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Neuron restorative potency of pisonia alba on animal models of cerebral ischemia/reperfusion-induced oxidative stress

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ABSTRACT

In this study, rats' brain ischemia/reperfusion-induced oxidative stress was tested for its ability to protect rats' neurons. By blocking the bilateral carotid arteries for 30 minutes, followed by one hour and four hours of reperfusion, male albino Wistar rats underwent global cerebral ischemia. At different times following reperfusion, the levels of malondialdehyde (MDA), glutathione peroxidase (GPx), glutathione reductase (GR), glutathione-s-transferase (GST), and hydrogen peroxide (H2O2) activity, as well as the amount of brain water, were analysed. MDA and hydrogen peroxide concentrations increased prior to ischemic changes, whereas GPx, GR, and GST activity decreased afterward. After treatment with P alba, the oxidative stress brought on by ischemia was significantly reduced. P alba treatment rapidly reversed and recovered to nearly normal levels in the groups pre-treated with methanolic extract (250 and 500 mg/kg, provided orally in single and double doses/day for 10 days). This effect was dose-dependent. P alba changed the cerebral water content in the rats who underwent ischemia and reperfusion. The histological alterations in the mice with cerebral ischemia further supported the neurodegeneration. The results of this study show that P alba protects neurons by reducing oxidative stress in rats suffering from global cerebral ischemia injury.

Keywords: Brain edema, global cerebral ischemia, histopathology, oxidative stress, *Pisonia alba*

INTRODUCTION

Stroke is the third leading cause of death, behind cancer and myocardial infarction, with cerebrovascular diseases predicted to overtake cancer as the leading killer in 2020. This is the main factor in the permanent impairment and disability-adjusted loss of independent living years in Western countries. According to the Framingham statistics, haemorrhages account for 14% of strokes, atherothrombotic and cardioembolic occlusions account for 87%, and other or unidentified causes account for 3%. [1] Risk factors for stroke include old age, hypertension, previous stroke or transient ischemic attack, diabetes, excessive cholesterol, smoking, and atrial fibrillation. High blood pressure is the most significant modifiable risk factor for stroke. [2] Changes in the antioxidant state of nerve tissue have been connected to both normal ageing and the pathophysiology of neurodegenerative diseases such amyotrophic lateral sclerosis, Parkinson's disease, and Alzheimer's disease. Oxidative stress has been connected to acute traumas like ischemic stroke in addition to chronic neuropathologies. [3-6] Numerous herbal extracts include polyphenolics, which include flavonoids and have been shown to be potent ROS scavengers, antioxidants, and neuroprotectors in vitro. [7]
Members of the Nyctaginaceae family include Pisonia umbellifera, Pisonia alba, and Pisonia alba spanoghe. It can be found on many of the Seychelles Islands that have undergone habitat restoration, and it is an important part of the habitat with a high level of biodiversity and a complex food web. The Seychelles warbler, a unique land bird that was saved from extinction through meticulous habitat management and transfer, was discovered to frequently nest on pisonia trees, demonstrating the importance of considering the entire island ecology. Other natural tree species cannot simply take the place of the Pisonia. The leaves can be eaten. Vegetables are made from the young leaves. In addition to being fed to animals, leaves are commonly utilised as rheumatism and arthritis treatments. The leaves are cooked and eaten for arthritis, they are a carminative and an antidote for snake bites, and they are used as an anti-diabetic in

traditional Indian medicine. The indigenous people also utilise the leaves as cattle feed. However, there is no evidence that it possesses anti-anxiety properties. The purpose of this investigation was to examine the analgesic and anti-inflammatory effects of different fractions of Pisonia alba root extract. (5-10). Present study was undertaken to evaluate the neuroprotective potential of methanolic extract of *P alba* in bilateral common carotid artery (BCA) occlusion induced global cerebral ischemia model in rats.

MATERIALS AND METHODS

Chemicals and Drugs

Glutathione (oxidized and reduced), nicotinamide adenine dinucleotide phosphate reduced (NADPH), 1-chloro-2,4-dinitrobenzene (CDNB), thiobarbituric acid (TBA), ethylenediaminetetraacetic acid (EDTA), and nitroblue tetrazoleum chloride (NBT), were purchased from Sigma Aldrich (St. Louis, MO, USA), SRL, Bombay and other chemicals were AR grade.

Animal

The National Institute of Mental Health and Neuro Science (NIMHANS), Bangalore provided male Wistar albino rats (250–300 g). Rats were kept in air-conditioned rooms in polypropylene cages. Water and regular rat food pellets were freely available.

Plant Material

The areal parts of *Pisonia alba* were collected in the month of May 2015 from …….. A herbarium specimen of the plant was deposited in the Department of Pharmacognosy. The plant was identified by……...,

Plant Extraction

P. alba's fresh stem portion was extracted using petroleum ether, chloroform, and methanol in that order. Chloroform extract and petroleum ether were thrown away. The residue was then extracted with methanol (yield: 8.9 g) over the course of 48 hours in a Soxhlet device. In a rotary vacuum evaporator, the methanol solvent was evaporated under decreasing pressure.

Experimental Protocol for Global Ischemia

The methodology was split into two groups: 1 hour reperfusion models and 4 hour reperfusion models. Each major group was further broken into six groups, each containing six Wistar male rats that had been given methanolic extract or vehicle for 10 days before to the experiment and were treated as follows:

Group I: Normal saline (10 ml/kg, orally), no ischemia. Group II: Normal saline (10 ml/kg, orally), bilateral carotid artery occlusion (BCAO) for 30 min and followed by 1 h and 4 h reperfusion individually (ischemic control). Group III: *P alba* (250 mg/kg, single dose/day, orally), BCAO for 30 min and followed by 1 h and 4 h reperfusion individually. Group IV: *P alba* (250 mg/kg, double dose/day, orally), BCAO for 30 min and followed by 1 h and 4 h reperfusion individually. Group V: *P alba* (500 mg/kg, single dose/day, orally), BCAO for 30 min and followed by 1 h and 4 h reperfusion individually. Group VI: *P alba* (500 mg/kg, double dose/day, orally), BCAO for 30 min and followed by 1 h and 4 h reperfusion individually.

Induction of Global Cerebral Ischemia and Reperfusion (I/R)

On a group of animals, bilateral carotid artery occlusion was carried out. Rats were put to sleep using 40 mg/kg of thiopentone sodium intravenously. A midline ventral incision in the neck was done while the animals were on their backs. The right and left common carotid arteries of the animal were found once the trachea was opened. The vagus nerve fibres were carefully separated from and preserved when both carotid arteries were exposed. By inserting a cotton thread beneath each carotid artery and tying a surgical knot on both arteries, ischemia was created for 30 minutes. After 30 minutes of total cerebral ischemia, the thread was withdrawn to allow for 1 hour and 4 hours of perfusion (blood flow) through the carotid arteries. Using a heated operating table, the rats' body temperatures were kept at 37.5% of normal during the procedure. The only difference between sham control animals and the experimental animals' surgical treatments was that the BCA was not blocked. Once the reperfusion period had passed, the mice were tested for their neuroprotective capacity before being put to death. The purpose of the brain dissection was to evaluate biochemical parameters, determine brain weight, examine the histology, and gauge the size of cerebral infracts.

Preparation of Post-Mitochondrial Supernatant & biological activity

The animals were immediately decapitated and put to death after BCAO. Their brains were taken out, rinsed in 0.9 percent saline that had been refrigerated, and then frozen for five minutes at 20°C. After being blotted on filter paper, the brain was weighed and homogenised in cold sodium phosphate buffer (0.1 M, pH 7.4) using a REMI tissue homogenizer. After centrifugation at 10,000 g for 20 minutes at 4°C, post-mitochondrial supernatant (PMS) was obtained from 10% (w/v) brain tissue and stored at -10°C for upcoming experiments. Malondialdehyde, glutathione peroxidase activity, glutathione reductase activity, glutathione-S-transferase activity, hydrogen peroxide, protein concentration, brain weight, and water contents were among the different biochemical parameters examined, along with histopathological research. [13, 14]

Statistical analysis

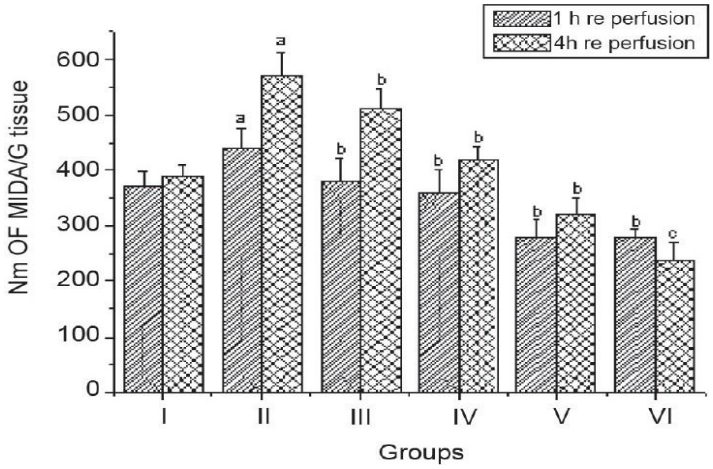
The results were expressed as mean ± S.E.M. Statistical difference between mean were determined by one-way analysis of variance (ANOVA), followed by Dunnett t-test. The diagrammatic representation of the data was performed by using; Microcal™ Origin® Version 6.0 (Origin 6.0 AddOn, Data analysis and Technical graphics) software was used for all statistical calculations. Differences were considered significant at $P<0.05$.

RESULTS

Effect of P alba on Biochemical Analysis

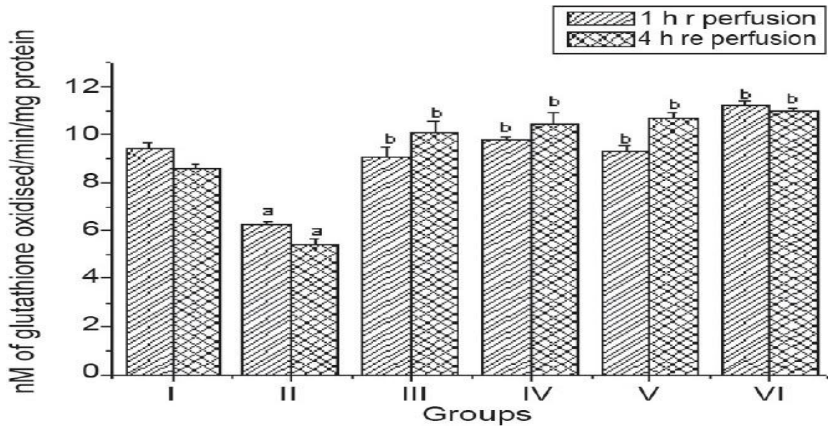
The biochemical results are showed in [Figure 1- 5]. The results showed that the cerebral ischemia and reperfusion

significantly decreased antioxidative activities (GPx, GR, and GST) and increased the level of lipid peroxidation (malondialdehyde content, an index of lipid peroxidation) and hydrogen peroxide (H2 O2) in the injured brain tissue of rats as compared with the sham control group. However, the pretreatment of rats with *P alba* (250, 500 mg/kg, single and double dose/day) was markedly increased GPx, GR, and GST activity. In contrast, MDA and H2 O2 content in the injured brain tissue of rats decreased significantly (P<0.01) in *P alba* extract treated group compared to ischemic control group. However, the accumulation of MDA and H2 O2 content was significantly lower in cerebral ischemia in extract treated animals. Interestingly, the double dose/day treated group (250 and 500 mg/kg) had significantly alters enzyme activities than the single dose/day treated group with methanolic extract of *P alba* in cerebral ischemia-subjected rats.



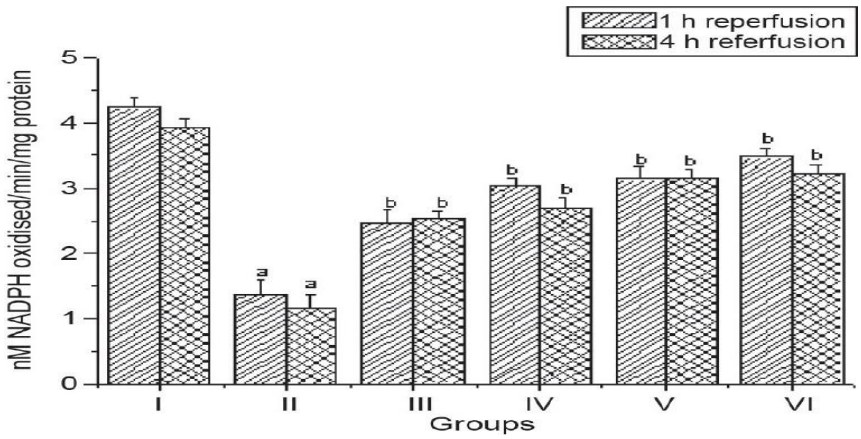
I: Sham control, no occlusion, II: ischemic control (normal saline, 10 ml/ kg, p.o.), III: *P alba* (250 mg/kg, single dose/day, p.o.) + ischemia, IV: *P alba* (250 mg/kg, double dose/day, p.o.) + ischemia, V: *P alba* (500 mg/kg, single dose/day, p.o.) + ischemia, VI: *P alba* (500 mg/kg, double dose/day, p.o.) + ischemia. Values are expressed as mean ± S.E.M., (n = 6). a = *P*<0.01 vs Sham control, b = *P*<0.01 vs ischemic control, by one-way analysis of variance (ANOVA), followed by Dunnett t-test.

Fig 1: Effect of *P alba* on MDA in rats subjected to global cerebral ischemia followed by reperfusion.



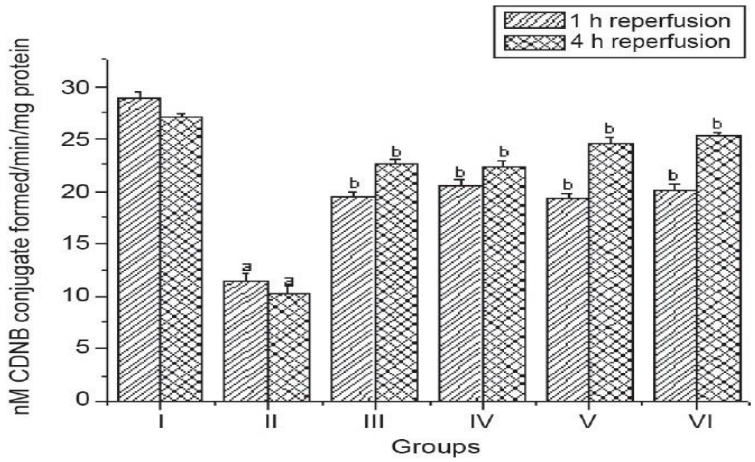
(a = P<0.01 and b = P<0.001 vs Sham control, c = P<0.01 and d = P<0.001 vs ischemic control (see the legends in Fig. 1)

Fig 2: Glutathione peroxidase (GPx)



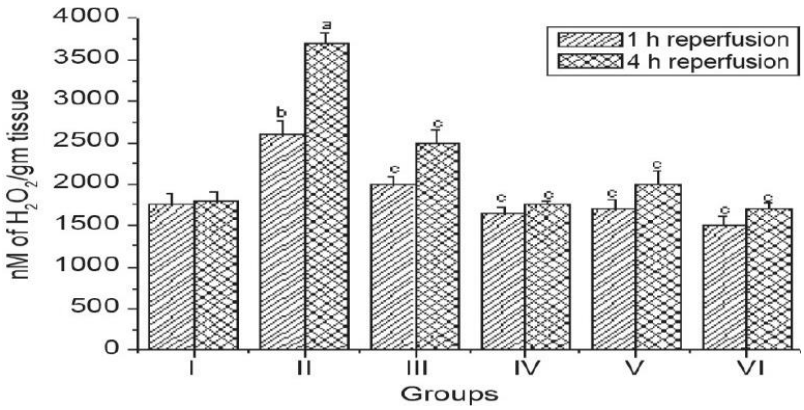
(a = P<0.001 vs Sham control, b = P<0.01 vs ischemic control (see the legends in Fig. 1)

Fig 3: Glutathione reductase (GR)



(a = P<0.001 vs Sham control, b = P<0.01 vs ischemic control (see the legends in Fig. 1)

Fig 4: Glutathione-S-transferase (GST)

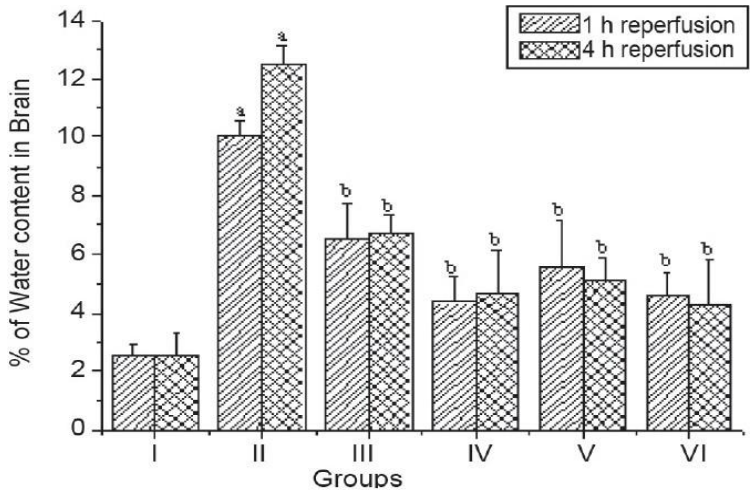


(a = *P*<0.001 vs Sham control, b = *P*<0.01 vs Sham control, c = *P*<0.01 vs ischemic control (see the legends in Fig. 1)

Fig 5: Hydrogen peroxide (H2O2)

Effect of P alba on Brain Weight and Water Content

In the ischemic/reperfusion control group, the amount of cerebral water (edoema) was considerably higher. Pretreatment with *P alba* (250, 500 mg/kg) resulted in a substantial (P<0.01) drop in water content, with a more than two-fold decrease in treated rats compared to the ischemia control group. However, in the treated groups, there was a considerable reduction in brain weight. [Figure 6].

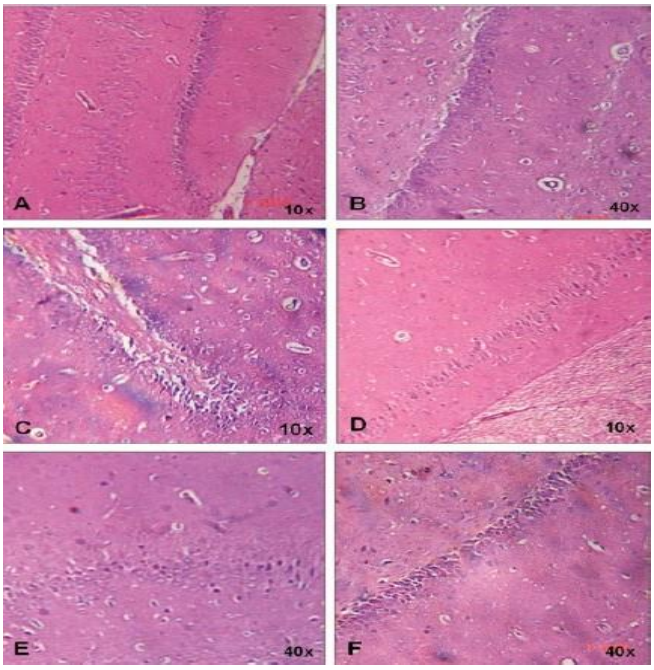


a = *P*<0.001 vs Sham control, b = *P*<0.01 vs ischemic control (see the legends in Fig 1).

Fig 6: Effect of methanolic extract of *P alba* on water content in brain in rats subjected to global cerebral ischemia followed by reperfusion.

Effect of *P alba* on Histopathology

The histopathological study, it was observed that section of brain tissue showing swollen neurons, dilated blood vessels with neuronal loss occurred in brain regions of I/R rats induced by BCAO for 30 min followed by 1 h and 4 h reperfusion in ischemic control group [Figure 7]b and [Figure 8]b. While no apparent morphological changes in sham control and brain section showing normal structure [Figure 7]a and [Figure 8]a. *P alba* (250 and 500 mg/kg) treated group of 1 h reperfusion brain section showed significantly prevented the neuron loss by compared with ischemic control group and in the other hand no significant difference between the doses of 250 mg/kg of *P alba* on 4 h reperfusion ischemic treated groups.



(A) Sham control, no ischemia (normal saline, 10 ml/kg, p.o.); (B) ischemic control (normal saline, 10 ml/kg, p.o.); (C) *P alba* (250 mg/kg, single dose/day, p.o.) + ischemia; (D) *P alba* (250 mg/kg, double dose/day, p.o.) + ischemia; (E) *P alba* (500 mg/kg, single doses/day, p.o.) + ischemia; (F) *P alba* (500 mg/kg, double doses/day, p.o.) + ischemia.

Fig 7: Histopathological photographs of coronal sections of brain after 30 min of occlusion and 1 h of reperfusion in bilateral common carotid arteries occluded rats.

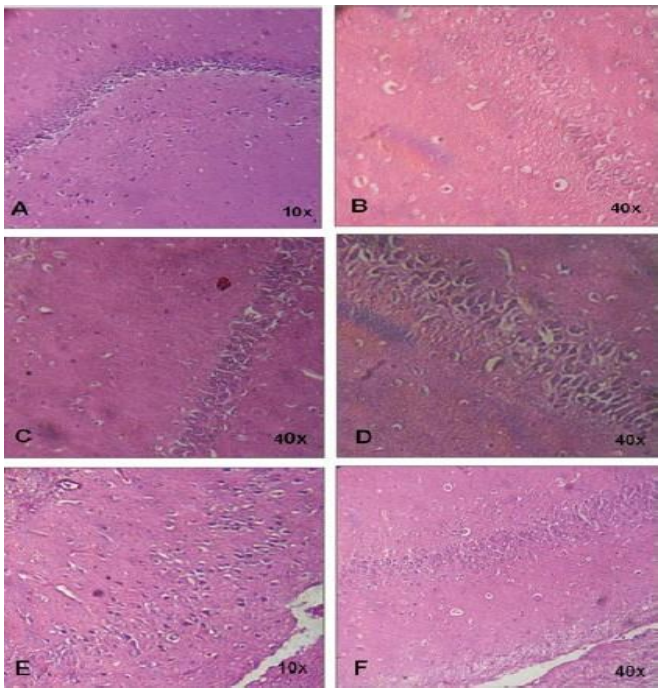


Fig 8: Histopathological photographs of coronal sections of brain after 30 min of occlusion and 4 h of reperfusion in bilateral common carotid arteries occluded rats (see the legends in Fig. 7).

DISCUSSION

In the current study, a stroke in rats was studied using a global ischemia model, and P alba was found to have therapeutic potential. We employed two different reperfusion (1 h and 4 h reperfusion) models because the degree of damage caused can vary substantially. Additionally, we wanted to evaluate the degree of brain damage brought on by both reperfusion models and the potential protective value of pretreatment with *P alba* methanolic extract.

It was found that P alba reduced the ischemia rats' decreased neurological deficit and impaired sensory and motor skills. The altered antioxidant enzymes appear to be restored by P alba activity, and the formation of MDA in the brain areas affected by BCA blockage is also reduced. The significance of ROS in the pathophysiology of I/R-induced oxidative stress in the brain was strongly supported by the available information. [15-18] Lipids are therefore the macromolecules that are most vulnerable to oxidative stress. According to the findings of our study, MDA generation in the rats' ischemic brain areas was dramatically increased after 1 and 4 hours of reperfusion. The findings showed that pretreatment with P alba significantly lowered the MDA level and prevented neuronal damage from spreading through a chain reaction of lipid peroxidation. Antioxidant therapy should keep GPx activity from declining by efficiently scavenging the surplus ROS. Both at high and low concentrations, GPx are involved in the detoxification of H 2 O 2. In this investigation, treatment of P alba substantially stopped the GPx activity from declining. [19,20]

An essential enzyme for preserving the levels of reduced glutathione inside of cells, glutathione reductase also eliminates free radicals. [21] Lack of glutathione reductase may both contribute to and reflect oxidative stress. The level of brain glutathione reductase was significantly reduced after ischemic injury. [22] In our study, it was also significantly lower in the I/R control group compared to the bogus control group. An increase in lipid peroxidation in the brain was associated with this deficiency. Glutathione system malfunction has been linked to a number of neurodegenerative diseases. [23] Thus, further evidence supports our decision to check the glutathione levels in the current investigation. The detoxification of oxidised catecholamine metabolites (o-quinone) is catalysed by glutathione-S-transferase, which may also act as an antioxidant to stop the onset of degenerative processes. [24] Hydrogen peroxide may therefore continue to exist longer after reperfusion and cause neuronal damage. In the groups that had previously had P alba treatment, the levels of H 2 O2 in the I/R rats' brains were noticeably reversed and returned to levels that were nearly normal. Because of increases in endogenous antioxidant defence enzymes, hydrogen peroxide may not cause neuronal damage. [25]

Peri-infarct depolarization happens as extracellular glutamate concentrations rise. Cells then swell as water moves, leading to cerebral edoema. In comparison to the ischemic control group, P alba also reversed effects on brain water content in the ischemia reperfusion mice. These findings suggest that it may have therapeutic benefit for treating stroke and other cerebrovascular illnesses. The histological variations between the treatment and ischemia control groups further support the neurodegenerative outcomes that have been reported. When the extract was taken twice daily, the brain damage was reversed and a 40% reduction in the death of neurons was achieved. It is hypothesised that P alba pretreatment could have a neuroprotective impact on the I/R-exposed brain. [26]

CONCLUSION

Methanolic extract of *P alba* could reduce neuronal loss of the ischemic brain tissue. *P alba* showed antioxidant activity in reperfusion induced oxidative stress.

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