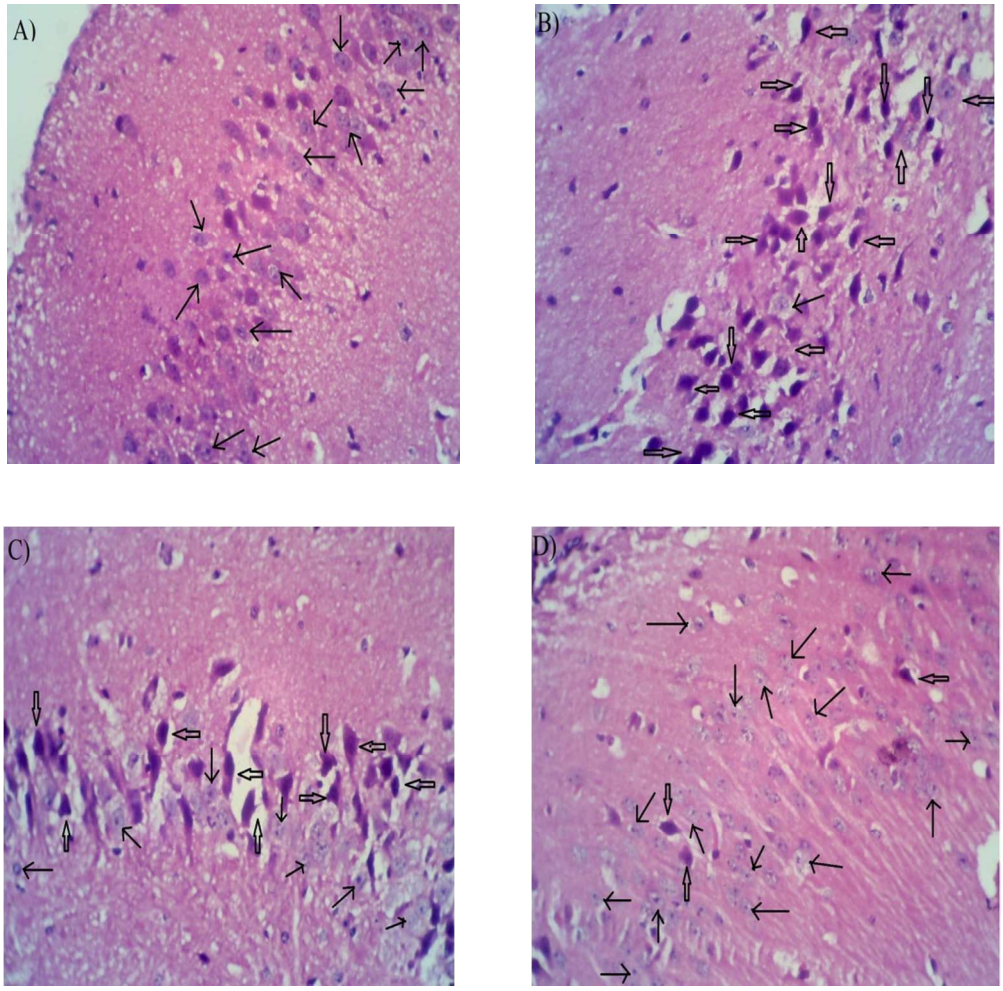


Figure 11: Effect of Lycopene on brain hippocampus of brain:

(A) Normal control, (B) Scopolamine (C) Lycopene 5 mg/kg + Scopolamine group (D) Lycopene 10 mg/kg + Scopolamine group. Arrow (→) indicates the presence of viable neuron cells, whereas thick (⇒) arrow indicates the population of degenerated neurons, pkynotic black neurons with condensed nucleus, the detail quantification of nerve cells and severe congestion in the blood capillaries.