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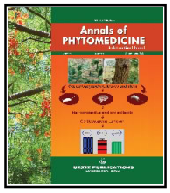

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# Neuroprotection of *Prunus avium* Linn. extract on cerebral ischemic stroke in rats associated with neuroendocrine effects

**Arpitaa Injamuri*, Hanish Singh Jayasingh Chellammal◆, Prakash V. Diwan* and Sumanth Yathavakilla***

*Department of Pharmacology and Pharmaceutical Chemistry, Faculty of Pharmacy, Universiti Teknologi MARA, Puncak Alam, Selangor-42300, Malaysia*

**School of Pharmacy, Anurag University (Formerly Lalitha College of Pharmacy), Venkatapur, Ghatkesar, Hyderabad-500 088, Telangana, India*

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**Abstract**

The fruits of *Prunus avium* Linn. protect from neurological diseases and produce a rejuvenating effect on the brain system, calming down nervous system disorientation and controls the insomnia and headache. The experiment is designed to evaluate the neuroprotective property of *P. avium* on cerebral ischemia in rat models. The neuroprotective effect of *P. avium* was studied in wistar rats of either sex by inducing ischemia through bilateral carotid artery occlusion. The ethanolic extract of *P. avium* (200 mg/kg and 400 mg/kg) were administered and evaluated for the neuroprotection. The biochemical parameters such as acetylcholinesterase, glutamate, corticosterone and antioxidant parameters were determined in the homogenate of brain. Open field and y-maze video tracking was determined to evaluate the behavioural learning and memory. The ethanolic extract of *P. avium* exhibited a significant decrease in AChE, glutamate, and corticosterone levels, restored the antioxidant enzyme levels and improved the learning and memory. The open field exploration and the alternation behaviour exhibited significant ($p$<0.001) improvement after the treatment with *P. avium* extracts. In biochemical parameters, the AChE and corticosterone were regulated significantly ($p$<0.001) in high dose treatment of *P. avium*. In conclusion, this study reveals the pharmacotherapeutic property and neuroendocrine regulation in cerebral ischemia induced rats and indicates the neuroprotective potential of *P. avium*.

## 1. Introduction

Cerebral stroke is a cerebrovascular disease ranking second lead cause of death. The common cause of stroke is occlusion of the bilateral carotid artery (BCCA), and the global mortality rate was 5.5 million cases in 2018 (Donkor, 2018). Ischemic stroke leads to neurological deficits, followed by the activation of the neurotransmission cascade involved in neurodegeneration, primarily by apoptosis. The most suitable animal model of ischemic stroke was the BCCA occlusion model in rats (Golubev, 2020). It is well characterized by cerebral ischemic reperfusion. The influence of pathological mechanisms such as excitotoxicity, inflammation, reactive oxygen species, peri-infarct depolarization and apoptosis are inevitable (Shekhar *et al.*, 2018). Excitotoxicity of glutamate (N-methyl-D-aspartate (NMDA)) receptor due to the accumulation of glutamate after ischemic/reperfusion. This plays a central role and, in turn, activates the hydroxyl radicals (OH), anion ($O_2$) radicals and hydrogen peroxide ($H_2O_2$) (Hou, 2020). The upturned mechanism of glutamate pool and its imbalance is believed to aggravate during ischemia. Sensory-motor ability is impaired in cerebral ischemia, lead to abrupt loss of neurological functions, inflammation, and oxidative stress leads to memory dysfunctions during ischemia (Maheshwari *et al.*, 2011). Moreover, it has been implicated that stress increases the expression of corticosterone during the cerebral ischemic stroke (De La Tremblaye *et al.*, 2014) and disturbs the neuroendocrine function. During ischemic stroke, metabolic energy is diminished, altering the functions of ATP-dependent membrane ionic pumps, leading to a rise in intracellular $Ca^{2+}$ and $Na^{+}$ concentrations to elevate the glutamate levels (He *et al.*, 2020). Drugs possess antioxidant properties, and anthocyanin-enriched extracts exert a free radical scavenging effect to minimize the pathological defects in CNS disorders and stroke (Parisi *et al.*, 2014). *Prunus* species contain high antioxidants and are found to improve learning and memory (Hanish Singh Jayasingh *et al.*, 2020). *P. avium* is commonly called as sweet cherry belonging to the family Rosaceae. It is native European tree and deciduous. It is also scattered in western Turkey, northwestern Africa and western Asia, with a small disjunct population in the western Himalayas. Traditionally, *P. avium*. is used for jaundice, kidney complaints, indigestion, diarrhoea, tuberculosis, bronchitis and rheumatism. This also has antioxidant and anti-inflammatory activity due to chemical constituents such as anthocyanins (Ademovic *et al.*, 2017; Dziadek *et al.*, 2019; Shahidi *et al.*, 2013). Herbs provide a natural rejuvenation and wellbeing and used for prevention of various ailments (Mehrotra, 2021). The fruits of *P. avium* and its species constitute sugars, organic acids, phenolic compounds, flavonoids, anthocyanins, and hydroxyl cinnamic acids (Dziadek *et al.*, 2019) and are responsible for various

**Corresponding author:** Dr. Hanish Singh Jayasingh Chellammal
*Department of Pharmacology and Pharmaceutical Chemistry, Faculty of Pharmacy, Universiti Teknologi MARA, Puncak Alam, Selangor-42300, Malaysia*
**E-mail:** hanishsinghjc@gmail.com
**Tel.:** +601115096775

biological effects (Sumathi *et al.*, 2019).This study was designed and investigated the neuroprotective activity of *P. avium* ethanol extract in cerebral ischemic and reperfusion-induced stroke and motor dysfunction in wistar rats by evaluating behavioral and biochemical parameters with stress related neuroendocrine (corticosterone) effects.

## 2. Materials and Methods

### 2.1 Drug source and extract preparation

The ripe unprocessed *P. avium* fruit was collected commercially and assured by Dr. Babashankar Rao, Associate Professor, Pharmacognosy, School of Pharmacy, Anurag Group of Institutions. A voucher sample was submitted at the Pharmacognosy Index of the Institute (COP: PA: 03). The shade dried fruits were separated from seeds exposed to homogenization using double-distilled solvent ethanol (99.9%). The homogenized extract was then filtered and evaporated using a rotary evaporator (Heidolph) to retain a copious consistent product, and the extract was stored on refrigeration in an airtight container. This extract was used to determine the neuroprotective activity in bilateral common carotid arteries (BCCA) occlusion induced animals treated with ethanolic extracts of *P. avium* (EEPA).

### 2.2 Experimental animals

Colony inherited strains of wistar rats, either sex, weighing 220-250 gm, were used for the neuropharmacological evaluations. The experimental rats were maintained under standard circumstances maintained at 23-25ºC, 12 h rotation of light-dark cycle and providedthe standard pellet diet (Tetragon LTD) with water *ad libitum*. Prior to the pharmacological studies the animals remained adapted for one week to the laboratory environment. The animals were considered as four groups encompassing six animals in each. Principles of animal handling have strictly adhered to institutes ethics committee. The experimental procedure has been duly approved by the institutional animal ethics committee (IAEC) of the School of Pharmacy, Anurag Group of Institutions Protocol No. I/IAEC/LCP/ 016/2012WR.

### 2.3 Study on toxicity (Acute)

Acute toxicity study evaluation was accomplished as per the organization of economic cooperation and development (OECD) guidelines, 423-annexure-d. Swiss albino mice (3 numbers, weight-22 to 25 gm) are selected and marked to permit individual identification and adopted to laboratory conditions for five days prior to dosing. A dose of 2000 mg/kg in aqueous form was prepared and administered to the animals, and food was withheld for 3-4 h. The animals were visually observed separately after drug dosing, and observations were made on autonomic system with tremors, convulsions, salivation and diarrhoea. The conditions on sleep and other gross behavioral changes (restlessness, aggressiveness, straub tail reaction, motor incoordination and respiration) or mortality are also observed (OECD, 2002). During the period of observation, if any toxic signs and symptoms were found, further the study was repeated with the lower dose of 300, 50 and 5 mg/kg

### 2.4 Experimental protocol and initiation of ischemia

The four groups of animals were subjected to 21 days of experimental period. After 20 days of treatment, the animals were subjected to behavioural studies and the animals were sacrificed on 21$^{st}$ day. The brain was isolated and homogenates were prepared in phosphate buffered saline at 10% concentration for assessing the antioxidant and biochemicals. Blood collection was performed by retro-orbital bleeding for assessing the plasma corticosterone. The design of experiment follows; Group I-Sham operated animals treated with saline (vehicle). Group II, the BCCA occlusion ischemia-induced animals and treated with vehicle. Group III-animal treated with 200 mg/kg of EEPA (low dose). Group IV-the rats treated with 400 mg/kg of EEPA (high dose). For the induction of ischemia, the rats were anaesthetized on 14$^{th}$ day with urethane at a 1.5 gm/kg dose of the body weight and a midline slit was performed in neck. By gently retracting the neck muscles, the common carotid artery was identified and BCCA was occluded for 30 min and reperfused for reflow of blood. Then the midline incision was sutured, betadine oinment (5%) was applied to avoid infection (Hajizadeh Moghaddam *et al.*, 2021).

### 2.5 Behavioural testing

#### 2.5.1 Open field orientation test

The probing behavior of rats was assessed by open-field test instrument consisting of 60 × 60 cm square shaped platform. The rat was positioned in the arena of open field which consist of brown linoleum. The floor was alienated into twelve partition squares. The animals were allowed to explore freely for five minutes. The counts in head dipping and line crossings were monitored and accounted (Hanish Singh *et al.*, 2009).

#### 2.5.2 Y-maze video tracking test

The Y maze instrument is aided with the software (VJ Instruments) used to measure rats' working memory (Spatial). The specifications of the maze are 400 mm long, 130 mm high, 50 mm wide and made of acrylic opaque fiber. Each rat was positioned from one end the arm and permitted to move spontaneously for 8 min. Rat tends to reconnoiter maze thoroughly, entering each arm sequentially. The sequence of arm entries and conceivable re-entry into the visited arm were recorded in tracking system. The successive accesses into three arms (percentage alteration) were monitored (Singh *et al.*, 2013).

### 2.6 Biochemical evaluations

#### 2.6.1. Estimation of antioxidant enzyme activity

Pyrogallol method was employed for determining superoxide dismutase (SOD) action (Marklund and Marklund, 1974). 80 µl of pyrogallol was diluted to 100 µl using hydrochloric acid (0.01 N) and added with 600 µl of buffer (Tris-HCl). Then mixed well and further added with 100 µl of DETPA and 100 µl of distilled water. Test samples, 50 µl were added with equal volume of pyrogallol. The reaction was started and read at 420 nm at persistent temperature of 25ºC for 3 min. Evaluation of catalase (CAT) effect was performed in 50 µl of test added with 50 µl of the substrate. To the mixture, 100 µl of 32.4 mM ammonium molybdate was added and read at 405 nm. The unit of the enzyme is defined as millimoles of $H_2O_2$ degraded/ min/milligram of protein (Goth, 1991). Glutathione peroxidase was measured in brain homogenate. To 0.1 ml enzyme (brain homogenate), reduced glutathione (0.2 ml), sodium azide (0.1 ml), hydrogen peroxide (0.1 ml) and 1 ml water is added. Then incubated for1.5min and 3min. After incubation 10% 1 ml trichloroacetic acid was added and centrifuged (3000 rpm for 15 min).1 ml of supernatant and 0. 5 ml of 5,5-dithio-bis-(2-nitrobenzoic acid) DTNB were added with

4 ml of phosphate solutionand read at 412 nm (Lawrence and Burk, 1976).

### 2.6.2 Estimation of acetylcholinesterase (AChE) enzyme

AChE was determined in the whole-brain and measured by the modified Ellman method. In this method. The principle involved in this method was the development of yellow colour after reacting with thiocholine and dithiobisnitrobenzoate ions. The rate of development of thiocholine from acetylcholine iodide from brain cholinesterase was read by using UV-spectrophotometer -Shimadzu (Ellman *et al.*, 1961).

### 2.6.3 Estimation of glutamate

The glutamate estimation is based on the principle of partition coefficients between the stationary cellulose phase with amino acids mobile solvent phase. Quantification and extraction are done by reacting with ninhydrin (triketohydrindene hydrate). Ninhydrin chemically interacts to the amino acid and releases $CO_2$, $NH_2$ and its subordinate aldehyde. This produces a chromophore called as Ruhemann's purple.The spots of the reactants were isolated and eluted with a mobile phase of 0.005% $CuSO_4$ in 75% ethanol. The absorbance was measured compared to a buffer at 515 nm (UV spectrometer), and the amounts were indicated as mmol/gm wet weight of tissue (Sunanda *et al.*, 2000).

### 2.6.4. Estimation of corticosterone activity

Corticosterone estimations were performed in plasma. In heparinized tube, the blood was collected and separated the plasma at 4ºC. The corticosterone quantification was performed using HPLC/UV system (SPD-20A, Shimadzu) with dexamethasone as internal standard (Woodward and Emery, 1987). 50 µl of plasma added with known amount of dexamethasone (1 µg) was extracted with 5 ml of dichloromethane (DCM). The obtained DCM portion was dried under liquid nitrogen and dispersed in 100 µl of the mobile phase. From this extract, 20 µl was injected to the system for quantification. Methanol: water (70:30) was used as mobile phase and the flow rate was maintained at 1.2 ml/min.to the detect the corticosterone at 250 on UV detector.

## 3. Results

### 3.1 Acute toxicity study

The drug *P. avium* is categorized as non-toxic. The extract has not exhibited any toxic effects or mortality when administered orally at a 2000 mg/kg dose in mice. As per the OECD classification, the $LD_{50}$ of 2000 mg/kg and overhead is unclassified and declared non-toxic.

### 3.2 Behavioural testing

### 3.2.1 Open field orientation test

In open field exploration and memory effect, the BCCA induced animals indicated a decrease in the number of crossings and head dips. The results were significant ($p$<0.001) once equated to the control animals. The administration of EEPA in Groups III and IV improved the open field exploration memory which is indicated by a greater number of head dips and crossings with significant differences ($p$<0.01), respectively (Table 1).

### 3.2.2. Video tracking study on Y-maze test

In the Y-maze evaluation, the Group II (BCCA-occlusion induced) animals showed the diminished proportion of alteration with a significant ($p$<0.01) decrease on comparison with control animals. EEPA (200 mg/kg and 400 mg/kg) improved the percentage alteration ($p$<0.05 and $p$<0.01) significantly. Moreover, it exhibited the dose-dependent rise in percentage alternation ($p$<0.05) when compared to the both doses of EEPA (Table 1).

**Table 1: Effect of *P. avium* on exploratory behaviour and Y-Maze**

| Group | Line crossing (counts/5 min) | Head dips alternation | Y-Maze |
|---|---|---|---|
| Group I | 69.5 ± 5.43 | 5.1 ± 0.47 | 51.90 ± 2.11 |
| Group II | 40.17 ± 3.21[a] | 2.5 ± 0.22[a] | 26.52 ± 1.53[a] |
| Group III | 54.83 ± 2.10[c] | 4.0 ± 0.36 [c] | 31.90 ± 1.76[b] |
| Group IV | 57.83 ± 1.40[c] | 4.5 ± 0.34[c] | 42.30 ± 3.82[c,d] |

Values are expressed as mean ± SEM of six animals. Superscript letters represent the statistical significance done by ANOVA, followed by Tukey's multiple comparison tests. [a] $p$<0.001 indicates the comparison of Group II with Group I. [b]$p$<0.05 indicates the significant difference on comparing Group II with III. [c] $p$<0.01 indicates comparison of Group II with IV. [d]$p$<0.05 indicates dose dependent significance on comparing Group IV with Group III.

### 3.3 Biochemical parameters

### 3.3.1 Antioxidants parameters

The induction of cerebral ischemia in Group II animals indicated the significant reduction of SOD enzyme with $p$<0.001. After the treatment of EEPA, the SOD was improved well in the 200 mg/kg and 400 mg/kg. There were a significant ($p$<0.01 and $p$<0.001) difference in improvement of SOD, respectively. The catalase levels were diminished after induction of BCCA occlusion in Group II ($p$<0.001). The treatment of 200 mg/kg and 400 mg/kg EEPA restored the reduced activity of catalase significantly ($p$<0.001) when compared with negative control group. There were a dose-dependent escalation of catalase was found between the two doses ($p$<0.05).The reduction of GPx in Group II animals after the ischemia was observed when compared to the normal control group. Upon treatment with two doses of EEPA, there was an increase in GPx enzyme significantly with $p$<0.01 and $p$<0.01 when associated to the negative control group (Table 2).

**Table 2: Effect of *P. avium* on antioxidant parameters**

| Group | Catalase (U/mg protein) | SOD (U/min/mg protein) | GPx |
|---|---|---|---|
| Group I | 1.68 ± 0.07 | 1.6 ± 0.11 | 56.27 ± 5.7 |
| Group II | 0.69 ± 0.05[a] | 0.9 ± 0.54[a] | 18.7 ± 0.95[a] |
| Group III | 0.89 ± 0.08[b] | 1.2 ± 0.07[c] | 30.46 ± 1.40[c] |
| Group IV | 1.12 ± 0.09[b,f] | 1.3 ± 0.06[b,d] | 31.8 ± 0.49[c] |

Values are expressed as mean ± SEM for six animals. Superscripts indicates the statistical significance performed by ANOVA, followed by Tukey's multiple comparison tests. [a] $p$<0.001 represents the assessment of Group II with Group I. [b] $p$<0.001 express the significant alteration on comparing Group II with Group III and

IV.[c]$p<0.01$express the comparison of Group II with Group III and IV, [d]$p<0.01$ and [f]$p<0.05$ indicates the dose dependent activity between Group III and IV.

### 3.3.2 Acetylcholinesterase enzyme

The induction of cerebral ischemia in ischemia induced group (Group II), elevated the AChE level significantly ($p<0.001$) on equated to the sham operated Group (Group I).The treatment of two doses (Group III and IV) of EEPA significantly ($p<0.001$) controlled the escalation of AChE. When compared the low dose with high dose (200 mg/kg with 400 mg/kg), there was a significant ($p<0.05$) difference is found in AChE. This indicated the dose dependent effect of EEPA. (Table 3).

### 3.3.3 Glutamate

In Group II, the ischemia induction reduced the glutamate level significantly ($p<0.001$) which was compared to Group I animals. 200 mg/kg and 400 mg/kg of EEPA treatment neurodegeneration induced rats significantly ($p<0.001$) improved the brain concentration of glutamate when compared to the ischemia induced group. It was merely equivalent to the normal control animals and express the property of regulating glutamate by EEPA. (Table 3).

### 3.3.4 Corticosterone

The ischemia induction in the negative control group (Group II) exhibited a elevated level of corticosterone significantly ($p<0.001$) when equated to control group (Group I). In the treatment Group IV (400 mg/kg EEPA), there was a substantial decrease in corticosterone with $p<0.001$ in comparison with the Group II. There was significant ($p<0.05$) dose dependent reduction of corticosterone when compared to the levels of low dose (200 mg/kg) treated animals and high dose (400 mg/kg) treated animals (Table3).

**Table 3: Effect of *P. avium* on AChE, glutamate and corticosterone**

| Group | AChE (µg/min/mg protein) | Glutamate (mmoles/gram wet tissue weight) | Corticosterone (ng/ml serum) |
|---|---|---|---|
| Group I | 10.77 ± 1.00 | 952.90 ± 36.82 | 140.2 ± 8.22 |
| Group II | 18.50 ± 1.52[a] | 1939.00 ± 38.57[a] | 239.3 ± 11.73[a] |
| Group III | 11.80 ± 0.83[b] | 1186.00 ± 49.91[b] | 220.6 ± 9.24 |
| Group IV | 12.15 ± 0.70[b,f] | 845.00 ± 77.85[b] | 165.7 ± 8.78[b,f] |

Values indicated as mean ± SEM of six animals. The statistical significance was substituted and derived by ANOVA, followed by Tukey's test (multiple comparison). [a] $p<0.001$ express the relation of Group II with Group I.[b] $p<0.001$ express the statistical difference on comparing Group II with III and IV. [f] $p<0.05$ reveals the dose dependent activity between III and IV group.

## 4. Discussion

Cerebral ischemic stroke occurs due to impediment of blood streaming to the brain, leading to various triggering of pathophysiological mechanisms. Increased reactive oxygen species, excitotoxicity, inflammation and its mediators, mitochondrial dysfunction, and ATP deprivation induces severe neuronal damage with major pathophysiological condition of ischemic stroke (Justin *et al.*, 2018). The damage to neurons also induces spatial learning and memory deficits and mediates neurodegeneration (Milot and Plamondon, 2011). The treatment with a drug possessing antioxidants and rejuvenating capacity that diminishes the neuronal deficits and prevents the memory impairment may be beneficial in treating cerebral ischemia.

Anthocyanins are flavonoids containing cyaniding 3-O-glucosides, delphinidin-3-O-â-glucopyranoside and cyaniding-3-O-D-arabinopyranoside, which are widely distributed in fruits and vegetables. Phytochemical compounds enriched with polyphenols and anthocyanins were reported to exhibit potential neuroprotective effects in AD and PD (Sekeroglu and Gezici, 2019). Previous studies have reported that anthocyanins have reduced the oxidative stress in certain neurodegenerative disorders such as Parkinson's and Alzheimer's condition (Yamakawa *et al.*, 2016) and also possess strong antioxidant properties (Gezici *et al.*, 2020). Various phytochemical extracts and their components are also highly effect in CNS related disorders (Uddin *et al.*, 2020).The ability of cyanidin 3 glucoside to cross blood-brain barrier (BBB) has provided central actions. *P. avium* (Rosaceae) was very rich in anthocyanins (Dziadek *et al.*, 2019). Ethanolic fruit extracts *P. avium* is explored in the present investigation and found neuroprotective. The extract exhibits neuroprotection, as evidenced by the restoration of antioxidants and its effect on AChE. The glutamate levels and the biochemical parameters were reversed and restored to near-normal levels in the groups pretreated with the drug. The animals in ischemic conditions show differential behavioral changes when compared to normal animals. Hippocampus is the key driving part of brain for the regulation of learning and memory. Hippocampal neurons are extremely vulnerable to low blood flow, ischemia and reperfusion-injury (Yang *et al.*, 2021).Therefore Y-maze and habitual open field test has been widely used for evaluating the memory impairment resulted from cerebral ischemia.Y-maze is used for the evaluation of spatial working and long-term memory. In our investigations, it was observed that the EEPA pretreatment has prevented the ischemia-induced memory impairment and motor incoordination. The treatment with EEPA restored to that of normal group. Open field exploration is used to evaluate behavioral memory, which is determined by observing the head dips and crossings. It was observed that the animals preserved with EEPA have significantly downregulated the impairment in exploratory behaviour compared with unadministered ischemic rats.The principal neurotransmitter glutamate is excitatory in nature on the brain. Higher glutamatergic transmission have been documented in the pathogenesis of cerebral ischemia (Yang *et al.*, 2015). During ischemia, glutamate can be released through two different mechanisms, either through the $ca^{2+}$ dependent and vesicular mode or through the glutamate transporters like NMDA receptors (Li *et al.*, 2017). The treatment with EEPA decreased glutamate levels in the ischemic animals.

The pathophysiology of cerebral ischemia and reperfusion-induced damage is characterized by increased buildup of reactive oxygen species causing depletion of reduced glutathione and a fall in cellular antioxidant enzyme levels (Song *et al.*, 2020). In this investigation, it was found that the drug administered groups with EEPA showed an improvement in enzymatic levels of cellular antioxidant biomarkers such as SOD, catalase and Gpx,in comparison to the negative control. The underlying mechanism for such an elevation of cellular antioxidant enzyme levels during the treatment with EEPA may be due to

anthocyanins in EEPA. Earlier studies have suggested that anthocyanins increase the expression of antioxidant enzymes by promoting the expression of the Nrf2 transcription factor, which controls the expression of antioxidant response element proteins (ARE), thereby increasing the expression of cellular antioxidant enzymes (Qu *et al.*, 2020). It is also suggested that hypothalamic-pituitary-adrenal (HPA) axis activation during ischemic conditions exerts adaptive changes; however, chronic activation leads to neurological disorders. Chronic elevation of cortisone exacerbates neurodegeneration in neurological disorders and age-related dementia, including stroke (Sugo *et al.*, 2002). It has been understood from previous studies that during cerebral ischemia, the increased expression of amyloid precursor protein (APP) and Aβ expression in some ischemic regions are noted, particularly in the hippocampus, thalamus and corpus callosum, which leads to memory impairment. The increased APP and Aβ expression abnormalities in certain neurotransmitters such as ACh, dopamine and serotonin (Alasmari *et al.*, 2018). The decrease in ACh level, a noteworthy neurotransmitter for memory and spatial behavior, is due to the abnormal function of enzyme AChE. The AChE hydrolyzes the available ACh and produces its deficiency. Previous studies in *P. avium* exhibited a neuroprotective effect in streptozotocin-induced neurodegeneration (Vinitha *et al.*, 2014). In the present research, it was found that the increase in the activity of AChE in cerebral ischemic animals when compared to untreated sham operated animals. The group of animals administered with EEPA have exhibited a remarkable reduction in the AChE activity compared to ischemic induced untreated animals. Based on the outcome and results of this investigation, it may be determined that EEPA possesses neuroprotective effects. It is highly evident from the reduction of AChE, glutamate and restored levels of SOD, GPx catalase levels with attenuation in motor exploratory dysfunctions. The anti-ischemic and cerebral neuroprotective effects may be due to the presence of anthocyanins and their property to scavenge the reactive oxygen species. These experimental outcomes suggest that *P. avium* has neuroprotective activity and can be used to treat cerebral ischemic stroke.

## 5. Conclusion

The study results conclude the neuroprotective potential of *P. avium* extract in bilateral carotid artery occlusion type of stroke. The reduction in corticosterone (stress related neuroendocrine hormone), acetylcholinesterase and turnover in glutamate with antioxidants played a pivotal role in the neuroprotective mechanisms, thus regulating stress and brain organic antioxidants. In conclusion, this research reports that *P. avium* could be a pharmacotherapeutic agent for neuroprotection in stroke and associated neurodegenerative disorders.

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## Conflict of interest

The authors declare no conflicts of interest revelent to this article.

## References

**Ademovic, Z.; Hodzic, S.; Halilic Zahirovic, Z.;Husejnagic, D.; Dzananovic, J.; Saric-Kundalic, B. and Suljagic, J. (2017).** Phenolic compounds, antioxidant and antimicrobial properties of the wild cherry (*Prunus avium* L.) stem. Acta Period. Technol., **48**:1-13. https://doi.org/10.2298/APT1748001A.

**Alasmari, F.; Alshammari, M.A.; Alasmari, A.F.; Alanazi, W.A. and Alhazzani, K. (2018).** Neuroinflammatory cytokines induce amyloid beta neurotoxicity through modulating amyloid precursor protein levels/metabolism. Biomed Res. Int., Article ID 3087475. https://doi.org/10.1155/2018/3087475.

**De La Tremblaye, P.B.; Raymond, J.; Milot, M.R.; Merali, Z. and Plamondon, H. (2014).** Evidence of lasting dysregulation of neuroendocrine and HPA axis function following global cerebral ischemia in male rats and the effect of antalarmin on plasma corticosterone level. Horm. Behav., **65**:273-284. https://doi.org/10.1016/j.yhbeh.2014.01.003

**Donkor, E.S. (2018).** Stroke in the 21st Century: A snapshot of the burden, epidemiology, and quality of life. Stroke Res. Treat., 3238165. https://doi.org/10.1155/2018/3238165

**Dziadek, K.; Kopec, A. and Tabaszewska, M. (2019).** Potential of sweet cherry (*Prunus avium* L.) by-products: Bioactive compounds and antioxidant activity of leaves and petioles. Eur. Food Res. Technol., 245, 763-772. https://doi.org/10.1007/s00217-018-3198-x

**Ellman, G.L.; Courtney, K.D.; Andres, V. and Featherstone, R.M. (1961).** A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem. Pharmacol., **7**: 88-95. https://doi.org/10.1016/0006-2952(61)90145-9

**Gezici, S.; Koçum, D.; Yayla, F.; Sekeroglu, N. and Khan, A.A. (2020).** Screening for *in vitro* antioxidant activities, polyphenolic contents and neuroprotective potentials of *Clinopodium serpyllifolium* subsp. *serpyllifolium* endemic to Turkey. Ann. Phytomed., **9**:181-186. https://doi.org/10.21276/ap.2020.9.1.23

**Golubev, A.M. (2020).** Models of ischemic stroke (Review). Gen. Reanimatol., **16**:59-72. https://doi.org/10.15360/1813-9779-2020-1-59-72

**Goth, L. (1991).** A simple method for determination of serum catalase activity and revision of reference range. Clin. Chim. Acta., **196**: 143-151. https://doi.org/10.1016/0009-8981(91)90067-M

**Hajizadeh Moghaddam, A.; Shirej Pour, Y.; Mokhtari Sangdehi, S.R. and Hasantabar, V. (2021).** Evaluation of hesperetin-loaded on multiple wall carbon nanotubes on cerebral ischemia/reperfusion injury in rats. Biomed. Pharmacother., 138. 111467. https://doi.org/10.1016/j.biopha.2021.111467.

**Hanish Singh, J.C.; Muralidharan, P.; Narsimha Reddy, Y.; Sathish Kumar, S. and Alagarsamy, V. (2009).** Anti-amnesic effects of *Evolvulus alsinoides* Linn. in amyloid β(25-35) induced neurodegeneration in mice. Pharmacologyonline, **1**:70-80.

**Hanish Singh Jayasingh, C.; Mohamed Mansor, M.; Afiq, A.; Yasothini, M.; Punitha, C. and Pavithiraa, C. (2020).** Neurocognitive effects of *Prunus domestica* fruit extract on scopolamine-induced amnesic mice. J. Appl. Pharm. Sci., **10**:59-66. https://doi.org/10.7324/JAPS.2020.10118

**He, Z.; Ning, N.; Zhou, Q.; Khoshnam, S.E. and Farzaneh, M. (2020).** Mitochondria as a therapeutic target for ischemic stroke. Free Radic. Biol. Med., **146**:45-58. https://doi.org/10.1016/j.freeradbiomed.2019.11.005

**Hou, S.T. (2020).** The regulatory and enzymatic functions of CRMPs in neuritogenesis, synaptic plasticity, and gene transcription. Neurochem. Int., **139**:104795. https://doi.org/10.1016/j.neuint.2020.104795.

**Justin, A.; Divakar, S. and Ramanathan, M. (2018).** Cerebral ischemia-induced inflammatory response and altered glutaminergic function mediated through brain AT1 and not AT2 receptor. Biomed. Pharmacother., 102, 947-958. https://doi.org/10.1016/j.biopha.2018.03.164

**Lawrence, R.A. and Burk, R.F. (1976).** Glutathione peroxidase activity in selenium-deficient rat liver. Biochem. Biophys. Res. Commun., **71**: 952-958. https://doi.org/10.1016/0006-291X(76)90747-6

**Li, L.; Xue, J.; Liu, R.; Li, X.; Lai, L.; Xie, J.; Huang, Z. and Huang, C. (2017).** Neuroprotective effects of genistein-32 -sodium sulfonate on focal cerebral ischemia in rats. Neurosci. Lett., **646**:43-48. https://doi.org/10.1016/j.neulet.2017.02.046.

**Maheshwari, A.; Badgujar, L.; Phukan, B.; Bodhankar, S.L. and Thakurdesai, P. (2011).** Protective effect of etoricoxib against middle cerebral artery occlusion induced transient focal cerebral ischemia in rats. Eur. J. Pharmacol., **667**: 230-237. https://doi.org/10.1016/j.ejphar.2011.05.030

**Marklund, S. and Marklund, G. (1974).** Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. Eur. J. Biochem., **47**:469-474. https://doi.org/10.1111/j.1432-1033.1974.tb03714.x

**Mehrotra, N. (2021).** Herbs that heal: Nature's pharmacy endowed remedies for better health. Ann. Phytomed., **10**:6-22. https://doi.org/10.21276/ap.2021.10.1.2.

**Milot, M.R. and Plamondon, H. (2011).** Changes in HPA reactivity and noradrenergic functions regulate spatial memory impairments at delayed time intervals following cerebral ischemia. Horm. Behav., **59**:594-604. https://doi.org/10.1016/j.yhbeh.2011.02.017

**OECD. (2002).** Test No. 420: Acute oral toxicity: Fixed dose procedure, OECD guideline for testing of chemicals, OECD guidelines for the testing of chemicals, Section 4. OECD. https://doi.org/10.1787/9789264070943-en.

**Parisi, O.I.; Puoci, F.; Restuccia, D.; Farina, G.; Iemma, F. and Picci, N. (2014).** Polyphenols and their formulations. Polyphenols in human health and disease., Elsevier, pp:29-45. https://doi.org/10.1016/B978-0-12-398456-2.00004-9.

**Qu, Z.; Sun, J.;Zhang, W.; Yu, J. and Zhuang, C. (2020).** Transcription factor NRf2 as a promising therapeutic target for Alzheimer's disease. Free Radic. Biol. Med., **159**:87-102. https://doi.org/10.1016/j.freeradbiomed.2020.06.028.

**Sekeroglu, N. and Gezici, S. (2019).** *Astragalus neurocarpus* Bioss. as a potential source of natural enzyme inhibitor associated with Alzheimer's and Parkinson diseases along with its rich polyphenolic content and antioxidant activities. Ann. Phytomed., **8**:82-87. https://doi.org/10.21276/ap.2019.8.1.9

**Shahidi, S.; Setareye, S. and Mahmoodi, M. (2013).** Effect of *Prunus domestica* L. (Mirabelle) on learning and memory in mice. Anc. Sci. Life., **32**: 139. https://doi.org/10.4103/0257-7941.122996.

**Shekhar, S.; Cunningham, M.W.; Pabbidi, M.R.; Wang, S.; Booz, G.W. and Fan, F. (2018).** Targeting vascular inflammation in ischemic stroke: Recent developments on novel immunomodulatory approaches. Eur. J. Pharmacol., **833**:531-544. https://doi.org/10.1016/j.ejphar.2018.06.028

**Singh, H.J.C., Syeda, T.U.B., Kakalij, R.M., Prasad, V.V.L.N. and Diwan, P. V. (2013).** Erythropoietin protects polychlorinated biphenyl (Aroclor 1254) induced neurotoxicity in mice. Eur. J. Pharmacol., **707**: 54-60. https://doi.org/10.1016/j.ejphar.2013.03.009

**Song, X.; Gong, Z.; Liu, Kaili.; Kou J.; Liu, B. and Liu, Kang. (2020).** Baicalin combats glutamate excitotoxicity *via* protecting glutamine synthetase from ROS-induced 20S proteasomal degradation. Redox Biol., **34**:101559. https://doi.org/10.1016/j.redox.2020.101559.

**Sugo, N.; Hurn, P.D.; Morahan, M.B.; Hattori, K.; Traystman, R.J. and DeVries, A.C. (2002).** Social stress exacerbates focal cerebral ischemia in mice. Stroke., **33**:1660-1664. https://doi.org/10.1161/01.STR.0000016967.76805.BF.

**Sumathi, S.; Suganya, K.; Vishali, A. and Padma, P.R. (2019).** Anticariogenic effect of *Vaccinium macrocarpon* Aiton against causative organisms of dental caries. Ann. Phytomed., **8**:99-104. https://doi.org/10.21276/ap.2019.8.2.11.

**Sunanda.; Rao, B.S.S. and Raju, T.R. (2000).** Restraint stress-induced alterations in the levels of biogenic amines, amino acids, and AChE activity in the hippocampus. Neurochem. Res., **25**:1547-1552. https://doi.org/10.1023/A:1026606201069.

**Uddin, M.I. A.; Vandana, A.V.; Kavya, G.K. and Syed, Y.H. (2020).** Systematic study on protective role of date palm (*Phoenix dactylifera* L.) on central nervous system disorders. Ann. Phytomed., **9**:58-65. https://doi.org/10.21276/ap.2020.9.1.7.

**Vinitha, E.; Singh, H.J.C.; Kakalij, R.M.; Kshirsagar, R.P.; Kumar, B.H. and Diwan, P. V. (2014).** Neuroprotective effect of *Prunus avium* on streptozotocin-induced neurotoxicity in mice. Biomed. Prev. Nutr., **4**:519-525. https://doi.org/10.1016/j.bionut.2014.08.004

**Woodward, C.J.H. and Emery, P.W. (1987).** Determination of plasma corticosterone using high-performance liquid chromatography. J. Chromatogr. B Biomed. Sci. Appl., **419**:280-284. https://doi.org/10.1016/0378-4347(87)80287-6.

**Yamakawa, M.Y.; Uchino, K.; Watanabe, Y.; Adachi, T.; Nakanishi, M.; Ichino, H.; Hongo, K.; Mizobata, T.; Kobayashi, S.; Nakashima, K. and Kawata, Y. (2016).** Anthocyanin suppresses the toxicity of Aâ deposits through diversion of molecular forms in *in vitro* and *in vivo* models of Alzheimer's disease. Nutr. Neurosci., **19**: 32-42. https://doi.org/10.1179/1476830515Y.0000000042.

**Yang, X.; Song, D.; Chen, L.; Xiao, H.; Ma, X.; Jiang, Q. and Cheng, O. (2021).** Curcumin promotes neurogenesis of hippocampal dentate gyrus via Wnt/â-catenin signal pathway following cerebral ischemia in mice. Brain Res., **1751**:147-197. https://doi.org/10.1016/j.brainres.2020.147197.

**Yang, Z.B.; Luo, X.J.; Ren, K.D.; Peng, J.J.; Tan, B.; Liu, B.; Lou, Z.; Xiong, X.M.; Zhang, X.J.; Ren, X. and Peng, J. (2015).** The beneficial effect of magnesium lithospermate B on cerebral ischemia-reperfusion injury in rats involves the regulation of miR-107/glutamate transporter 1 pathway. Eur. J. Pharmacol., **766**:91-98. https://doi.org/10.1016/j.ejphar.2015.09.042.

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