Neuroprotective effect of *Prunusavium*on on cerebral ischemic stroke in rats

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ABSTRACT

In this study we use anthocyanins which have both COX-2 inhibitory action and antioxidant activity. Anthocyanins are richly concentrated as pigments in berries. They belong to class of flavonoids, they are water soluble vacuolar pigments that may appear red, purple or blue according to P^H. Anthocyanins obtained from berries have therapeutic uses in cancer, aging, and neurological diseases, inflammation, diabetes, bacterial infections, fibrocystic diseases the present work is aimed at evaluating the cyclooxygenase-II inhibitory activity and antioxidant activity of anthocyanins obtained from *Prunusavium*in on cerebral ischemic stroke model in rat.

KEY WORDS: *Prunusavium*, cerebral ischemic stroke, neuroprotective activity.

1. INTRODUCTION

Stroke is clinically defined as a condition where there is decreased supply of blood to the brain due to formation of thrombosis, embolism or blockade of the artery supplying blood to brain, this result in decrease of supply of oxygen and glucose to the brain cells which leads to necrosis of brain cells. The decreased supply of blood to brain leading to reduced CBF causes metabolic and functional deficit which is defined as ischemia. Ischemia causes brain damage by activating the ischemic cascade, which progresses to local depletion of oxygen or glucose, causing failure of production of high energy phosphate compounds, like adenine triphosphate (ATP). This adversely affects energy-dependent processes necessary for tissue cell survival, and sets off a series of interrelated events culminating in cellular injury and death. The extent of damage usually depends on duration, severity, and location of ischemia.

Prunusavium (family: rosaceae) is a deciduous tree growing to 15–42 m tall, with a trunk up to 1.5 m in diameter. The bark is smooth purplish-brown with prominent horizontal grey-brown lenticels on young trees, becoming thick dark blackish-brown and fissured on old trees. The leaves are alternate, simple ovoid-acute, 7–14 cm long and 4–7 cm broad, glabrous matt or sub-shiny green above, variably finely downy beneath, with a serrated margin and an acuminate tip, with a green or reddish petiole 2–4.5 cm long bearing two to five small red glands. Medicine can be prepared from the stalks of the drupes that is astringent, antitussive, and diuretic.

2. MATERIALS AND METHODS

2.1. Collection and authentication of plant material: plant material was collected from the plant nursery and it was identified and authenticated by Dr.M.Raghu Ram, Department of Botany, Acharya Nagarjuna University, Guntur.

2.2. Preaparation of ethanolic Extract of *Prunusavium:* The fruits were shade dried until they are free from moisture and the seeds are separated from the fruits then subjected to homogenization using ethanol as solvent. The extract is then evaporated using rots evaporator to obtain a thick consistent product. The % yield obtained was 51.8gms and the extract was stored in a cool place in air tight container.

2.3. Grouping of animals and Treatment schedule: Male Wistar rats of weight (200-250gm) were divided into following groups each consisting of six animals.

Group A- Vehicle group.

Group B – MCAO.

Group C – MCAO+EEPA 200 mg/kg.

Group D – MCAO+EEPA 400 mg/kg.

2.4. Open field habituation: The exploratory behavior of rat was evaluated by open field habituation memory test. Rat was placed in an open field whose brown linoleum floor was divided into twelve equal squares by white lines and left to explore it freely for five minutes. The number of line crossings and head dipping were counted.

2.5. Y-MAZE task: Video tracking system (VJ, Instruments) in Y maze is assisted with software was used to measure the spatial working memory in mice. The maze is made of gray plastic. Each arm is 40 cm long, 14 cm high, 4 cm wide at the bottom, 10 cm wide at the top, and converged at an equal angle. Each mouse was placed at the end of one arm and allowed to move freely through the maze 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 % age alteration is obtained by the assistance of the software. Alteration is defined as the successive entries into the three arms, on overlapping triplet sets.

3. RESULTS AND DISCUSSION

3.1. Effect of EEPA on exploratory behavior in open field: In the open field habituation memory test the animals induced with ischemia by MCAO indicated a reduction in the line crossings and head dippings significantly (P<0.001) when compared to the control animals. The treatment of EEPA in group III and IV protected the open field habituation memory by increasing the activity of head dipping and line crossings with significant difference (P<0.05) and (P<0.01) respectively.

3.2. Effect of EEPA on Y-maze test: In the y-maze test, the group II animals indicated the impaired percentage alteration with significant (P<0.01) reduction on comparing with the control animals. In the treatment group, EEPA at the doses of 200mg/kg and 400mg/kg improved the percentage alteration significantly (P<0.01). Moreover there was dose dependent increase in activity was found in comparison with the two doses of EEPA.

Table.1.Results

Group	Line Crossing No of line crossing	Nose poking No of nose poking	Y-MAZE % alteration
Group I (vehicle)	5.533 ± 69.5	0.5773 ± 5.167	2.115 ±51.90
Group II (MCAO)	3.219 ± 50.17[a]	0.2236 ± 2.500	1.533 ±26.5
GROUP III (MCAO+EEPA 200mg/kg)	2.120 ± 55.83[d]	0.3651 ± 5.000	1.767 ± 31.90
GROUP IV (MCAO+EEPA 400mg/kg)	2.120 ± 55.83[d]	0.3651 ± 5.000	1.767 ± 31.90

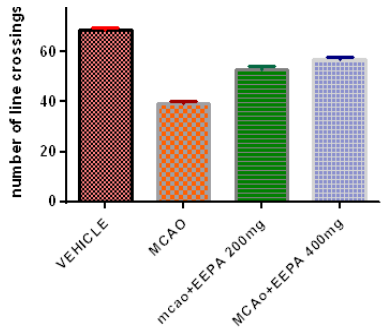

Figure.1.Effect of EEPA on line crossings in open field habituations

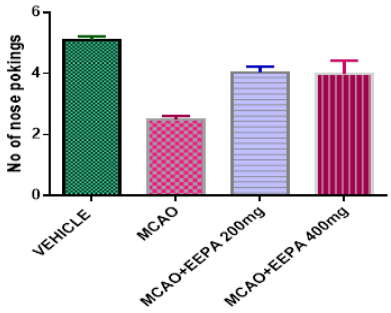

Figure.2. Effect of EEPA on nose pokings in open field habituations

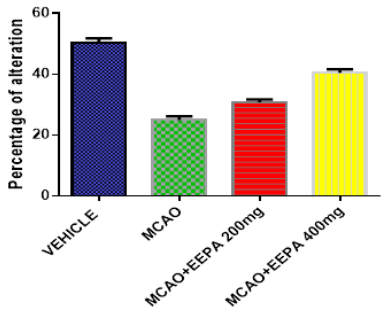

Figure.3. Effect of EEPA on exploratory behavior in Y-Maze

Table.2.Effect of EEPA on AchEand glutamate

Group	ACHE (µ mol/mg)	Glutamate (µ mol/mg)
Group I (vehicle)	0.08285 ± 1.181	36.82 ± 952.9
Group II (MCAO)	0.9773 ± 2.192	38.57 ± 1939.0
GROUP III (MCAO+EEPA 200mg/kg)	0.08663 ± 1.79	59.91 ± 1188
Group IV (MCAO +EEPA 400mg/kg)	0.8735 ± 1.252	77.85 ± 855

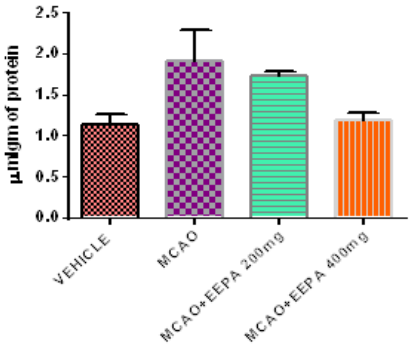

Figure.4.Effect of EEPA on ACHE

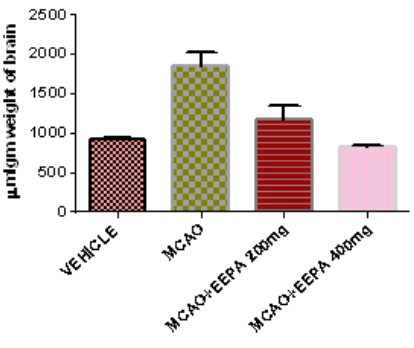

Figure.5. .Effect of EEPA on glutamate

DISCUSSION

Cerebral ischemic stroke occurs due to obstruction in blood supply to the brain which leads to cascade of physicochemical mechanisms like increase in reactive oxygen species, release of inflammatory mediators, excitotoxicity, mitochondrial dysfunction and deprived ATP levels causing severe neuronal damage. The damage to neurons also leads to spatial learning and memory. The treatment with a drug that decreases the neuronal damage and inhibits memory impairment will be useful in cerebral ischemia. Anthocyanins are a type of flavinoids rich in cyaniding. From the previous studies, it has been observed that anthocyanins have decreased oxidative stress in certain neurodegenerative diseases such as Alzheimer's and Parkinson's. The ability of Cyanidin 3 Glucoside to cross BBB has provided evidence for its action centrally. *Prunusavium* a member of rosaceae was found to be rich in anthocyanins. Ethanolic extract of fruits of *Prunusavium* are used in present investigation. The extract exhibits neuroportection as is evident from the restortation of antioxidants and its effect on ACHE, Y-maze is used for the evaluation of spatial working and long term memory It was observed that the pretreatment with EEPA has prevented the ischemia induced memory impairment and motor in coordination and restored as that of normal animal. Open field test is used for the evaluation of exploratory behavior which is determined by observing the nose poking and line crossings. It was observed that the animals which were treated with EEPA have significantly prevented impairment in exploratory behavior when compared with untreated ischemic animals.

Glutamate is the principle excitatory neurotransmitter in the brain and an increased glutematergic transmission has been recorded in the pathogenesis of cerebral ischemia, the treatment with EEPA leads to decrease in glutamate levels in the ischemic animals. The decrease in ACHE level, which is a major neurotransmitter for memory and spatial behavior, is due to the increased activity of enzyme ACHE which hydrolysis the available Ach and causes its deficiency. In the present study, the increase in the activity of ACHE has been found in ischemic animals when compared to the normal animals. The animals treated with EEPA have shown a significant decrease in the ACHE activity when compared to untreated ischemic animals.

4. CONCLUSION

Based on the results of this study, it's revealed and concluded neuroprotective effect which is evident from the reduction of ACHE, glutamate levels and restoration of SOD, GPx, catalase levels and also attenuation of motor dysfunction. It was evident that, the ethanolic extract of *Prunusavium* decreased the formation of free radical production in the brain of MCAO induced rats and exerts neuroprotction in ischemic conditions and further studies are required to reveal the effect of principal bioactive compound in molecular aspects of ACHE inhibiton and from the turnover of stress harmone. The neuroprotective effect can be well correlated with the presence of anthocyanin which is the principal component in *Prunusavium***.**

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