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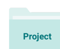

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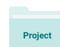

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Original article

Neuroprotective effect of *Prunus avium* on streptozotocin induced neurotoxicity in mice

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ABSTRACT

Objective: To evaluate the anti-amnesic and neuroprotective activity of ethanolic extract of *Prunus avium* (EEPA) on streptozotocin (STZ) induced neurotoxicity in mice.
Methods: The mice were pre-treated with EEPA at selective doses (200, 400 mg/kg, p.o.) for a period of 3 weeks followed by intracerebroventricular injection (ICV) of STZ (0.5 mg/kg). Neurobehavioral-alterations were evaluated using Y-maze and elevated plus maze. Biochemical markers, such as acetylcholinesterase (AChE), corticosterone, thiobarbituric reactive substances (TBARS), tissue nitrite, antioxidants, such as superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase were estimated.
Results: Obtain results revealed those 28 days of treatment with EEPA was effective in averting neurotoxicity. EEPA supplementation significantly reduced AChE, corticosterone, TBARS, tissue nitrite levels and ameliorated the deficits in learning and memory impairment with increased levels of antioxidants.
Conclusions: These results envisage that ethanolic extract of *Prunus avium* exhibit cognitive improvement which is most likely related, at least in part, to its antioxidant and neuroprotective activity. Further studies are suggested to evaluate the isolated bioactive *Prunus avium* fruits to identify the molecular mechanism involved in modulation of cholinergic transmission.

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1. Introduction

Alzheimer's disease (AD) type of dementia is associated with neurodegeneration due to accumulation of neurofibrillary tangles, senile plaque deposits and neuroinflammation leading to progressive decline in cognition. The neurotransmitter acetylcholine imperatively plays a role in the hippocampus of brain for learning and memory, and in contrast, the loss of cholinergic activity correlates with impairment of cognition. The amyloid hypothesis emphasizes that increased beta amyloid (Aβ) production or failure of Aβ clearance which induces gradual Aβ accumulation, result in the formation of plaques. Aβ aggregates induce changes in calcium influx [1], increases oxidative stress [2], and activates inflammatory mediators, such as microglia and astrocytes [3,4]. Activated microglia cells in AD release a variety of pro-inflammatory mediators, such as cytokines, reactive oxygen species (ROS), and nitric oxide which can contribute to neuronal dysfunction and cell death [5].

Oxidative stress is one of the major causes of cell damage in central nervous system, and is seen in many neurodegenerative disorders, including AD. The free radical produced during oxidative stress causes oxidation of DNA and RNA indicated by elevated levels of 8-hydroxyl-2-deoxyguanosine and 8-hydroxy guanosine [6,7], increased levels of protein carbonyl residues [8,9] and the lipid peroxidation (LPO) is marked by higher levels of thiobarbituric acid reacting substances (TBARS), malondialdehyde (MDA), 4-hydroxy *trans* neoneal (HNE) and isoprostrane [10,11]. Impaired insulin signaling leads to increased oxidative stress (Agarwal et al., 2010). Cholinergic neurons are among the earliest to succumb to the neurotoxic actions. Oxidative stress also increases the expression of AChE, decreases the activity of choline acetyltransferase, the enzyme that synthesizes acetylcholine activity [12]. Moreover, oxidative stress stimulates hypothalamic–pituitary–adrenal (HPA) axis, which release the glucocorticoids from adrenal cortex. Dysregulation of the HPA axis results in elevated circulating cortisol levels [13–15], which leads to shrinkage in hippocampal volume and memory impairment.

Prunus avium is a deciduous tree belonging to the family Rosaceae, commonly called wild cherry. The fruit of *P. avium* is a drupe, bright red to dark purple, edible, variably sweet to astringent and bitter to eat. The fruits are rich sources for flavonoids. The

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medicine prepared from the stalks of the fruits possesses diuretic, astringent, anti-tussive property. Fruits have been reported for its antioxidant activity, antiproliferative and anti-cancer properties, anti-inflammatory effects, protection against cardiovascular diseases and retarding of aging process [16–18]. Herein, the study was designed to evaluate the anti-amnesic and neuroprotective activity of EEPA on STZ induced neurotoxicity in mice. Pharmacological investigations were carried out by assessing the behavioural parameters, such as Y-maze in video tracking system, elevated plus maze and open field exploration. The biochemical changes in brain were evaluated by estimating the corticosterone level, AChE level and tissue nitrite and antioxidant enzymes after the treatment with EEPA.

2. Materials and methods

2.1. Drugs and reagents

Streptozotocin, 5,5′-dithiobis (2-nitrobenzoic acid), eserine, acetylthiocholine iodide, reduced glutathione, NADPH, pyrogallol, dexamethasone and DPPH were procured from Sigma Aldrich, USA. All other chemicals were purchased from S. D Fine chemicals Pvt LTD, India.

2.2. Plant material and extraction

The *P. avium* fruits were collected from commercial distributor, Hyderabad and authenticated by pharmacognosist Dr. Babashankar Rao, School of Pharmacy, Hyderabad and a voucher specimen (No: LCP/COG/3256) was deposited in department of Pharmacognosy. The obtained fruits were deseeded and shade dried. An amount of 1 kg of dried sample was homogenized with sufficient amount of double distilled absolute ethanol (99.9%) at room temperature. After homogenization, it was filtered and concentrated using rotary vacuum evaporator (Heidolph: 5690005000). The resultant thick consistent drug material was placed in an airtight container; the yield was noted as 5% and stored in refrigerator to avoid microbial contamination.

2.3. Experimental design

2.3.1. In vitro evaluation

2.3.1.1. DPPH radical scavenging activity assay. The free radical scavenging activity of crude extracts on 2, 2-di-phenyl-2-picrylhydrazyl (DPPH•) radical was measured by reduction of DPPH• to DPPHH (Di-phenyl picryl hydrazine) [19,20]. IC_{50} value was determined as the inhibitory concentration of extract that could scavenge 50% of the DPPH radicals. Ascorbic acid was used as positive reference.

2.3.1.2. Reducing power assay. An aliquot of the extract of *P. avium* (125 μL) was mixed with125 μL of sodium phosphate buffer (0.2 M, pH 6.6) and 125 μL of 1% $K_3Fe(CN)_6$ followed by incubation at 50 °C for 20 min. After adding 125 μL of 10% trichloroacetic acid, the mixture was centrifuged at 3750 *g* for 10 min. The supernatant solution (100 μL) was mixed with 100 μL of double distilled water and 20 μL of 1% ferric chloride to react for 10 min. Subsequently, the absorbance was measured at 700 nm. The EC_{50} value is the concentration of sample at which the absorbance is 0.5. L-Ascorbic acid was used as thr positive reference [21].

2.3.1.3. Assay of nitric oxide scavenging activity. Nitric oxide scavenging activity can be estimated by the use of Griess Ilosvoy reaction [22]. Scavengers of nitric oxide compete with oxygen leading to reduced production of nitrite ions. Inhibition of nitrite formation by the EEPA extract and the standard antioxidant ascorbic acid were calculated relative to the control. Inhibition data (percentage inhibition) were linearized against the concentrations of each extract and standard antioxidant. IC_{50}, which is an inhibitory concentration of extract required to reduce 50% of the nitric oxide formation, was determined.

2.3.2. In vivo pharmacological study

2.3.2.1. Experimental animals. Male Swiss albino mice, weighing 20–25 g, at the age 4–5 weeks were used in the study and given standard laboratory pellet chow diet; Provimi limited (India), provided water *ad libitum* and were kept under standard conditions at 23–25 °C, 35 to 60% humidity, and 12 h light/dark cycle. The mice were acclimatized to the laboratory conditions a week prior to experiment. The experimental protocol (No: I/IAEC/LCP/001/2012/SM/24) was duly approved by institutional animal ethics committee (IAEC).

2.3.2.2. Acute toxicological studies. The procedure was followed by using OECD 423 annexure (D) acute toxic class method [23]. The acute toxic class method is a stepwise procedure with three animals of a single sex per step. The starting dose level of EEPA was 2000 mg/kg body weight p.o. using water as vehicle. Drug was administered to overnight fasted female mice. Food was withheld for a further 3–4 hours after administration of EEPA and observed for signs of toxicity.

2.3.2.3. Induction of neurotoxicity and grouping. Neurotoxicity was induced by ICV injection of STZ (0.5 mg/kg) by identifying the bregma point on skull; in brief, bregma point was identified according to [24,25]. Approximately 1–3 mm rostral to the line is drawn through anterior base of ears, then at 45° angle, the needle was inserted 2 mm lateral to midline and STZ was injected. Animals were divided into four groups of each six. Group I was treated with ICV injection of phosphate buffered saline (PBS) alone; group II was injected with two doses of STZ (0.5 mg/kg) ICV injection bilaterally in PBS. The second dose was administered after 48 hours of the first dose. Group III and group IV were pre-treated with EEPA 200 mg/kg and 400 mg/kg p.o., respectively for 21 days. On 21st day, group III and group IV were injected with STZ ICV and the second dose was administered after 48 hours of the first dose and the treatment with EEPA was continued for 28 days. All the behavioural parameters are evaluated two days before termination of the study. On 28th day, the animals were sacrificed and the brains was isolated for biochemical estimations.

2.4. Behavioural evaluation

2.4.1. Video tracking in Y-Maze

Video tracking (VJ, Instruments) in Y-maze is assisted with software was used to measure the spatial working memory in mice for 8 minutes. Mice tend to explore maze systematically, entering each arm in turn. The series of arm entries, including possible returns into the same arm is recorded by using the video tracking system and the percentage alteration is obtained by the assistance of the software. Alteration is defined as the successive entries into the three arms, on overlapping triplet sets. [26].

2.4.2. Elevated plus maze test

This task has been widely validated to measure anxiety-like behaviour in rodents. The entire maze is elevated to height of 50 cm above the floor level. Mice were housed in pair for 10 days prior to testing in the apparatus. During this time, the mice are handled by the investigator on alternate days to reduce the stress. One hour after oral administration of the extract, each mouse was placed in the center of the maze facing towards one of the open arm. During

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a five minutes session, the following parameters were noted: number of entries into open arm, and the time spent in the open arm was calculated. [27–29].

2.4.3. Open field habituation

The exploratory behaviour of the mice was evaluated by open field habituation task method. Mice was placed in a 40 cm × 50 cm × 60 cm open field whose brown linoleum floor was divided into 12 equal squares by white lines and left to explore it freely for 5 minutes. The number of squares crossings and head dips were counted [30].

2.5. Biochemical estimations

Mice brains were isolated washed with ice-cold phosphate buffer to remove blood and homogenized with 10% phosphate buffer saline solution. The homogenate and the resultant supernatant were used for further biochemical estimations, such as AChE, NO and antioxidants. Blood samples were collected by retro-orbital bleeding for the estimation of plasma corticosterone levels.

2.5.1. Estimation of acetylcholinesterase (AChE) activity

The AChE activity was measured in brain tissue by the reaction of thiocholine with dithiobisnitrobenzoate ions ([31]; [32]). The rate of formation of thiocholine from acetylcholine iodide in the presence of brain cholinesterase was measured using a spectrophotometer (Shimadzu 1800) at a wavelength of 412 nm.

2.5.2. Estimation of corticosterone

Blood was collected from retro-orbital sinus in tubes containing heparin and centrifuged at 1000 × *g* for 20 min at 4 °C HPLC/UV system was used for quantification of plasma corticosterone using dexamethasone as an internal standard. Plasma was separated; 50 μL of plasma containing known quantity of dexamethasone (1 μg) was extracted with 5 mL of dichloromethane (DCM). The DCM extract was evaporated to dryness and dissolved in 100 μL of mobile phase. Then, 20 μL of extract was injected into HPLC (Shimadzu SPD 20AD) system for quantification. Mobile phase consisted of methanol:water (70:30) at a flow rate of 1.2 mL/min and corticosterone was detected at 250 nm using UV detector [33,34].

2.5.3. Estimation of nitrite levels

Nitrite and nitrate determinations in biological material are increasingly being used as markers of NO production. Nitric oxide production was quantified by measuring nitrite, a stable oxidation end-product of NO [35].

Lipid peroxidation was estimated by measuring the levels of thiobarbituric acid reactive substances (TBARS) in tissues by the method of [36]. The pink chromogen produced by the reaction of thiobarbituric acid with malondialdehyde, a secondary product of lipid peroxidation was estimated at 532 nm.

2.5.4. Antioxidant activity

2.5.4.1. Thiobarbituric acid reactive substances. Lipid peroxidation was estimated by measuring the levels of thiobarbituric acid reactive substances (TBARS) in tissues by the method of [36]. The pink chromogen produced by the reaction of thiobarbituric acid with malondialdehyde, a secondary product of lipid peroxidation, was estimated at 532 nm.

2.5.4.2. Superoxide dismutase. Superoxide dismutase (SOD) activity was determined by pyrogallol oxidation method [37]. One unit SOD activity is defined as the amount of enzyme that inhibits the rate of auto-oxidation of pyrogallol by 50%. The reaction is initiated by adding pyrogallol and the change in optical density was recorded at 420 nm.

2.5.4.3. Glutathione peroxidase (GPx). GPx was determined on the basis of the oxidation of glutathione to oxidized glutathione catalyzed by glutathione peroxidase, which is then coupled to their cycling of oxidized glutathione back to glutathione utilizing glutathione reductase and nicotinamide adenine dinucleotide phosphate (NADPH). Then, decrease in NADPH absorbance measured at 340 nm during the oxidation of NADPH to NADP+ is indicative of glutathione peroxidase activity [38].

2.5.4.4. Catalase. The rate of decomposition of H_2O_2 to water and molecular oxygen is proportional to the activity of catalase. The sample containing catalase is incubated in the presence of a known concentration of H_2O_2. After incubation for exactly one minute, the reaction is stopped with ammonium molybdate. The amount of H_2O_2 remaining in the reaction is then determined by the oxidative coupling reaction between molybdate and H_2O_2 [39].

3. Results

3.1. Acute toxicity

The drug EEPA was found to be non-toxic, and the LD_{50} of 2000 mg/kg and above is said to be unclassified according to OECD 423. Hence, (200 mg/kg) and (400 mg/kg) of this dose were selected for further study.

3.2. In vitro antioxidant assays

3.2.1. DPPH radical scavenging activity assay

The extract exhibited potent radical scavenging activity in a concentration-dependent manner. The IC_{50} value of the extract was 28.37 μg/mL as opposed to that of standard ascorbic acid (5.52 μg/mL). The results were shown in the Table 1.

3.2.2. Reducing power

The reducing power of a substance is associated with its potential antioxidant activity. To measure the reducing power of EEPA, its ability to transform Fe^{3+} into Fe^{2+} was investigated. Fe^{2+} formation can be monitored by measuring Prussian blue formation at 700 nm. The EC_{50} value of the extract was 454.4 μg/mL as opposed to that of standard ascorbic acid (16.09 μg/mL). The results were shown in the Table 1.

3.2.3. Assay of nitric oxide scavenging activity

EEPA showed a strong nitric oxide scavenging activity, which was comparable to the standards ascorbic acid. The IC_{50} value of the extract was 93.6 μg/mL as opposed to that of standard ascorbic acid (6.18 μg/mL). The results were shown in the Table 1.

3.2.4. Behavioural estimations

3.2.4.1. Effect of EEPA on Y-maze. Effects of EEPA on percentage spontaneous alteration are shown in Table 2. Administration of STZ significantly ($P < 0.001$) decreased the percentage spontaneous alteration in negative control group when compared with the vehicle treated group. The group treated with 200 and 400 mg/kg EEPA increased the percentage spontaneous alteration and showed the significance ($P < 0.01$ and $P < 0.001$), respectively. A dose-dependent increase in percentage alterations was found on administration of EEPA with significance of $P < 0.05$.

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Table 1
In vitro antioxidant assays.

Extract	IC_{50} (μg of dried extract/mL) scavenging ability on DPPH radicals	Reducing power	IC_{50} (μg of dried extract/mL) scavenging of nitric oxide
Ascorbic acid	5.52 ± 0.17	16.09 ± 1.25	6.18 ± 0.07
Ethanolic extract of *Prunus avium*	28.37 ± 0.02[a]	454.4 ± 14.45[a]	93.6 ± 1.35[a]

Values are expressed as mean ± SEM. Superscript letters represents the statistical significance done by unpaired t test.
[a] $P < 0.001$ indicates the significance on comparison of ethanolic extract of *Prunus avium* with ascorbic acid.

Table 2
Effect of EEPA on behavioural activity.

Groups	Y-Maze	Elevated plus maze		Open field exploration	
	Percentage alterations	Number of open arm entries	Time spent on open arm	Line crossings	Head dips
Group I (ICV PBS)	44.61 ± 1.93	7.16 ± 0.40	14.5 ± 0.80	137.0 ± 10.43	20.00 ± 2.95
Group II (ICV–STZ 0.5 mg/kg)	19.40 ± 1.01[a]	2.83 ± 0.30[a]	4.16 ± 0.30[c]	80.50 ± 5.41[a]	4.83 ± 0.70[b]
Group III (ICV–STZ + EEPA 200 mg/kg)	32.32 ± 2.15[e]	10.67 ± 0.55[d]	29.50 ± 3.01[d]	119.0 ± 6.76[e]	19.50 ± 3[e]
Group IV (ICV–STZ + EEPA 400 mg/kg)	41.49 ± 3.43[d,f]	12.83 ± 0.54[d,f]	41 ± 3.81[d,f]	123.8 ± 6.37[e]	18.67 ± 2.89[e]

Values are expressed as mean ± SEM of 6 animals. Superscript letters represents the statistical significance done by ANOVA, followed by Tukey's multiple comparison tests.
[a] $P < 0.001$.
[b] $P < 0.01$.
[c] $P < 0.05$ indicates the significance on comparison of group II with group I.
[d] $P < 0.001$.
[e] $P < 0.01$, indicates the significance on comparison of group III and IV with group II.
[f] $P < 0.05$ indicates the dose-dependent significance on comparing group III and IV.

3.2.4.2. Effect of EEPA on elevated plus maze. STZ administered mice showed a significant reduction ($P < 0.001$ and $P < 0.05$) in the time spent in the open arms and number of entries into the open arms when compared to vehicle group. The group treated with 200 and 400 mg/kg EEPA exhibited significant increase in the number of entries into open arm and time spent in open arm ($P < 0.001$) when compared with vehicle treated group. A dose-dependent activity was found on administration of EEPA with significance of $P < 0.05$. The results were shown in the Table 2.

3.2.4.3. Effect of EEPA on open field habituation. The exploratory behaviour i.e., the number of line crossings and head dipping's decreased in STZ treated group ($P < 0.001$, and $P < 0.01$) respectively in comparison with the control group. The number of line crossings and head dipping's increased significantly ($P < 0.01$ and $P < 0.01$) in both 200 mg/kg and 400 mg/kg EEPA treated groups respectively and indicates the improved open field habituation memory. The results were shown in the Table 2.

3.2.5. Biochemical estimations

3.2.5.1. Effect of EEPA on AChE activity. The effect of EEPA on AChE is shown in Fig. 1. Administration of STZ significantly ($P < 0.001$) increased the AChE activity when compared to the vehicle treated control group. Treatment of EEPA significantly ($P < 0.001$) attenuated the raise in enzyme level in both 200 and 400 mg/kg treated mice. A dose-dependent decrease in AChE was found on administration of EEPA with significance of $P < 0.01$.

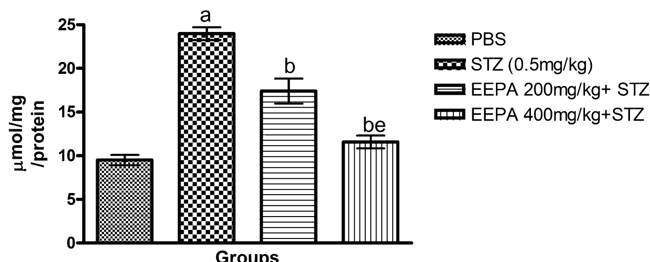

Fig. 1. Effect of EEPA on AChE enzyme level.

3.2.5.2. Effect of EEPA on corticosterone. Administration of STZ significantly ($P < 0.001$) increased the corticosterone level when compared with the control group. Treatment with EEPA 400 mg/kg significantly ($P < 0.01$) attenuated the increase in corticosterone level induced by STZ. Results are shown in Fig. 2. A dose-dependent decrease in corticosterone was found on administration of EEPA with significance of $P < 0.05$.

3.2.5.3. Effect of EEPA on tissue nitrite. Administration of STZ significantly ($P < 0.001$) increased tissue nitrite levels when compared with the control group. Treatment with EEPA 200 and 400 mg/kg significantly ($P < 0.001$) attenuated the increase in tissue nitrite induced by STZ. Results are shown in Fig. 3. A dose-dependent decrease in tissue nitrite was found on administration of EEPA with significance of $P < 0.01$.

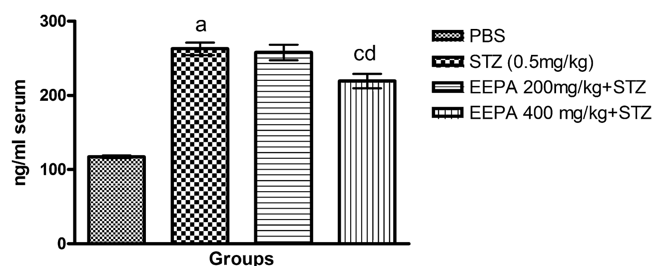

Fig. 2. Effect of EEPA on Corticosterone level.

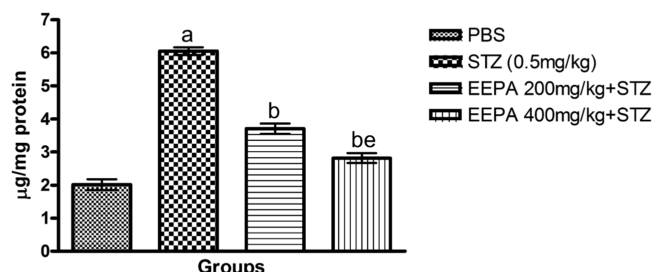

Fig. 3. Effect of EEPA on Tissue nitrite level.

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3.2.6. Antioxidant activities

3.2.6.1. Effect of EEPA on TBARS. Administration of STZ significantly ($P < 0.001$) increased TBARS when compared with the control group. Treatment with EEPA 200 and 400 mg/kg significantly ($P < 0.01$) attenuated the increase in TBARS induced by STZ. Results are tabulated in Table 3. A dose-dependent decrease in TBARS was found on administration of EEPA with significance of $P < 0.01$.

3.2.6.2. Effect of EEPA on superoxide dismutase. Administration of STZ significantly ($P < 0.001$) decreased the activity of SOD when compared to the vehicle treated control group. Treatment with EEPA 200 and 400 mg/kg doses showed a significant increase ($P < 0.01$ and $P < 0.001$) in activity of SOD, respectively compared to negative control group. The results are shown in Table 3. A dose-dependent increase in SOD was found on administration of EEPA with significance of $P < 0.01$.

3.2.6.3. Effect of EEPA on glutathione peroxidase (GPx). Administration of STZ significantly ($P < 0.001$) decreased the activity of GPx when compared to the vehicle treated control group. The two groups of animals treated with EEPA 200 mg/kg and 400 mg/kg doses showed a significant increase ($P < 0.01$ and $P < 0.001$) in activity of glutathione peroxidase, respectively compared to STZ treated group. The results are shown in Table 3. A dose-dependent increase in GPx was found on administration of EEPA with significance of $P < 0.01$.

3.2.6.4. Effect of EEPA on catalase. Administration of STZ significantly ($P < 0.001$) decreased the activity of catalase when compared to the vehicle treated control group. The two groups of animals treated with EEPA 200 mg/kg and 400 mg/kg doses showed a significant increase ($P < 0.001$) in activity of catalase compared to STZ treated group. The results are shown in Table 3.

4. Discussion

Oxidative stress is one of the foremost criteria for initiation and progression of AD and other neurodegenerative disorders. The excessive free radicals produced react with lipids, proteins, and nucleic acids leading to lipid peroxidation, protein oxidation and DNA oxidation. Oxidative damage to the cellular components result in alteration of the membrane properties, such as fluidity, ion transport, enzyme activities and mitochondrial dysfunction, all this eventually results in cell death. In the present study, antioxidant property of EEPA was assessed against various in vitro models. DPPH is a stable free radical and in order to attain stability it accepts an electron or hydrogen radical [40]. The reduction capability of DPPH radical is determined by the decrease in absorbance at 517 nm induced by antioxidants. The experimental data revealed that the *P. avium* extract possess free radical scavenging effects. Nitric oxide formed during their reduction with oxygen or with superoxide, such as NO_2, N_2O_4, N_3O_4 is very reactive. These radicals are responsible for altering the structure and functional behaviour of many cellular components. The EEPA showed better activity in competing with oxygen to react with nitric oxide and thus inhibited the generation of anions. In the measurement of the reducing ability, it has been investigated from the Fe^{3+}–Fe^{2+} transformation. Fe^{3+} reduction is often used as an indicator of electron donating activity, which is an important mechanism of phenolic antioxidant action and can be strongly correlated with other antioxidant properties [41]. The results from various free radicals scavenging systems reveal that EEPA has significant antioxidant activity, which was further confirmed with in vivo assays. Induction of neurotoxicity by STZ in mice resulted in excessive generation of free radicals, which, in turn, caused oxidative damages to membrane's lipid, and protein levels which was indicated by increased levels of TBARS and decreased levels of GPx, SOD and catalase in this study. This oxidative neuronal injury in ICV–STZ mice is consistent with previous reports ([42–44]; [45]). SOD converts superoxide into H_2O_2 [46,47], and catalase, which is available at extremely low levels in the brain and removes H_2O_2 in the form of H_2O. Moreover, in our study, *P. avium* supplementation significantly counteracted all the change in the markers of oxidative damage. This may be due to EEPA free radical scavenging potential which is evident from increased SOD, catalase and GPx levels in brain.

Long-term potentiation (LTP) is a phenomenon responsible for the cellular mechanism of learning and memory processes. The Y-maze is a simple test for measuring spatial recognition memory that is based on discrimination of novelty versus familiarity in the three arms. The ability to alternate requires that the mice know which arm they have already visited. In the study with behavioural activity, administration of STZ significantly decreased the percentage spontaneous alteration when compared with the vehicle treated group, which is an index for neurobehavioral toxicity. As reported by previous studies, the possible mechanism could be that STZ if injected ICV in mice impairs cerebral glucose turn over, energy metabolism and increases generation of free radicals, leading to cognitive dysfunction [48]. Supplementation with *P. avium* extract improved percentage spontaneous alteration and indicates the improvement of LTP. Elevated plus maze test has been widely validated to measure anxiety-like behaviour in rodents. Study results indicate that ICV injection of STZ enhanced the anxiety-like behaviour of mice. Based on the fact that ICV injection of STZ causes:

- abnormalities in metabolic pathways, which are under control of the insulin signaling cascade by desensitization of neuronal insulin receptor;
- reduced cerebral energy metabolism;
- deficiency in the cholinergic transmission, it is possible to postulate that the anxiogenic-like action induced by STZ involves one or more of these events [49].

This fact could indicate that the anxious behaviour occurs in the progression of dementia in the sporadic dementia of Alzheimer's type (SDAT) model. In this study, the group treated with *P. avium* exhibited significant increase in the number of entries into open arm and time spent in open arm when compared with vehicle treated group revealing that the drug possesses anxiolytic potential.

Open field test allows evaluating habituation memory through measurement of the exploratory behaviour (crossings and head dips). The treatment with *P. avium* extract decreased the cognitive deficits in STZ injected mice. These results are consistent with the favourable effect on cognition in open field habituation memory.

Evidence implicates that the brain cholinergic system, which plays an important role in learning and memory, is vulnerable to oxidative damage and pathogenesis of AD [50–52]. STZ induced neurotoxicity by ICV administration in mice causes a cholinergic deficiency, followed by decreased choline acetyltransferase (ChAT) activity, and increase in AChE activity in the hippocampus and leads to cognitive decline [43,53,54]. In our study, EEPA supplementation significantly decreased the AChE and ameliorated the deficits of learning and memory in ICV–STZ induced dementia. This suggests that EEPA possesses AChE inhibitory activity and it may be due to the potential antioxidant effect by increasing the level of antioxidant defense system in the cholinergic neurons of the brain and thereby improves learning and memory deficits. It had been postulated that neuroprotective effect of flavonoids are mediated by an ability to protect neurodegeneration, augment existing neuronal function, stimulate neuronal regeneration and induce neurogenesis in aged (19 months) Fischer 344 rats [55,56].

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Table 3
Effect of EEPA on antioxidant parameters.

Groups	Superoxide dismutase (U/mg protein)	Glutathione peroxidase (U/mg protein)	Catalase (U/mg protein)	TBARS (nM/mg protein)
Group I (ICV PBS)	18.44 ± 0.89	28.85 ± 1.09	1.32 ± 0.05	1.34 ± 0.05
Group II (ICV–STZ 0.5 mg/kg)	5.95 ± 1.13[a]	16.35 ± 1.36[a]	0.45 ± 0.06[a]	4.91 ± 0.22[a]
Group III (ICV–STZ + EEPA 200 mg/kg)	11.05 ± 0.48[b]	22.16 ± 0.41[b]	1.14 ± 0.05[c]	3.68 ± 0.29[b]
Group IV (ICV–STZ + EEPA 400 mg/kg)	15.5 ± 0.86[c,d]	27.04 ± 0.52[c,d]	1.28 ± 0.03[c]	2.49 ± 0.18[c,d]

Values are expressed as mean ± SEM of 6 animals. Superscript letters represents the statistical significance done by ANOVA, followed by Tukey's multiple comparison tests.
[a] $P < 0.001$, indicates the significance on comparison of group II with group I.
[b] $P < 0.01$.
[c] $P < 0.001$, indicates the significance on comparison of group III and IV with group II.
[d] $P < 0.01$, indicates the dose-dependent significance on comparing group III and IV.

HPA axis, which is a major component of the stress response, is activated during oxidative stress. Hypothalamus releases corticotrophin-releasing factor (CRF), which in turn stimulates the release of adrenocorticotropic hormone (ACTH) from the pituitary gland during stressful conditions. Excessive corticosterone is released due to increase in plasma ACTH levels, which impairs the function and integrity of the hippocampus. In the present investigation, it was found that injection of STZ, increased the level of corticosterone and the treatment with *P. avium* extract, attenuated it, which indicates the aptitude to inhibit stress induced corticosterone and improves the cognitive function.

In our study besides increased free radicals, there was an increase in level of brain nitrite following STZ administration. Treatment with EEPA, a polyphenolic compound, decreased the levels of nitric oxide and the mechanism might be by inhibiting iNOS transcription or decreasing iNOS and NOS activity [57,58]. Thus from these studies of pharmacological screening, it was found that the EEPA possess antioxidant, anti-amnesic and neuroprotective effect to improve cognition.

5. Conclusion

In the present investigation, behavioural pattern was precisely improved in Y-maze and elevated plus maze depicted the anti-amnesic property of *P. avium*. Principal reduction in AChE, corticosterone along with escalated levels of antioxidant enzymes possibly contributed neuroprotection against STZ induced neurotoxicity. Further studies have to be emphasized in APP/PS1 transgenic mice for isolated bioactive molecule from the fruits of *P. avium* to identify the molecular mechanism involved in modulation of cholinergic transmission.

Disclosure of interest

The authors declare that they have no conflicts of interest concerning this article.

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