



NEUROPROTECTIVE EFFECT OF METHANOLIC EXTRACT OF PASSIFLORA NEPALENSIS AGAINST OXIDATIVE STRESS IN MIDDLE CEREBRAL ARTERY OCCLUSION MODEL OF STROKE IN RATS

ABSTRACT

The present study was designed to evaluate the protective role of alcoholic extract of *Passiflora nepalensis* against oxidative stress in middle cerebral artery occlusion model of stroke in rat. Rats were pretreated for thirty days by methanolic extract of *Passiflora nepalensis* in two dose of 75 and 150 mg/kg BW p.o., the treated rats were subjected to oxidative stress by occlusion of middle cerebral artery. The changes in the biochemical parameters like TBARS, reduced glutathione, superoxide dismutase, and catalase were estimated in the brain homogenate. The methanolic extract of *Passiflora nepalensis* significantly reduced the oxidative stress induced by middle cerebral artery occlusion model of stroke in rat. This is the first study to demonstrate the protective effect of *Passiflora nepalensis* against ischemia reperfusion injury. The decrease in the markers of oxidative stress and that the antioxidant property of *Passiflora nepalensis* can be one of the mechanism involved in the neuronal protection.

Keywords: middle cerebral artery, oxidative stress, *Passiflora nepalensis*, Stroke

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INTRODUCTION

Stroke is the second leading cause of death throughout the world and considered the most common cause of disability in adults.¹ The World Health Organisation (WHO) defines stroke as “rapidly developing signs of focal or global disturbance of cerebral function, lasting longer than 24 h (unless interrupted by death) with no apparent non-vascular cause”. Cerebrovascular diseases include some of the most common devastating disorders such as ischemic stroke, hemorrhagic stroke, cerebrovascular anomalies etc. They causes 2 lacks deaths each year and are major cause of disability. The incidence of cerebrovascular diseases increases with age and number of strokes. Cerebral ischemia is causes by a reduction in blood flow that last longer than several seconds. When the blood

flow is quickly restored, brain tissue can be recovered fully and patients’ symptoms are only transient. So it is called as transient ischemic attack.

The stroke has occurred if the neurological signs and symptoms last for several hours. In more severe instances global hypoxic ischemia causes wide spread brain injury and infarctions in various regions in the brain.^{2,3} Reperfusion injury is a distinct entity from the primary ischemic injury; the oxygen arriving with blood circulation, although necessary for alleviating the ischemic status, may be harmful and worsen the damage. Excessive generation of reactive oxygen species (ROS) is believed to be the main culprit in the causation of reperfusion injury.

At the present, state of knowledge of treatment of ischemic stroke not adequate and the allopathic drugs for treatment of strokes are not sufficient and not much benefited. So, natural products (medicinal plants based products) probably represent an ideal source to develop safe and effective agents for management of stroke and desire scientific proofs. The scientifically validated flavonoids⁴ and Ginkgo biloba⁵, Ginseng⁶ and have reported successfully recovery from ischemia / reperfusion injuries in the brain.

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Table 1. Level of TBARS, GSH, SOD and Catalase in brain homogenate

Groups	Parameters			
	TBARS nmole/g wet wt	GSH $\mu\text{g/g}$ wet wt	SOD I.U/mg protein	CATALASE I.U/mg protein
BL	239.93 \pm 5.6	171.3 \pm 8.0	17.6 \pm 2.8	66.3 \pm 4.6
C	226.6 \pm 1.2	167.7 \pm 5.8	16.2 \pm 0.1	64.4 \pm 2.9
I	653.1 \pm 2.8*	102.8 \pm 7.6*	10.8 \pm 0.7*	45.1 \pm 6.1*
IR	207.9 \pm 3.0*	98.3 \pm 10.3*	12.9 \pm 1.2*	58.4 \pm 0.9*
P1 BL	243.5 \pm 9.1*	416.3 \pm 3.8*	24.5 \pm 0.3*	88.5 \pm 2.1*
P1 IR	219.7 \pm 10.6*	163.1 \pm 4.5*	15.6 \pm 1.0*	60.8 \pm 1.6*
P2 BL	202.4 \pm 6.3*	267.7 \pm 3.7*	11.1 \pm 0.2*	74.3 \pm 1.9*
P2 IR	282.1 \pm 10.1*	186.7 \pm 20.3*	10.2 \pm 0.4*	68.8 \pm 2.8*

All values are expressed as Mean \pm SE (n=10). * $p < 0.001$ vs BL * $p < 0.001$ vs IR (One way ANOVA)

- BL** Vehicle (saline) treated rats
- C** Vehicle-treated rat subjected surgical procedure to 24 hours perfusion
- I** Vehicle-treated rat subjected to 2 hours ischemia
- IR** Vehicle-treated rat subjected to 2 hours ischemia + 24 hours perfusion
- P1 BL** Rats treated with 75mg/kg of methanolic extract of *Passiflora nepalensis*
- P1 IR** 75mg/kg of methanolic extract of *Passiflora nepalensis* treated rat subjected to IR
- P2 BL** Rats treated with 150mg/kg of methanolic extract of *Passiflora nepalensis*
- P2 IR** 150mg/kg of crude *Passiflora nepalensis* treated rat subjected to IR

The genus *Passiflora* consists of 500 species which are mostly found in warm and tropical regions. *Passiflora* comes from Latin word "Passio" that was first time discovered by Spanish discoverers in 1529 and was described as a symbol for "Passion of Christ".^{7,8} *Passiflora* is used widely in traditional medicine in West India, Mexico, Netherland, South America, Italia, and Argentina. One of species of this genus named as *Passifloranepalensis* (Passifloraceae) is more popular than its other species in Eastern India. *P. nepalensis* is used in folklore medicine for treatment of hypertension and inflammation.^{9,10} *Passiflora* contains several compounds including alkaloids, phenols, glycosyl flavonoids and cyanogenic compounds. One of the most important glycosyl flavonoid vitexin has been isolated recently from the methanolic extract of *Passiflora nepalensis* Walp. and it showed antioxidant effect.¹¹ Hence, the present study was designed to investigate the neuroprotective effect of methanolic extract of the *Passiflora nepalensis* in middle cerebral artery occlusion model of stroke in rats.

MATERIAL AND METHODS

Extraction

The whole parts were dried in shade and powdered (no. 60 mesh) and 100 g of the dried powder was soxhlet extracted successively with petroleum ether, chloroform, and methanol. The weight of methanolic extract after drying was calculated as 18 g.

Animals

Albino male Wistar rats procured from the central animal facility at the parent Institute, were group-housed in polypropylene cages with not more than five animals per cage. They were maintained under standard laboratory conditions with a natural dark–light cycle and allowed free access to standard dry rat diet. And tap water *ad libitum*. All experimental procedures described in rats

were reviewed and approved by the Institutional Animal Ethics Committee.

Experimental

Rats were initially divided into eight groups consisting of six rats each, i.e. sham (BL), vehicle-saline ©, vehicle-treated middle cerebral artery occluded group (IR), 75 and 150 mg/kg p.o., methaolic extract treated group (P1BL and P1BL), and 75 and 150 mg/kg p.o., methaolic extract treated middle cerebral artery-occluded groups (P1IR and P1IR). The methanolic extract of *Passiflora nepalensis* were pretreated to rats for thirty days and after 24 h of last dose the rats were subjected to middle cerebral artery occlusion to induce cerebral ischemia. After 2 h of reperfusion, rats were euthanized for estimation of oxidative stress markers TBARS and reduced glutathione, catalase and Superoxide dismutase in whole brain tissue.

Occlusion of middle cerebral artery to induce cerebral ischemia

Rats were anesthetized with chloral hydrate, 400 mg/kg i.p., dissolved in distilled water. The body temperature was maintained around 37 °C throughout the surgical procedure using a heating lamp and the thermo controlled base of the operating table. A midline incision was made and the right common carotid artery, external carotid artery and internal carotid artery were exposed under an operative magnifying glass. A 4.0 monofilament nylon thread with its tip rounded by rapid heating, by bringing it near a flame, was used to occlude the middle cerebral artery. The filament was advanced from the external carotid artery into the lumen of the internal carotid artery until resistance was felt, which ensured the occlusion of the origin of the middle cerebral artery. The nylon filament was allowed to remain in place for 2 h, after which it was gently retracted so as to allow reperfusion of the ischemic region.¹²

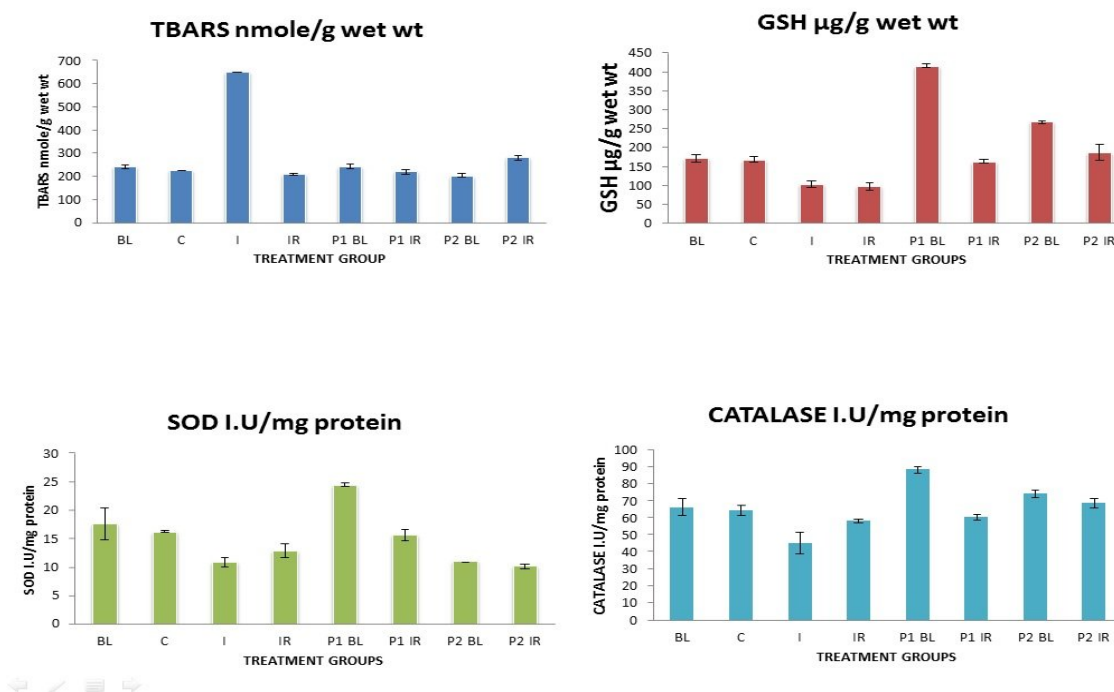


Figure 1. Level of TBARS, GSH, SOD and Catalase in brain homogenate

Biochemical parameters

The following biochemical parameters were estimated in the brain homogenate.

Thiobarbituric acid reactive substances (TBARS)

TBARS levels in the brain were measured by a method of Ohkawa *et al.*¹³. Brain were homogenized in 10% trichloro acetic acid in 4° C. 0.2 ml homogenate was pipetted into a test tube, followed by the adding of 0.2 ml of 8.1% sodium dodecyl sulfate (SDS), 1.5 ml of 20% acetic acid (pH-3.5) and 1.5 ml of 0.8% TBA. Tubes were heated for 60 min at 95 °C and then chilled in ice. Double distilled water (1.0 ml) and n-butanol: pyridine (15:1 v/v) combination (5.0 ml) was added to the test tubes and centrifuge at 4000 x g for 10 min. The absorbance of formed colour in organic layer was measured at 532 nm. Data are expressed as nmole of TBARS/g wet wt.

Reduced glutathione (GSH)

GSH was estimated by the method of Ellman.¹⁴ The reaction mixture contained 0.1 ml of supernatant, 2.0 ml of 0.3 M phosphate buffer (pH-8.4), 0.4 ml of double distilled water and 0.5 ml of DTNB (5,5 dithiobis-2-nitrobenzoic acid). The reaction mixture was incubated for 10 min and the absorbance was measured at 412 nm. Data are expressed as mole/g wet wt.

Superoxide dismutase (SOD)

SOD levels in the brains were determined by the method of McCord & Firdovich modified by Kakkare *et al.*¹⁵ A sample (0.6 ml) was added to sodium pyrophosphate buffer (pH-8.3) followed by the addition of 0.1 ml of 186 M phenazinemethosulphate, 0.3 ml of 300 mM nitrobluetetrazolium and 0.2 ml of 780 M NADH. The reaction mixture was incubated for 90 s at 30 °C and stopped the reaction by adding 1 ml of acetic acid.

n-Butanol (4 ml) was then added and centrifuged at 3000 x g for 10 min. The absorbance of organic layer was measured at 560 nm. Data are expressed as units per mg protein.

Estimation of catalase (CAT)

Catalase was estimated by the method described by Aebi.¹⁶ Sample was added to a 3 ml cuvette that contained 1.95 ml of 50 mM phosphate buffer (pH-7.0). Then 1 ml of 30 mM hydrogen peroxide was added and changes in absorbance were followed for 30 s at 240 nm at an interval of 15 s. Data are expressed as units per mg protein.

Estimation of protein

Protein estimation for the tissue sample of SOD and catalase were done by the method of Bradford.¹⁷ Sample was added up to 20 µL with double distilled water, 50 µL in NaOH and 1 ml of Bradford reagent and kept aside for 10 min after vortexing. The absorbance was measured at 595 nm.

Statistical analysis

All values are expressed as mean±SEM for 6 animals in each group. Data for various biochemical parameters were analyzed using analysis of variance (ANOVA) (GraphPad Version 3.06, La Jolla, CA, USA). Significance is set at p<0.05.

RESULTS

The results of biochemical estimation of TBARS, GSH, SOD and Catalase were presented in table 1 and figure 1

TBARS

There was a significant increase in the level of TBARS (P<0.001) in brain homogenate of group I when compared with group C and BL rats. There was

significant ($P < 0.001$) decrease in the level of TBARS in group P1IR and P2IR in comparison to the group IR.

GSH, SOD and CAT

There was significant ($P < 0.0001$) decrease in the level of GSH, SOD and CAT in brain homogenate of rats in group I when compared with group C and BL rats. There was significant ($P < 0.0001$) increase in the level of GSH, SOD and CAT in group P1IR and P2IR in comparison to the group IR.

DISCUSSION

Stroke causes brain injury in millions of people worldwide each year Read et al.¹⁸ Despite the enormity of the problem, there is no approved therapy that can reduce infarct size or neurological disability. Recently, both animal as well as human studies have provided evidence that the oxidative damage to membrane lipids and proteins is increased during ischemia and reperfusion Mason et al.¹⁹ Exploration of oxidative stress is important

to evolve neuroprotective strategies so as to enhance neuronal survival after cerebral ischemia.

The level of TBARS was significantly attenuated by methanolic extract of *Passiflora nepalensis* treatment. The antioxidant levels were significantly lower in the ischemic group which increased more in the methanolic extract of passifloranepalensis treatment group in both the dose. In summary, *Passiflora nepalensis* treatment of middle cerebral artery-occluded rats caused a decrease in oxidative stress, which was associated with a increased antioxidant property of extract may be the mechanism involved in neuronal protection.

CONCLUSION

This is the first study to demonstrate the protective effect of *Passiflora nepalensis* against cerebral ischemic reperfusion injury. The decrease in the markers of oxidative stress and that the antioxidant property of *Passiflora nepalensis* can be one of the mechanisms involved in the neuronal protection.

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