Int. J. Morphol.,
42(3):876-890, 2024.

Neurotrophic Factor Mediated Neurorestorative Effect of *Pongamia pinnata* Against Transient Cerebral Hypoperfusion and Reperfusion in Rats: Preliminary Histological and Molecular Level Evidence

Efecto Neurorestaurador Mediado por Factor Neurotrófico de *Pongamia pinnata* Contra la Hipoperfusión y Reperfusión Cerebral Transitoria en Ratas: Evidencia Preliminar de un Estudio a Nivel Histológico y Molecular

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SUGANITHA, B.; JAGADEESAN, V.; SIVANESAN, S.; PRATHAP, L.; MUNDUGARU, R.; ALZERWI, N.; SARASWATHI, I. & SAIKARTHIK, J. Neurotrophic factor mediated neurorestorative effect of *Pongamia pinnata* against transient cerebral hypoperfusion and reperfusion in rats: A preliminary histological and molecular level evidence. *Int. J. Morphol., 42(3)*:876-890, 2024.

SUMMARY: Stroke is the leading cause of acquired physical disability in adults and second leading cause of mortality throughout the world. Treatment strategies to curb the effects of stroke would be of great benefit. *Pongamia pinnata* is a recent attraction in medicine, owing to its abundant medicinal benefits with minimal side effects. The present study aimed to examine acute and subacute effect of *Pongamia pinnata* leaf extract on transient cerebral hypoperfusion and reperfusion (tCHR) in Wistar rats. 24 adult Wistar rats (12 each for acute and subacute study) were divided in to four groups each viz normal control group, tCHR + NS group, tCHR + 200mg/kg bw and tCHR + 400mg/kg bw groups. Cerebral ischemia induction was carried out by bilateral common carotid artery occlusion and reperfusion. Ethanolic extract of *Pongamia pinnata* leaves were orally administered for 7 days and 21 days after the surgical procedure for acute and subacute study respectively. Behavioural analysis, histological assessment, and estimation of mRNA levels of HIF-1, GDNF, BDNF and NF-kB were performed. In both acute and subacute study, there was significant improvement in the beam walking assay, neuronal count, decreased neuronal damage in histological sections and higher mRNA expression of BDNF and GDNF in the treatment groups. There was no significant difference in the expression of HIF1 and NF-kB. Thus, *Pongamia pinnata* has excellent neurorestorative property reversing many of the effects of ischemic stroke induced by tCHR in rats with the underlying mechanism being an improvement in the expression of neurotrophic factors GDNF and BDNF.

KEY WORDS: Stroke; *Pongamia pinnata*; Cerebral cortex; Neurorestorative.

INTRODUCTION

Cardiovascular and neurodegenerative diseases are considered to be the leading cause of global health concern. Stroke has become one of the most prevalent and swiftly progressing neurological disease cause debilitation and death (GBD 2016 Neurology Collaborators, 2019). Factors that contribute to stroke includes high blood pressure, diabetes, cardiovascular disorders, inflammatory conditions (e.g.: viral infections), high cholesterol levels, age and gender factors, ethnicity, and genetics. Other contributory factors include, depression, anxiety, unhealthy lifestyle habits, pollution, medicines that cause clotting in an increased rate and various other factors (Boehme *et al*., 2017). The most prevalent cause of brain ischemia, responsible for approximately 60 % to 70 % of transient ischemic attacks (TIAs) and stroke cases, is the embolization of a clot that originated in the heart or a major artery (DeSai & Hays Shapshak, 2023). Cerebral

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Received: 2024-01-03 Accepted: 2024-02-23

ischemia could also be head trauma related. Trauma that caused the brain injury could cause disruption to cerebral blood flow though, in traumatic brain injury (TBI) patients, the risk of haemorrhagic stroke is more than that of ischemic stroke (Qu *et al*., 2022). Histological analysis indicates ischemic damage in nearly 60 % of fatal traumatic brain injuries, even in the absence of large vessel occlusions (Graham & Adams, 1971).

Cerebral ischemia and head trauma results in acute brain injury which stands as a prominent cause of mortality in industrialized nations and contribute to long-term motor and cognitive impairments among survivors (Roger *et al*., 2011). Despite significant progress in comprehending the mechanisms underlying the onset of brain injury, progression of damage, and the involvement of inflammatory processes, universally effective treatments still remain elusive (Iadecola & Anrather, 2011). Research has been targeted to explore a cure to such diseases that are generally life threatening. Studies and reports observed that there is an emerging need for herbal based treatment options for better survival and recovery. Hence considerable focus has been currently shifting towards herbs and herbal by-products that can be used as medicines (Manigauha *et al*., 2009).

Pongamia pinnata is more commonly referred to as Indian beech tree, pongam oil tree and karanja. *Pongamia pinnata* has numerous pharmacological properties like antioxidant, anti-lipid oxidative, anti-inflammatory, anti-diarrhoeal, anti-hyperglycaemic, anti-nociceptive and various other properties. Significant phytochemical component include Furano flavonoids and chalcones which exert a neuroprotective activity in various neurodegenerative diseases (Manigauha *et al*., 2009; Yadav *et al*., 2011). Medical benefits of *Pongamia pinnata* include treatment of skin diseases, piles, and wounds (Al Muqarrabun *et al*., 2013). Significant phytochemical component identified from the plant includes Furano flavonoids and chalcones which exhibits antioxidant property and exerts neuroprotective effect in various neurodegenerative diseases (Manigauha *et al*., 2009; Yadav *et al*., 2011). Studies have been conducted using the various parts of the *Pongamia pinnata* plant material highlighting its medicinal properties including its protective effects against cisplatin and gentamycin induced nephrotoxicity, Alzheimer's disease, and anticonvulsant property (Shirwaikar *et al*., 2003; Manigauha *et al*., 2009; Al Muqarrabun *et al*., 2013; Saini *et al*., 2017). Raghavendra *et al*. (2007) had demonstrated the anti-oxidative stress and cognition enhancing effect of *Pongamia pinnata* root extract against acute cerebral ischemia and reperfusion. However, there is no available research on the neurorestorative potential of *Pongamia pinnata* against stroke induced by transient cerebral ischemia.

Thus, the current study focuses on the neurorestorative potential of *Pongamia pinnata* against transient cerebral hypoperfusion and reperfusion in rat model. Behavioural analysis to assess functional improvement, comparison between Cresyl violet and haematoxylin eosin staining and neuronal count for increased clarity in the neuronal structure in normal, pathological, and treated brain sections, molecular analysis of the transcriptional factors and neurotrophic factors (GDNF, BDNF, NF-kB and HIF-1) were employed to assess the neurorestorative potential of ethanolic extract of *Pongamia pinnata* leaves. Thus, the current study aims to evaluate the neurorestorative potential of *Pongamia pinnata* on stroke induced rat models, on a behavioural, histopathological, and molecular level.

MATERIAL AND METHOD

Plant extract preparation: The plant extract was derived from mature green leaves of P. pinnata. It was collected from Bangalore Medical College & Research Institute Campus. The plant was identified and authenticated at the Herbarium of the Vishveswarapura College of Science. Voucher number of the specimen was B-00010. The P. pinnata leaves were collected and dried under the shade at room temperature. The dried leaves were powdered and used for Soxhlet extraction using 70 % ethanol to obtain ethanolic extract of *Pongamia pinnata* leaves.

Animals: Male Wistar rats, with age range between 10 and 12 weeks and weighing 280-320 grams were used in the study. Rats were fed with a commercial rat pellet diet provided by MASS BIOTECH, CHENNAI, and were provided with food and water *ad libitum*. The animals were maintained in a natural light and dark cycle environment under 25 – 20 ºC with humidity of 50 to 55 %.

Ethical statement: The experiment was conducted following the guidelines by the Institutional Animal Ethical Committee (IAEC) approval of Saveetha Dental College, Chennai, India (BRULAC/SDCH/SIMATS/IAEC/08-2022/138).

Study Design

Bilateral common carotid artery occlusion: Transient cerebral hypoperfusion followed by reperfusion was done by performing Bilateral Common Carotid Artery Occlusion (BCCAO) (Speetzen *et al*., 2013; Mundugaru *et al*., 2018). Rats were anaesthetized with injection of 0.3 of ketamine & xylazine, following which their crown of head was shaved and cleaned with 70 % alcohol, and continuously monitored for spontaneous breathing. The skin incision was done on the ventral aspect of neck and both the carotid arteries were

visualized in the surgical field. Both the common carotid arteries were ligated simultaneously and was left for 60 minutes and then allowed for reperfusion (Iwasaki *et al*., 1989). Continuous monitoring was done to ensure the BCCAO. Under the aseptic condition the incision was sutured with 2.0 silk sutures. The animals were placed in a dry cage separately with the assistance of heat pad and the body temperature was maintained at 37 ºC throughout the period of ischemia and recovery from anaesthesia. All possible efforts were taken to minimalize the suffering of the animals. Temperature was monitored periodically. The neurological examination was performed for each rat 24 h after the surgical procedure according to Bederson *et al*. (1986).

Post-surgery, the rats were orally administered with the plant extract, at the dosage of 200 mg/kg body weight and 400 mg/kg body weight for groups 3 and 4 respectively and normal saline for groups 1 and 2 every day till the day they were euthanized (Table I).

One set of rats (3 in each group) were sacrificed 7 days after the beginning of the study to study the acute effect of the plant extract on induced stroke and another set on 21 days after the beginning for subacute effect. Behavioural analysis (beam walking assay) was done a day before sacrificing of the animals. After euthanasia histological assessment and mRNA expression analysis of candidate genes using Real time- Polymerase chain reaction (RT-PCR) was performed (Fig. 1).

Experimental Groups

The animals were randomly divided in to 4 groups with 3 animals each (Table I).

Group 1 (normal control): The rats in this group were not subjected to any surgical procedure and administered normal saline (control + NS).

Group 2 (negative control): The rats were subjected to bilateral carotid artery occlusion followed by reperfusion, to cause transient global cerebral ischemia and observe the changes that takes place during an ischemic stroke sequence and are orally administered with normal saline (BCCAO/R + NS).

Table I. Distribution of Groups.

Groups	Details	No. of Animals	
		Acute	Subacute
G1	Positive Control	3	3
G2	Negative Control – Stroke Induced Model	3	3
G3	Experimental Study 1 (Dosage 1 – 200 mg/kg)	3	3
G4	Experimental Study 2 (Dosage 2 – 400 mg/kg)	3	3
	Total Number of Animals	24	

Group 3: The rats were subjected to the BCCAO/R, and then orally administered with ethanolic extract of *Pongamia pinnata* leaves at a dosage of 200 mg/kg body weight (BCCAO/R + 200 mg/kg bw) daily till the day of sacrifice.

Group 4: The rats were subjected to the BCCAO/R, and then orally administered with ethanolic extract of *Pongamia pinnata* leaves at a dosage of 400mg/kg body weight (BCCAO/R + 400mg/kg bw) daily till the day of sacrifice.

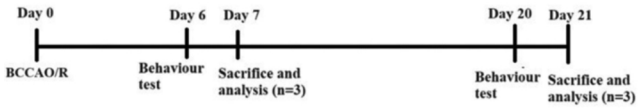

Fig. 1. The study involved two phases- Acute study and Subacute study. 3 animals in each group were sacrificed at the end of 7 days for acute treatment. Another set of 3 animals were sacrificed at the end of 21 days for subacute study.

Behavioural study: Beam walking assay was performed in all animals following specified guidelines to study their balance and locomotion. The task involved the training of rats to traverse a narrow wooden beam measuring 4 cm in width and 105 cm in length. This beam was elevated 80 cm above the ground by wooden supports fixed at both ends. A ramp was present at the "starting" end, while a platform, along with the home cage of the tested rat, was positioned at the opposite end. To cushion potential falls, 10-cm thick sawdust litters were placed beneath the beam. The starting area, designated by a 20-cm section, featured a drawn horizontal line 20 cm away from the starting zone. During the test, a rat was positioned at the starting zone, facing its home cage, and a stopwatch was initiated as the animal was released. The time taken to initiate the beam crossing and the overall duration of the beam crossing were measured. 2 minutes was set as the maximum time allowed to complete the task. All rats were pretrained a day before the test (Zhang *et al*., 2015).

Histopathology of brain: On the designated day the rats were sacrificed using CO_2 chamber (Fig. 1) (Yousefzadeh *et al*., 2021). After careful dissection, the brains were fixed in 10 % formalin and processed for paraffin embedding. The motor cortex of the rats was used for the histopathological study. Each sample of brain was cut in to 10 coronal sections of thickness 5 µm using rotary microtome. The right side of the brain sections were used for histopathological assessment and the left side for target gene estimation. The paraffin embedded brain sections (5 sections each) were stained with Hematoxylin and Eosin and 0.5 % Cresyl violet following standard protocol (Feldman & Wolfe, 2014).

The H&E and Cresyl violet stained sections of cerebral cortex area were focused under 40x and 10x objective lens and the neurons were observed with a microscope (Olympus CX31 light microscope). Photomicrographs of the Cresyl violet stained sections of cerebral cortex were scrutinized, and cell counting was carried out with ImageJ software 1.45. The number of viable cells were counted within a 1mm2 area. Cells which were well rounded and with absence of pyknosis were considered as live cells (Jayakumar *et al*., 2017). The average of the cells counted from each of the five sections were used as the mean value (Sarshoori *et al*., 2014).

Real time PCR analysis. Traditionally RT-PCR involves two steps: the RT reaction and PCR amplification. CFX96 Touch Real Time PCR Detection system was used for this analysis. The following primers in Table II were used for the gene expression.

Primer Designing was the initial step which was done by synthesizing the primers of the target gene. The next step was that of RNA extraction. The brain sections were used to extract the total RNA present in the given model, and this was carried out using the standard protocol with Trizol, which was then dissolved using DEPC-treated deionized water and quantified with a spectrophotometer. Enrichment was done for the RNAs with polyadenylated tails. The next step was quantitative Real time PCR wherein the targets were amplified and then set for quantification of gene expression was performed to obtain the fold changes of the targets. Cycle threshold (Ct) values were obtained for each sample and, the result analysis was performed to depict the comparative levels of mRNA expression in treated groups and effect of *Pongamia pinnata* in animals (Wong & Medrano, 2005; Mo *et al*., 2012; Hua *et al*., 2018).

Statistical Analysis: Data are presented as mean ± standard error of mean (SEM). Comparison between groups were done using one way ANOVA followed Tukey's post-hoc test. P value less than 0.01 was considered to be statistically significant. All the statistical analysis were performed using the software GraphPad Prism 8.4.3 (GraphPad Software, Inc USA).

RESULTS

Pongamia pinnata improves motor function in transient cerebral hypoperfusion and reperfusion in rats

One-way ANOVA showed significant difference between the studied groups in the time taken to traverse the beam in both acute ($F=211.5$, $P<0.0001$) and subacute study ($F=208.2$, $P<0.0001$). In the beam walking assay, the ischemia induced rats without treatment took significantly longer duration to traverse the beam in both acute and subacute study (11.67±0.33 and 10.9±0.03 seconds respectively). Treatment with the plant extract reduced the time duration significantly in both the acute and subacute studies with no significant differences between the normal control group (group 1) and both the treatment groups in the subacute study ($P=0.0845$ and 0.2907) (Figs. 2A,B) (Supplementary Tables S1 & S2).

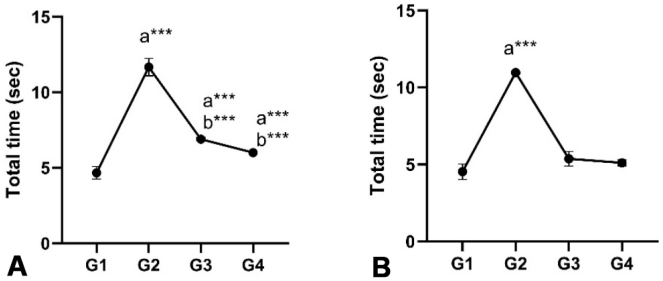

Fig. 2. Beam walking test for acute (A) and sub-acute (B) study- The time taken by each group to complete the beam walking test is plotted in X axis (sec). a - Significant when compared to control group (G1), b - significant when compared to negative control group (G2), ab - significant when compared to both G1 and G2 group. *** - indicates $P< 0.001$.

***Pongamia pinnata* reduces neuronal damage in the ischemic cortex of transient cerebral hypoperfusion and reperfusion in rats** There was significant difference in neuronal count between the groups in acute ($F=692.7$, $P<0.0001$) and subacute study ($F=539.9$, $P<0.0001$). The neuronal count was significantly lower in the tCHR rats when compared to the normal control rats in both the studies ($P<0.0001$) (Supplementary table S2). The neuronal count was significantly higher in both the treatment groups when compared to NS treated tCHR rats with $P<0.0001$ (Figs. 3A,B) (Supplementary Tables S3 & S4).

Table II. Forward and reverse primers.

Markers	Forward Primers	Reverse Primers
BDNF	AATAATGTCTGACCCCAGTGCC	CTGAGGGAACCCGGTCTCAT
GDNF	GCGCTGACCAGTGACTCCAA	GCGACCTTTCCCTCTGGAAT
NF-KB	F:5'GGTGGAGTTTGGGAAGGATTTG 3'	R:5'TTTTCTCCGAAGCTGAACAAACAC
H1F-1α	5'-GGTGCTAACAGATGATGGTGAC-3'	5'-GGCTCATAACCCATCAACT CAG-3'
B Actin	ACAACCTTCTTGCAGCTCCTC	CTGACCCATACCCACCATCAC

Table S1: Beam walking assay in acute study.

Tukey's multiple comparisons test	Mean 1	SEM1	Mean 2	SEM2	Mean Diff.	SE of diff.	95.00 % CI of diff.	Adjusted P Value
G1(Sham+NS) vs. G2 (BCCAO/R+NS)	4.667	0.2404	11.67	0.3333	-7.000	0.2963	-7.949 to -6.051	<0.0001
G1(Sham+NS) vs. G3(BCCAO/R +200 mg)	4.667	0.2404	6.900	0.05774	-2.233	0.2963	-3.182 to -1.285	0.0003
G1(Sham+NS) vs. G4(BCCAO/R+400 mg)	4.667	0.2404	6.000	0.05774	-1.333	0.2963	-2.282 to -0.3846	0.0086
G2 (BCCAO/R+NS) vs. G3(BCCAO/R+ 200 mg)	11.67	0.3333	6.900	0.05774	4.767	0.2963	3.818 to 5.715	<0.0001
G2 (BCCAO/R+NS) vs. G4(BCCAO/R+400 mg)	11.67	0.3333	6.000	0.05774	5.667	0.2963	4.718 to 6.615	<0.0001
G3(BCCAO/R +200 mg) vs. G4(BCCAO/R+400 mg)	6.900	0.05774	6.000	0.05774	0.9000	0.2963	-0.04877 to 1.849	0.0631

Table S2: Beam walking assay in subacute study.

Tukey's multiple comparisons test	Mean 1	SEM1	Mean 2	SEM2	Mean Diff.	SE of diff.	95.00% CI of diff.	Adjusted P Value
G1(Sham+NS) vs. G2 (BCCAO/R+NS)	4.533	0.2906	10.97	0.03333	-6.433	0.2944	-7.376 to -5.491	<0.0001
G1(Sham+NS) vs. G3(BCCAO/R +200 mg)	4.533	0.2906	5.367	0.2728	-0.8333	0.2944	-1.776 to 0.1094	0.0845
G1(Sham+NS) vs. G4(BCCAO/R+400 mg)	4.533	0.2906	5.100	0.1155	-0.5667	0.2944	-1.509 to 0.3761	0.2907
G2 (BCCAO/R+NS) vs. G3(BCCAO/R+ 200 mg)	10.97	0.03333	5.367	0.2728	5.600	0.2944	4.657 to 6.543	<0.0001
G2 (BCCAO/R+NS) vs. G4(BCCAO/R+400 mg)	10.97	0.03333	5.100	0.1155	5.867	0.2944	4.924 to 6.809	<0.0001
G3(BCCAO/R +200 mg) vs. G4(BCCAO/R+400 mg)	5.367	0.2728	5.100	0.1155	0.2667	0.2944	-0.6761 to 1.209	0.8025

Table S3: Neuronal count in acute study.

Tukey's multiple comparisons test	Mean 1	SEM1	Mean 2	SEM2	Mean Diff.	SE of diff.	95.00% CI of diff.	Adjusted P Value
G1(Sham+NS) vs. G2 (BCCAO/R+NS)	93.00	0.9504	11.43	0.3283	81.57	1.803	75.79 to 87.34	<0.0001
G1(Sham+NS) vs. G3(BCCAO/R +200 mg)	93.00	0.9504	55.67	1.506	37.33	1.803	31.56 to 43.11	<0.0001
G1(Sham+NS) vs. G4(BCCAO/R+400 mg)	93.00	0.9504	60.87	1.795	32.13	1.803	26.36 to 37.91	<0.0001
G2 (BCCAO/R+NS) vs. G3(BCCAO/R+ 200 mg)	11.43	0.3283	55.67	1.506	-44.23	1.803	-50.01 to -38.46	<0.0001
G2 (BCCAO/R+NS) vs. G4(BCCAO/R+400 mg)	11.43	0.3283	60.87	1.795	-49.43	1.803	-55.21 to -43.66	<0.0001
G3(BCCAO/R +200 mg) vs. G4(BCCAO/R+400 mg)	55.67	1.506	60.87	1.795	-5.200	1.803	-10.97 to 0.5731	0.0783

Table S4: Neuronal count in subacute study.

Tukey's multiple comparisons test	Mean 1	SEM1	Mean 2	SEM2	Mean Diff.	SE of diff.	95.00% CI of diff.	Adjusted P Value
G1(Sham+NS) vs. G2 (BCCAO/R+NS)	93.44	0.5722	17.67	0.3383	75.77	2.042	69.23 to 82.31	<0.0001
G1(Sham+NS) vs. G3(BCCAO/R +200 mg)	93.44	0.5722	62.30	2.701	31.14	2.042	24.60 to 37.67	<0.0001
G1(Sham+NS) vs. G4(BCCAO/R+400 mg)	93.44	0.5722	82.97	0.7755	10.47	2.042	3.929 to 17.00	0.0040
G2 (BCCAO/R+NS) vs. G3(BCCAO/R+ 200 mg)	17.67	0.3383	62.30	2.701	-44.63	2.042	-51.17 to -38.10	<0.0001
G2 (BCCAO/R+NS) vs. G4(BCCAO/R+400 mg)	17.67	0.3383	82.97	0.7755	-65.30	2.042	-71.84 to -58.77	<0.0001
G3(BCCAO/R +200 mg) vs. G4(BCCAO/R+400 mg)	62.30	2.701	82.97	0.7755	-20.67	2.042	-27.21 to -14.13	<0.0001

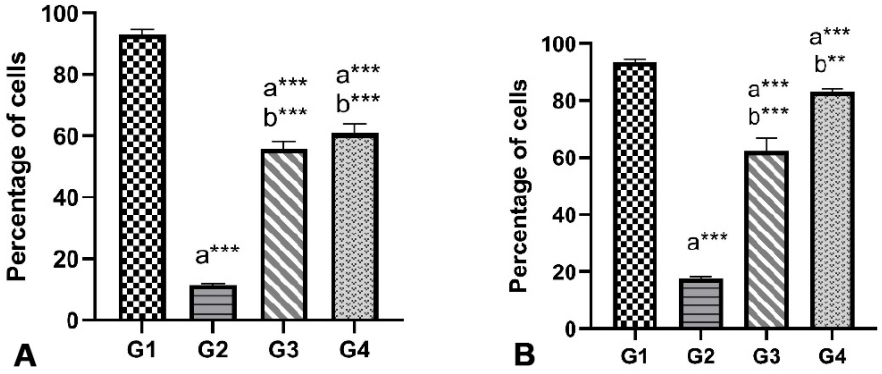

Fig. 3. Neuronal Cell counting using Crysl Violet staining for acute (A) subacute (B) study.The number of neurons with normal cell morphology is plotted in X axis. a - significant when compared to control group (G1), b - significant when compared to negative control group (G2). *** - indicates P< 0.001.

Pongamia pinnata alleviates histopathological alterations in the ischemic cortex of transient cerebral hypoperfusion and reperfusion in rats

The photomicrographs of sections stained with Cresyl violet, showed characteristic changes in the neuronal cell bodies (Figs. 4 and 5). In both acute and subacute study, G1 (normal control rats) showed viable cells. Normal neuronal cells were seen as well-rounded cells with complete nucleus and absence of pyknosis. Whereas G2 (BCCAO/R + NS) exhibited a wide area of degeneration with intercellular oedema. The cellular structure was not visible which simulates the state of the neuronal tissue after an ischemic stroke. Interestingly, G3 (BCCAO/R + 200 mg/kg bw), showed intercellular oedema with congested blood vessels. Few areas of mild degeneration were visible. In G4 (BCCAO/R + 400 mg/kg bw), the amount of intercellular oedema and cellular degeneration was comparatively decreased than G3, implying the beneficial effects of the ethanolic extract of *Pongamia pinnata* leaves.

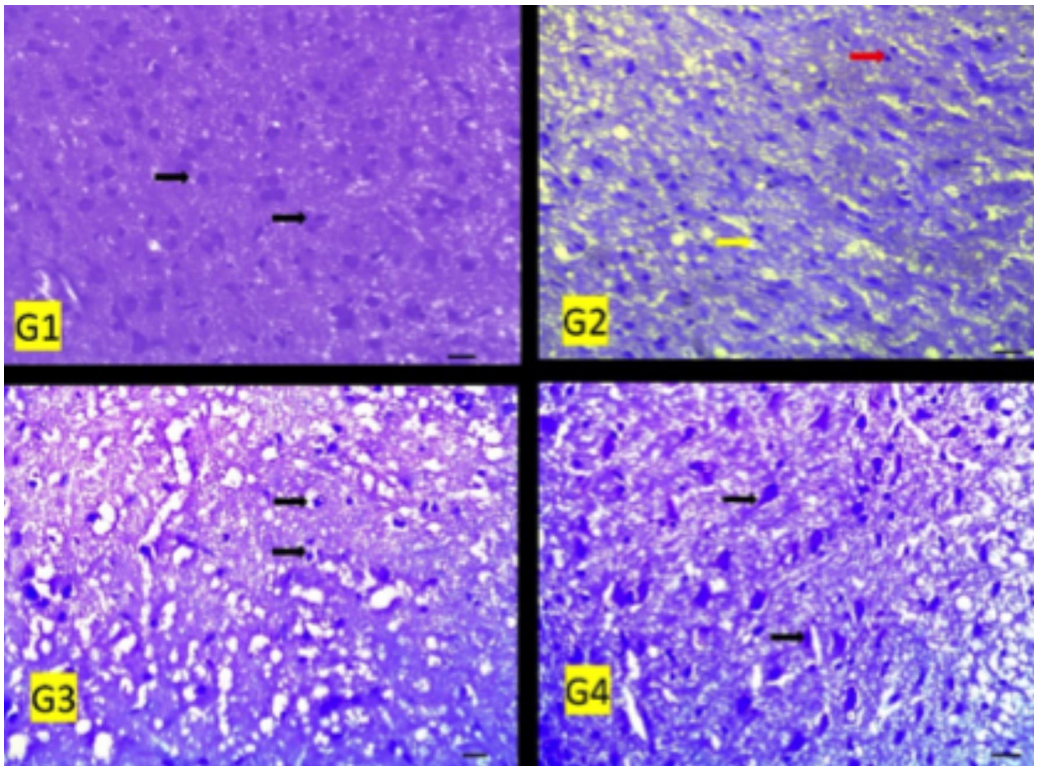

Fig. 4. Representative photographs of Cresyl violet stain in rat cerebrum-Acute study (400x).

G1- Control group, G2- Negative control group, G3- Treated with 200 mg/kg for 7 days, G4- Treated with 400 mg/kg for 7 days. Black arrow shows neurons with normal morphology. Red arrow indicates eosinophilic neurons with pyknotic nuclei. Yellow arrow indicates the increase peri-cellular space and edema. (Scale 20 µm).

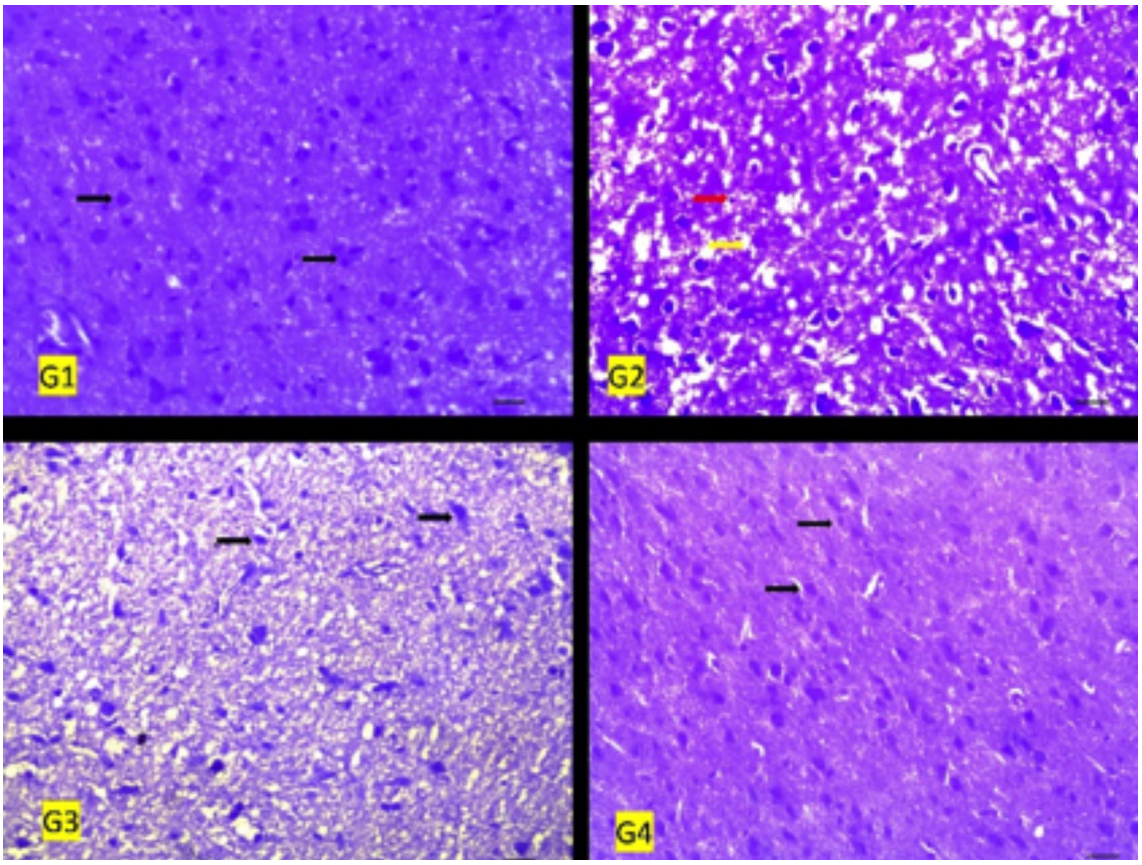

Fig. 5. Representative photographs of Cresyl violet stain in rat cerebrum-Subacute study (400x). G1- Control group, G2- Negative control group, G3- Treated with 200 mg/kg for 7 days, G4- Treated with 400mg/ kg for 7 days. Black arrow shows neurons with normal morphology. Red arrow indicates eosinophilic neurons with pyknotic nuclei. Yellow arrow indicates the increase peri-cellular space and edema. (Scale 20 μm).

Photomicrographs of sections stained with H&E showed relatively similar results as those stained with Cresyl violet (Figs. 6 and 7). G1 showed normal histological structure of the rat brain. Well-rounded cells with absence of pyknosis were noted. In G2, the photomicrographs of stroke induced rats showed neurons with cell shrinkage, presence of nuclear pyknosis and increased intercellular space. In G3, there were some edematous and degenerative areas. The number of normal neurons markedly increased. In G4, presence of dilated and congested blood vessels & vacuolations, were also found with more neurons with intact morphology.

The improvement in neuronal cell count was more pronounced in the subacute study than in the acute study.

Pongamia pinnata promotes the expression of neurotrophic factors in the ischemic cortex of transient cerebral hypoperfusion and reperfusion in rats

One-way ANOVA showed significant difference in BDNF gene expression between groups in acute ($F=114.5$, $P<0.0001$) and subacute study ($F=69.83$, $P<0.0001$). Similarly significant difference was found in GDNF gene expression between groups ($F=70.11$, $P<0.0001$ for acute study and $F=62.65$, $P<0.0001$ for subacute study). In both the acute and subacute study, tCHR rats showed significantly lower BDNF and GDNF gene expression when compared to normal control rats and the treatment group rats ($P<0.0001$). There was no significant difference in BDNF and GDNF gene expression between normal control rats and low dose and high dose treated rats in acute (BDNF - $P=0.3276$ and 0.0925, GDNF – $P=0.6474$ and 0.9999 respectively) and subacute study (BDNF - $P=0.3019$ and 0.3376, GDNF – $P=0.1122$ and 0.9997 respectively) (Figs. 8A-D and Figs. 9A-D), Supplementary Tables S5-8). There were marginal changes in the gene expression of the transcriptional factors NF-kB and HIF1 which was not statistically significant in both acute (HIF1 - $F=1.148$, $P=0.3871$, NF-kB - $F=0.5106$, $P=0.6861$) and subacute study (HIF1 - $F=0.1901$, $P=0.9002$, NF-kB - $F=0.08771$, $P=0.9648$) (Supplementary Table S9-12).

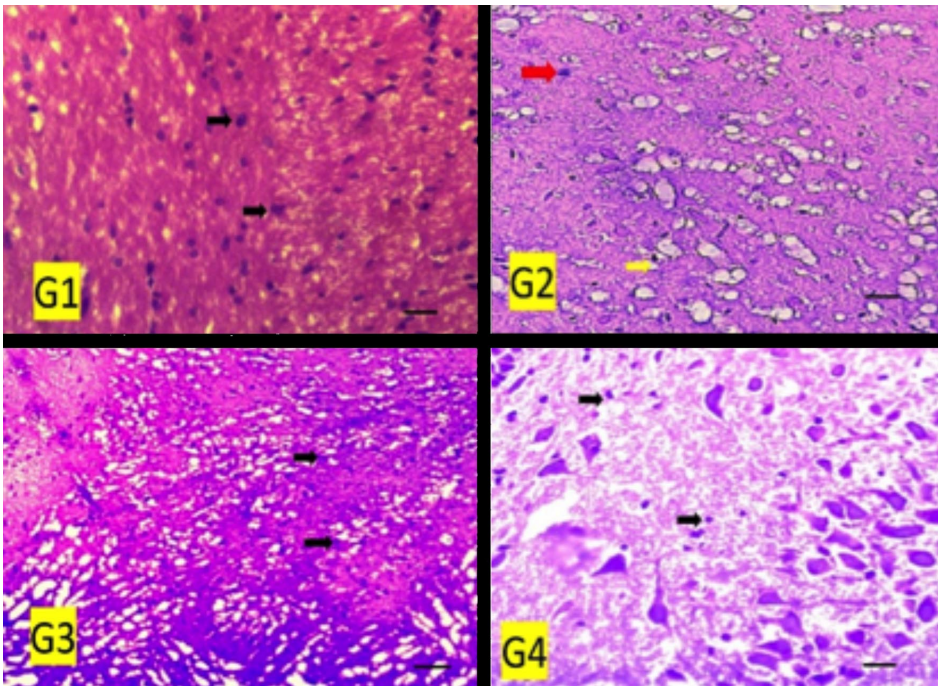

Fig. 6. Representative photographs of Haematoxylin and Eosin stain in rat cerebrum-Acute study (400x). G1- Control group, G2- Negative control group, G3- Treated with 200 mg/kg for 7 days, G4- Treated with 400mg/ kg for 7 days. Black arrow shows neurons with normal morphology. Red arrow indicates eosinophilic neurons with pyknotic nuclei. Yellow arrow indicates the increase peri-cellular space and edema. (Scale 20 µm)

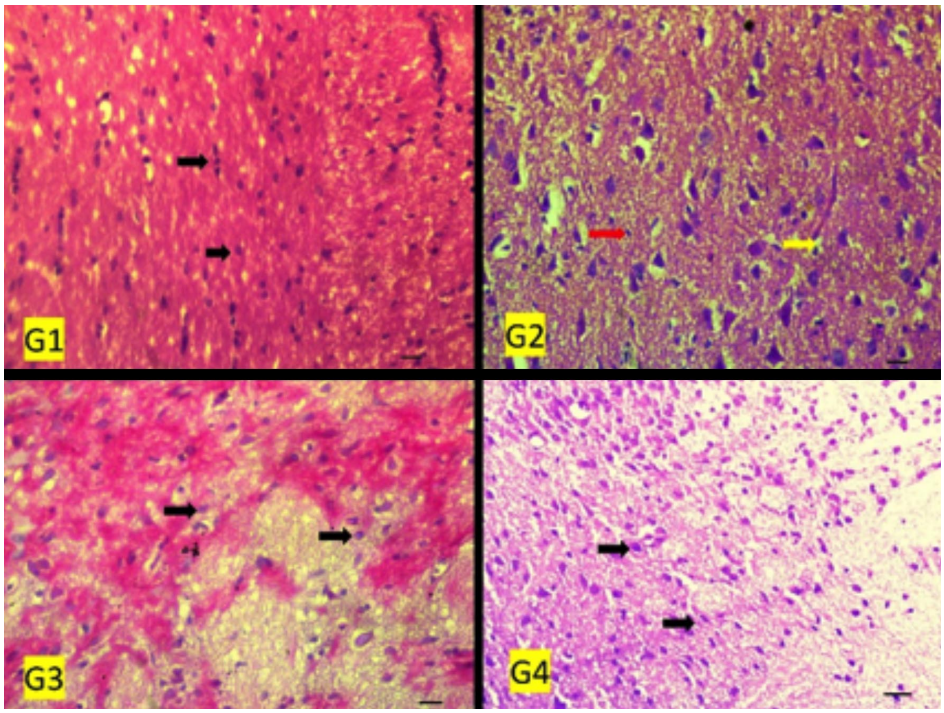

Fig. 7. Representative photographs of Haematoxylin and Eosin stain in rat cerebrum-Subacute study (400x). G1- Control group, G2- Negative control group, G3- Treated with 200 mg/kg for 7 days, G4- Treated with 400mg/ kg for 7 days. Black arrow shows neurons with normal morphology. Red arrow indicates eosinophilic neurons with pyknotic nuclei. Yellow arrow indicates the increase peri-cellular space and edema. (Scale 20 µm)

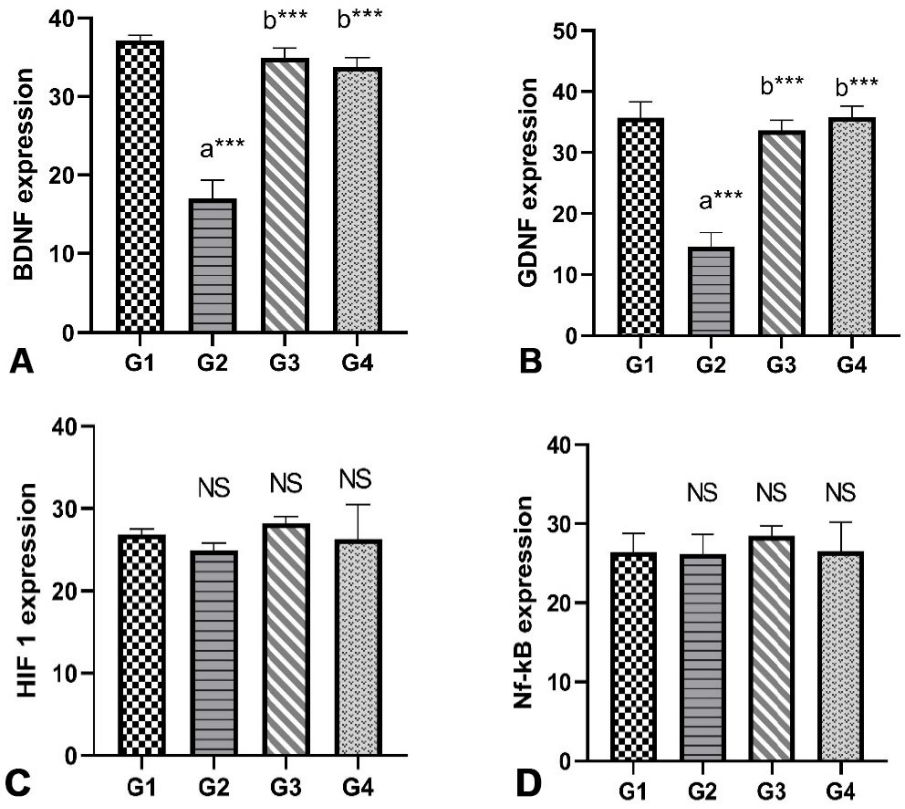

Fig. 8. Expression of BDNF (A), GDNF (B), HIF 1 (C) and Nf-kB (D) in acute study. The level of expression is expressed on the X axis. a - significant when compared to control group (G1), b - significant when compared to negative control group (G2), ab - significant when compared to both G1 and G2 group. *** - indicates P< 0.001; NS- Not significant

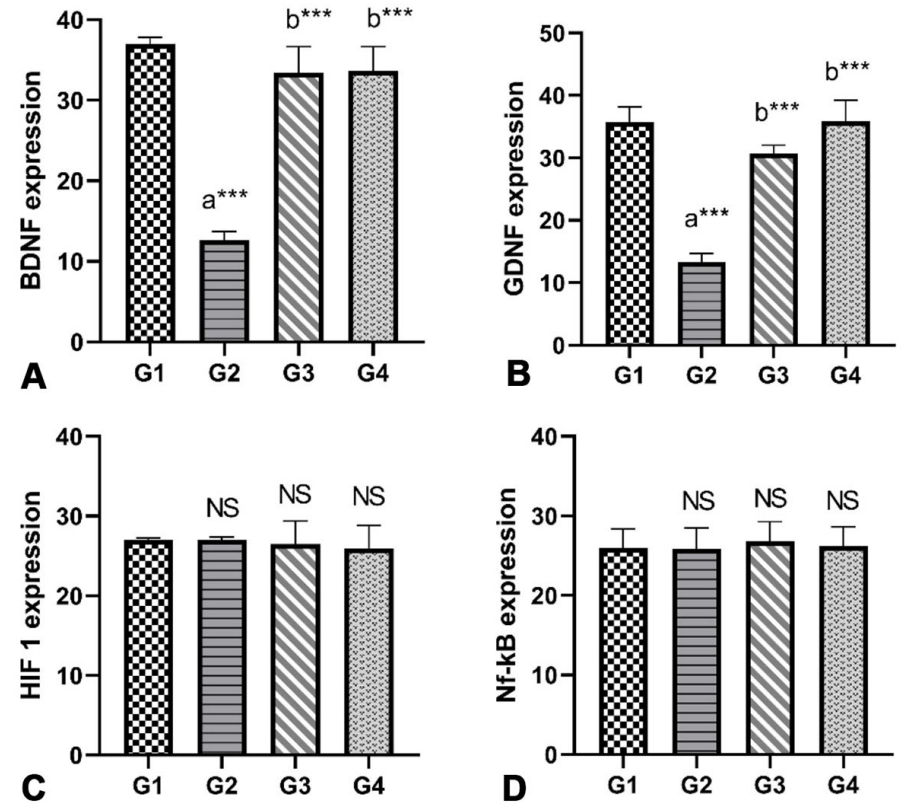

Fig. 9. Expression of BDNF (A), GDNF (B), HIF 1 (C) and Nf-kB (D) in Subacute study. The level of expression is expressed on the X axis. a - significant when compared to control group (G1), b - significant when compared to negative control group (G2), ab -significant when compared to both G1 and G2 group. *** - indicates P< 0.001; NS- Not significant

Table S5: BDNF expression in acute study.

Tukey's multiple comparisons test	Mean 1	SEM 1	Mean 2	SEM 2	Mean Diff.	SE of diff.	95.00 % CI of diff.	Adjusted P Value
G1 (Sham+NS) vs. G2 (BCCAO/R+NS)	37.17	0.3789	16.98	1.359	20.19	1.224	16.27 to 24.11	<0.0001
G1 (Sham+NS) vs. G3 (BCCAO/R + 200 mg)	37.17	0.3789	34.93	0.7162	2.240	1.224	-1.681 to 6.161	0.3276
G1 (Sham+NS) vs. G4 (BCCAO/R+400 mg)	37.17	0.3789	33.78	0.7041	3.387	1.224	-0.5340 to 7.307	0.0925
G2 (BCCAO/R+NS) vs. G3 (BCCAO/R + 200 mg)	16.98	1.359	34.93	0.7162	-17.95	1.224	-21.87 to -14.03	<0.0001
G2 (BCCAO/R+NS) vs. G4 (BCCAO/R+400 mg)	16.98	1.359	33.78	0.7041	-16.80	1.224	-20.72 to -12.88	<0.0001
G3 (BCCAO/R + 200 mg) vs. G4 (BCCAO/R+400 mg)	34.93	0.7162	33.78	0.7041	1.147	1.224	-2.774 to 5.067	0.7870

Table S6: BDNF expression in subacute study.

Tukey's multiple comparisons test	Mean 1	SEM 1	Mean 2	SEM 2	Mean Diff.	SE of diff.	95.00 % CI of diff.	Adjusted P Value
G1 (Sham+NS) vs. G2 (BCCAO/R+NS)	37.03	0.4612	12.64	0.6103	24.39	1.887	18.34 to 30.43	<0.0001
G1 (Sham+NS) vs. G3 (BCCAO/R + 200 mg)	37.03	0.4612	33.45	1.856	3.577	1.887	-2.467 to 9.620	0.3019
G1 (Sham+NS) vs. G4 (BCCAO/R+400 mg)	37.03	0.4612	33.62	1.759	3.407	1.887	-2.637 to 9.450	0.3376
G2 (BCCAO/R+NS) vs. G3 (BCCAO/R + 200 mg)	12.64	0.6103	33.45	1.856	-20.81	1.887	-26.85 to -14.77	<0.0001
G2 (BCCAO/R+NS) vs. G4 (BCCAO/R+400 mg)	12.64	0.6103	33.62	1.759	-20.98	1.887	-27.02 to -14.94	<0.0001
G3 (BCCAO/R + 200 mg) vs. G4 (BCCAO/R+400 mg)	33.45	1.856	33.62	1.759	-0.1700	1.887	-6.214 to 5.874	0.9997

Table S7: GDNF expression in acute study.

Tukey's multiple comparisons test	Mean 1	SEM 1	Mean 2	SEM 2	Mean Diff.	SE of diff.	95.00 % CI of diff.	Adjusted P Value
G1 (Sham+NS) vs. G2 (BCCAO/R+NS)	35.71	1.506	14.59	1.329	21.13	1.738	15.56 to 26.69	<0.0001
G1 (Sham+NS) vs. G3 (BCCAO/R + 200 mg)	35.71	1.506	33.64	0.9619	2.073	1.738	-3.491 to 7.638	0.6474
G1 (Sham+NS) vs. G4 (BCCAO/R+400 mg)	35.71	1.506	35.84	1.039	-0.1233	1.738	-5.688 to 5.441	0.9999
G2 (BCCAO/R+NS) vs. G3 (BCCAO/R + 200 mg)	14.59	1.329	33.64	0.9619	-19.05	1.738	-24.62 to -13.49	<0.0001
G2 (BCCAO/R+NS) vs. G4 (BCCAO/R+400 mg)	14.59	1.329	35.84	1.039	-21.25	1.738	-26.81 to -15.69	<0.0001
G3 (BCCAO/R + 200mg) vs. G4 (BCCAO/R+400 mg)	33.64	0.9619	35.84	1.039	-2.197	1.738	-7.761 to 3.368	0.6077

Table S8: GDNF expression in subacute study.

Tukey's multiple comparisons test	Mean 1	SEM 1	Mean 2	SEM 2	Mean Diff.	SE of diff.	95.00 % CI of diff.	Adjusted P Value
G1 (Sham+NS) vs. G2 (BCCAO/R+NS)	35.68	1.452	13.25	0.8637	22.43	1.908	16.32 to 28.54	<0.0001
G1 (Sham+NS) vs. G3 (BCCAO/R + 200 mg)	35.68	1.452	30.66	0.7957	5.017	1.908	-1.094 to 11.13	0.1122
G1 (Sham+NS) vs. G4 (BCCAO/R+400 mg)	35.68	1.452	35.85	1.948	-0.1700	1.908	-6.281 to 5.941	0.9997
G2 (BCCAO/R+NS) vs. G3 (BCCAO/R + 200 mg)	13.25	0.8637	30.66	0.7957	-17.41	1.908	-23.52 to -11.30	<0.0001
G2 (BCCAO/R+NS) vs. G4 (BCCAO/R+400 mg)	13.25	0.8637	35.85	1.948	-22.60	1.908	-28.71 to -16.49	<0.0001
G3 (BCCAO/R + 200 mg) vs. G4 (BCCAO/R+400 mg)	30.66	0.7957	35.85	1.948	-5.187	1.908	-11.30 to 0.9241	0.0990

Table S9: NF-kB expression in acute study.

Tukey's multiple comparisons test	Mean 1	SEM 1	Mean 2	SEM 2	Mean Diff.	SE of diff.	95.00 % CI of diff.	Adjusted P Value
G1(Sham+NS) vs. G2 (BCCAO/R+NS)	26.39	1.383	26.15	1.442	0.2367	2.113	-6.529 to 7.003	0.9995
G1(Sham+NS) vs. G3(BCCAO/R +200 mg)	26.39	1.383	28.47	0.7194	-2.087	2.113	-8.853 to 4.679	0.7604
G1(Sham+NS) vs. G4(BCCAO/R+400 mg)	26.39	1.383	26.56	2.102	-0.1700	2.113	-6.936 to 6.596	0.9998
G2 (BCCAO/R+NS) vs. G3(BCCAO/R + 200 mg)	26.15	1.442	28.47	0.7194	-2.323	2.113	-9.089 to 4.443	0.6996
G2 (BCCAO/R+NS) vs. G4(BCCAO/R+400 mg)	26.15	1.442	26.56	2.102	-0.4067	2.113	-7.173 to 6.359	0.9973
G3(BCCAO/R + 200 mg) vs. G4(BCCAO/R+400 mg)	28.47	0.7194	26.56	2.102	1.917	2.113	-4.849 to 8.683	0.8018

Table S10: NF-kB expression in subacute study.

Tukey's multiple comparisons test	Mean 1	SEM 1	Mean 2	SEM 2	Mean Diff.	SE of diff.	95.00 % CI of diff.	Adjusted P Value
G1(Sham+NS) vs. G2 (BCCAO/R+NS)	26.01	1.345	25.83	1.532	0.1733	2.013	-6.273 to 6.620	0.9998
G1(Sham+NS) vs. G3(BCCAO/R +200 mg)	26.01	1.345	26.80	1.428	-0.7933	2.013	-7.240 to 5.653	0.9778
G1(Sham+NS) vs. G4(BCCAO/R+400 mg)	26.01	1.345	26.26	1.382	-0.2567	2.013	-6.703 to 6.190	0.9992
G2 (BCCAO/R+NS) vs. G3(BCCAO/R+ 200 mg)	25.83	1.532	26.80	1.428	-0.9667	2.013	-7.413 to 5.480	0.9614
G2 (BCCAO/R+NS) vs. G4(BCCAO/R+400 mg)	25.83	1.532	26.26	1.382	-0.4300	2.013	-6.877 to 6.017	0.9963
G3(BCCAO/R + 200 mg) vs. G4(BCCAO/R+400 mg)	26.80	1.428	26.26	1.382	0.5367	2.013	-5.910 to 6.983	0.9928

Table S11: HIF1 expression in acute study.

Tukey's multiple comparisons test	Mean 1	SEM 1	Mean 2	SEM 2	Mean Diff.	SE of diff.	95.00 % CI of diff.	Adjusted P Value
G1(Sham+NS) vs. G2 (BCCAO/R+NS)	26.90	0.3698	24.94	0.5021	1.957	1.808	-3.832 to 7.745	0.7091
G1(Sham+NS) vs. G3(BCCAO/R +200 mg)	26.90	0.3698	28.24	0.4669	-1.347	1.808	-7.135 to 4.442	0.8763
G1(Sham+NS) vs. G4(BCCAO/R+400 mg)	26.90	0.3698	26.31	2.435	0.5833	1.808	-5.205 to 6.372	0.9875
G2 (BCCAO/R+NS) vs. G3(BCCAO/R+ 200 mg)	24.94	0.5021	28.24	0.4669	-3.303	1.808	-9.092 to 2.485	0.3284
G2 (BCCAO/R+NS) vs. G4(BCCAO/R+400 mg)	24.94	0.5021	26.31	2.435	-1.373	1.808	-7.162 to 4.415	0.8701
G3(BCCAO/R + 200 mg) vs. G4(BCCAO/R+400 mg)	28.24	0.4669	26.31	2.435	1.930	1.808	-3.858 to 7.718	0.7172

Table S12: HIF1 expression in subacute study.

Tukey's multiple comparisons test	Mean 1	SEM 1	Mean 2	SEM 2	Mean Diff.	SE of diff.	95.00 % CI of diff.	Adjusted P Value
G1(Sham+NS) vs. G2 (BCCAO/R+NS)	27.06	0.1041	26.98	0.2378	0.08333	1.679	-5.293 to 5.459	>0.9999
G1(Sham+NS) vs. G3(BCCAO/R +200 mg)	27.06	0.1041	26.49	1.664	0.5667	1.679	-4.809 to 5.943	0.9858
G1(Sham+NS) vs. G4(BCCAO/R+400 mg)	27.06	0.1041	25.94	1.673	1.123	1.679	-4.253 to 6.499	0.9058
G2 (BCCAO/R+NS) vs. G3(BCCAO/R+ 200 mg)	26.98	0.2378	26.49	1.664	0.4833	1.679	-4.893 to 5.859	0.9910
G2 (BCCAO/R+NS) vs. G4(BCCAO/R+400 mg)	26.98	0.2378	25.94	1.673	1.040	1.679	-4.336 to 6.416	0.9230
G3(BCCAO/R + 200 mg) vs. G4(BCCAO/R+400 mg)	26.49	1.664	25.94	1.673	0.5567	1.679	-4.819 to 5.933	0.9865

DISCUSSION

The current study was designed to analyze the neurorestorative potential of P.pinnata leaf extract on transient cerebral hypoperfusion and reperfusion by BCCAO in both acute and subacute setting. The underlying mechanism of action of P.pinnata in restoring neural health in tCHR was assessed by studying the expression of GDNF, BDNF, NF-kB and HIF1. Acute and subacute effect of P.pinnata in structural and functional changes in tCHR rats were studied using histopathological analysis and behavioural test. The results of the present study showed that tCHR rats took significantly higher time to traverse the beam in the beam walking test implying functional and motor impairment. Motor deficit and functional impairment is a well-documented feature of cerebral ischemia (Janac *et al*., 2006). Thus, the result of the present study is in accordance with other published studies demonstrating motor and locomotion impairment caused by cerebral hypoperfusion and reperfusion (Jeon *et al*., 2016; Ya *et al*., 2017; Singh *et al*., 2018). The treatment group rats in subacute study showed better motor coordination and locomotion on par with that of the normal control rats. This observation implies functional improvement highlighting the neurorestorative effect of P.pinnata leaf extract.

The present study has demonstrated restorative effects of P.pinnata leaf extract on the histoarchitectural changes and neuronal damage caused by tCHR in rats. Treatment with P.pinnata prevented neuronal damage significantly in both acute and subacute study in a dose dependent manner. Treatment with the extract for 21 days restored the neuronal count comparable to that of the normal control rats (Figs. 3A,B). Cerebral ischemia leading to neuronal necrosis and apoptosis is a well-known fact as was observed in the present study by significantly lower neuronal count and higher structural damage to the cytoarchitecture of the brain sections in tCHR rats (Figs. 3 and 4A,B). In the pathogenesis of ischemic stroke, different cells of the central nervous system undergo various alterations. Neurons exhibit morphological changes whereas the axons and cell bodies disintegrate. Glial cells and neurons undergo cytoplasmic swelling and disappearance of nucleolus. In penumbra, the ischemic neurons show changes like Nissl's bodies' disintegration. The other cells like glial cells, astrocytes and microglia also exhibit one or the other morphological changes. The permeability of BBB is exceedingly increased during a stroke sequence. This leads to infiltration of immune cells like macrophages, monocytes, and leukocytes in the site of ischemic lesions (Wahul *et al*., 2018; Liu *et al*., 2020). An abrupt interruption in the cerebral blood flow, causes depletion of oxygen and glucose stores from the brain tissues causing a derangement of ion homeostasis, acid-base imbalance, ATP synthesis, and increased energy deficiency. Cerebral ischemia also triggers neuronal cell depolarization and glutamate release, due to oxygen and glucose deficiency leading to neuronal excitotoxicity, apoptosis, and necrosis (Wahul *et al*., 2018; Barthels & Das, 2020; Qin *et al*., 2022). In the present study, P.pinnata treated tCHR rats showed marked improvement in ischemic structural damage of neurons induced by tCHR. There was lesser nuclear pyknosis, vacuolation, oedematous and degenerative areas, and cellular shrinkage in the treatment groups compared to the tCHR rats pointing to the beneficial effect of P.pinnata leaf extract on ischemic neuronal damage.

Activation of various signalling pathways takes place during the pathological transitions occurring in cerebral ischemia. Energy deficiency in turn leads to mitochondrial dysfunction and oxidative stress-induced damage. In the presence of oxidative stress there occurs release of free radicals which leads to lipid peroxidation promoting neuronal damage. This in turn causes a rapid influx of neurotoxic and neurotropic factors to exert either neuroprotective or detrimental effects on ischemic brain tissues. These pathways occur either separately or simultaneously in the stroke sequence (Wahul *et al*., 2018; Qin *et al*., 2022; Ojo *et al*., 2023). In our attempt to elucidate the mechanism underlying the neurorestorative effect of P.pinnata, the current study found that treatment with P.pinnata in tCHR rats resulted in the restoration of gene expression of neurotrophic factors BDNF and GDNF. This effect was noticeable in both acute and subacute study in a dose dependent manner. Treatments using recombinant GDNF, and molecular genetic methods have demonstrated that GDNF could offer brain protection and promote neuronal survival after ischemic stroke. In ischemic stroke, substantial interest has focused on the potential protective effects of GDNF owing to its upregulated production as well as expression of its receptors RET and GFRa-1. GDNF was also shown to protect against neuronal loss through attenuating the NMDA-induced cell death and calcium influx. GDNF could reduce oxidative stress and protect neurons via transcriptional regulation of glutathione synthesis (Kustova *et al*., 2022; Zhang *et al*., 2022). BDNF on the other hand plays a significant role in the prognosis, pathogenesis, and rehabilitation of stroke. It is well established that low levels of circulating BDNF are associated with a high risk of stroke and poor recovery, while BDNF expression in the brain is acutely stimulated by a stroke (Liu *et al*., 2020; Kustova *et al*., 2022). Thus, the results of the current study show that P.pinnata leaf extract prevents ischemic neuronal damage by promoting the expression of neurotrophic factors BDNF and GDNF. However, further studies are needed to elucidate other underlying mechanisms behind the neurorestorative action of the plant extract.

The signalling pathways that increase BDNF and GDNF during stroke treatment include the Hippo signalling pathway, Sonic Hedgehog (SHH) pathway, nuclear factor erythroid 2-related factor 2 (Nrf2)/antioxidant response element (ARE) pathway, hypoxia-inducible factor-1a (HIF-1a) pathway, PI3K/AKT pathway, JAK/STAT pathway, and AMPK pathway (Eskandari *et al*., 2021). The phytochemistry of *Pongamia pinnata* leaf ethanol extract reveals the presence of various bioactive compounds including phenolic compounds, tannins, flavonoids, terpenoids, alkaloids, and fatty acid esters (Al Muqarrabun *et al*., 2013). Flavonoids such as quercetin and kaempferol found in the leaf extract of P.pinnata were found to enhance the neurotrophic factors BDNF and GDNF by modulation of signalling pathways, including the phosphatidylinositol 3-kinase (PI3K)/Akt and mitogen-activated protein kinases (MAPK)/extracellular signal-regulated kinases (ERK) pathways (Marzouk *et al*., 2008; Moosavi *et al*., 2015; Farzaei *et al*., 2018). Terpenoids were isolated from P.pinnata leaf extract which were found to have neuroprotective effect by modulating PI3K/Akt pathway (Xu *et al*., 2022). Fatty acid esters have neurotrophic effects through the modulation of signalling pathways involved in neuronal survival, growth, proliferation, and differentiation (Katsuki & Okuda, 1995; Makino *et al*., 2010; Hosseini *et al*., 2018). These esters activate intracellular signalling pathways such as MAPK/ERK, PI3K/Akt, and cyclic adenosine monophosphate response element-binding protein (CREB) phosphorylation, Nrf2 pathway, which upregulates antioxidant genes and protects against oxidative stress (Moosavi *et al*., 2015; Lin-Holderer *et al*., 2016).

Wide brain damage is associated with an increase of HIF-1a production. This increase is initiated in the preconditioning-hypoxia sequence induced by neuronal damage. HIF-1a level, as the master regulator of the cellular response to hypoxia, is tightly controlled through synthesis and degradation. HIF-1a protein accumulation during hypoxia is a result from inhibition of its oxygen-dependent degradation by (pVHL) pathway (Koh *et al*., 2008; Dong *et al*., 2022). NF-kB activation takes place as an acute response to injury in stroke, participates in blood–brain barrier disruption, inflammation, and contributes to neuronal cell death. NF- kB activation during preconditioning causes induction of IkB, so that when the subsequent occlusive event occurs, NF-kB activity is reduced and therefore so is neuronal loss (Harari & Liao, 2010). However, in the current study, qPCR could not find any significant changes in HIF1 and NFKB between the studied groups indicating that the underlying mechanism behind the neurorestorative effect of P.pinnata leaf extract is mainly by promoting neurotrophic factors in ischemic brain.

CONCLUSION AND FUTURE DIRECTIONS

The result of the current study shows that *Pongamia pinnata* has excellent neurorestorative property reversing many of the effects of ischemic stroke induced by transient cerebral hypoperfusion - reperfusion injury in rats. Oral administration of the leaf extract of *Pongamia pinnata* caused improvement in motor function and histoarchitectural damage of ischemic brain induced by transient cerebral hypoperfusion and reperfusion in rats both 7 days and 21 days after ischemia. The underlying mechanism behind this neurorestorative effect was found to be an improvement in the expression of neurotrophic factors GDNF and BDNF.

Our initial evaluation addressing stroke induced by transient cerebral hypoperfusion and reperfusion using *Pongamia pinnata* leaf extract has proven to be promising with the functional, histological, and molecular level evidence that P. pinnata possesses neurorestorative properties. We hypothesise that the phytochemicals in the extract exert the neurorestorative effect by possibly modulating the signalling pathways viz PI3K/Akt, MAPK/ERK, CREB phosphorylation, Nrf2 pathways. Further studies aimed at isolating active ingredients from the plant extract and analysing molecular signalling modulation and neurogenic potential are needed to further our understanding of the neurorestorative effect of *Pongamia pinnata* and to provide visibility to propose *Pongamia pinnata* to be a potential adjunct in treating ischemic Stroke.

ACKNOWLEDGEMENTS. We would like to thank Dr. Ananthi, ClinbioScience, Mr.Manjunath, Philips India Ltd, Dr.Vasudha K, Prof & Head Aims & Rc, Dr.Ananda Ramajayan, Associate Prof . Aims & RC, India in supporting us to draft this manuscript and to carry the study forward successfully.

SUGANITHA, B.; JAGADEESAN, V.; SIVANESAN, S.; PRATHAP, L.; MUNDUGARU, R.; ALZERWI, N.; SARASWATHI, I. & SAIKARTHIK, J. Efecto neurorestaurador mediado por factor neurotrófico de *Pongamia pinnata* contra la hipoperfusión y reperfusión cerebral transitoria en ratas: Evidencia preliminar de un estudio a nivel histológico y molecular. *Int. J. Morphol., 42(3)*:876-890, 2024.

RESUMEN: El ataque cerebrovascular es la principal causa de discapacidad física adquirida en adultos y la segunda causa de mortalidad en todo el mundo. Las estrategias de tratamiento para frenar los efectos del ataque cerebrovascular serían de gran beneficio. *Pongamia pinnata* es una atracción reciente en la medicina, debido a sus abundantes beneficios medicinales con mínimos efectos secundarios. El presente estudio tuvo como objetivo examinar el efecto agudo y subagudo del extracto de hoja de *Pongamia pinnata* sobre la hipoperfusión y reperfusión cerebral

transitoria (tCHR) en ratas Wistar. Se dividieron 24 ratas Wistar adultas (12 cada una para el estudio agudo y subagudo) en cuatro grupos, el grupo control normal, el grupo tCHR + NS, los grupos tCHR + 200 mg/kg de peso corporal y tCHR + 400 mg/kg de peso corporal. La inducción de la isquemia cerebral se llevó a cabo mediante oclusión y reperfusión bilateral de la arteria carótida común. El extracto etanólico de hojas de *Pongamia pinnata* se administró por vía oral durante 7 días y 21 días después del procedimiento quirúrgico para estudio agudo y subagudo respectivamente. Se realizaron análisis de comportamiento, evaluación histológica y estimación de los niveles de ARNm de HIF-1, GDNF, BDNF y NF-kB. Tanto en el estudio agudo como en el subagudo, hubo una mejora significativa en el ensayo de desplazamiento del haz, el recuento neuronal, una disminución del daño neuronal en las secciones histológicas y una mayor expresión de ARNm de BDNF y GDNF en los grupos con tratamiento. No hubo diferencias significativas en la expresión de HIF1 y NF-kB. Por lo tanto, *Pongamia pinnata* tiene una excelente propiedad neurorestauradora que revierte muchos de los efectos del ataque cerebrovascular isquémico inducido por tCHR en ratas, siendo el mecanismo subyacente una mejora en la expresión de los factores neurotróficos GDNF y BDNF.

PALABRAS CLAVE: Ataque cerebrovascular; *Pongamia pinnata*; Corteza cerebral; Neurorestaurador.

REFERENCES

Al Muqarrabun, L. M. R.; Ahmat, N.; Ruzaina, S. A. S.; Ismail, N. H. & Sahidin, I. Medicinal uses, phytochemistry and pharmacology of *Pongamia pinnata* (L.) Pierre: a review. *J. Ethnopharmacol., 150(2)*:395-420, 2013.

Barthels, D. & Das, H. Current advances in ischemic stroke research and therapies. *Biochim. Biophys. Acta Mol. Basis Dis., 1866(4)*:165260, 2020.

Bederson, J. B.; Pitts, L. H.; Tsuji, M.; Nishimura, M.; Davis, R. & Bartkowski, H. Rat middle cerebral artery occlusion: evaluation of the model and development of a neurologic examination. *Stroke, 17(3)*:472-6, 1986.

Boehme, A. K.; Esenwa, C. & Elkind, M. S. V. Stroke risk factors, genetics, and prevention. *Circ. Res., 120(3)*:472-95, 2017.

DeSai, C. & Hays Shapshak, A. *Cerebral Ischemia*. In: StatPearls [Internet]. Treasure Island (FL), StatPearls Publishing, 2023. Available from: https://www.ncbi.nlm.nih.gov/books/NBK560510/

Dong, P.; Li, Q. & Han, H. HIF-1a in cerebral ischemia. *Mol. Med. Rep., 25(2)*:41, 2022.

Eskandari, S.; Sajadimajd, S.; Alaei, L.; Soheilikhah, Z.; Derakhshankhah, H. & Bahrami, G. Targeting common signaling pathways for the treatment of stroke and Alzheimer's: a comprehensive review. *Neurotox. Res., 39(5)*:1589-612, 2021.

Farzaei, M. H.; Tewari, D.; Momtaz, S.; Argüelles, S. & Nabavi, S. M. Targeting ERK signaling pathway by polyphenols as novel therapeutic strategy for neurodegeneration. *Food Chem. Toxicol., 120*:183-95, 2018.

Feldman, A. T. & Wolfe, D. Tissue processing and hematoxylin and eosin staining. *Methods Mol. Biol., 1180*:31-43, 2014.

GBD 2016 Neurology Collaborators. Global, regional, and national burden of neurological disorders, 1990-2016: a systematic analysis for the Global Burden of Disease Study 2016. *Lancet Neurol., 18(5)*:459-80, 2019.

Graham, D. I. & Adams, J. H. Ischaemic brain damage in fatal head injuries. *Lancet, 1(7693)*:265-6, 1971.

Harari, O. A. & Liao, J. K. NF-kB and innate immunity in ischemic stroke. *Ann. N. Y. Acad. Sci., 1207*:32-40, 2010.

Hosseini, R.; Moosavi, F.; Silva, T.; Rajaian, H.; Hosseini, S. Y.; Bina, S.; Saso, L.; Miri, R.; Borges, F. & Firuzi, O. Modulation of ERK1/2 and Akt pathways involved in the neurotrophic action of caffeic acid alkyl esters. *Molecules, 23(12)*:3340, 2018.

Hua, R.; Yu, S.; Liu, M. & Li, H. A PCR-based method for RNA probes and applications in neuroscience. *Front. Neurosci., 12*:266, 2018.

Iadecola, C. & Anrather, J. Stroke research at a crossroad: asking the brain for directions. *Nat. Neurosci., 14(11)*:1363-8, 2011.

Iwasaki, Y.; Ito, S.; Suzuki, M.; Nagahori, T.; Yamamoto, T. & Konno, H. Forebrain ischemia induced by temporary bilateral common carotid occlusion in normotensive rats. *J. Neurol. Sci., 90(2)*:155-65, 1989.

Janac, B.; Radenovic, L.; Selakovic, V. & Prolic, Z. Time course of motor behavior changes in Mongolian gerbils submitted to different durations of cerebral ischemia. *Behav. Brain Res., 175(2)*:362-73, 2006.

Jayakumar, S.; Raghunath, G.; Ilango, S.; Vijayakumar, J. & Vijayaraghavan, R. Effect of Fluoxetine on the Hippocampus of Wistar Albino Rats in Cold Restraint Stress Model. *J. Clin. Diagn. Res., 11(6)*:AF01-AF06, 2017.

Jeon, W. K.; Kim, M. S.; Lee, J. & Han, J. S. P2-036: Protective effect of Ginkgo Biloba Extract (EGB761) against chronic cerebral hypoperfusion by modulating neuroinflammation and the cholinergic system. *Alzheimer Dement., 12*:P621-P622, 2016.

Katsuki, H. & Okuda, S. Arachidonic acid as a neurotoxic and neurotrophic substance. *Prog. Neurobiol., 46(6)*:607-36, 1995.

Koh, M. Y.; Spivak-Kroizman, T. R. & Powis, G. HIF-1 regulation: not so easy come, easy go. *Trends Biochem. Sci., 33(11)*:526-34, 2008.

Kustova, A. O.; Gavrish, M. S.; Sergeeva, M. A.; Avlasenko, D. A.; Kiseleva, A. O.; Epifanova, E. A.; Babaev, A. A.; Mishchenko, T. A. & Vedunova, M. V. The influence of neurotrophic factors BDNF and GDNF overexpression on the functional state of mice and their adaptation to audiogenic seizures. *Brain Sci., 12(8)*:1039, 2022.

Lin-Holderer, J.; Li, L.; Gruneberg, D.; Marti, H. H. & Kunze, R. Fumaric acid esters promote neuronal survival upon ischemic stress through activation of the Nrf2 but not HIF-1 signaling pathway. *Neuropharmacology, 105*:228-40, 2016.

Liu, W.; Wang, X.; O'Connor, M.; Wang, G. & Han, F. Brain-derived neurotrophic factor and its potential therapeutic role in stroke comorbidities. *Neural Plast., 2020*:1969482, 2020.

Makino, A.; Iinuma, M.; Fukumitsu, H.; Soumiya, H.; Furukawa, Y. & Furukawa, S. 2-Decenoic acid ethyl ester possesses neurotrophin-like activities to facilitate intracellular signals and increase synapse-specific proteins in neurons cultured from embryonic rat brain. *Biomed. Res., 31(6)*:379-86, 2010.

Manigauha, A.; Patel, S.; Monga, J. & Ali, H. Evaluation of anticonvulsant activity of *Pongamia pinnata* Linn in experimental animals. *Int. J. PharmTech Res., 1(4)*:1119-21, 2009.

Marzouk, M. S.; Ibrahim, M. T.; El-Gindi, O. R. & Abou Bakr, M. S. Isoflavonoid glycosides and rotenoids from *Pongamia pinnata* leaves. *Z. Naturforsch. C. J. Biosci., 63(1-2)*:1-7, 2008.

Mo, Y.; Wan, R. & Zhang, Q. Application of reverse transcription-PCR and real-time PCR in nanotoxicity research. *Methods Mol. Biol., 926*:99-112, 2012.

Moosavi, F.; Hosseini, R.; Saso, L. & Firuzi, O. Modulation of neurotrophic signaling pathways by polyphenols. *Drug Des. Devel. Ther., 10*:23-42, 2015.

Mundugaru, R.; Sivanesan, S.; Udaykumar, P.; Vidyadhara, D.; Prabhu, S. N. & Ravishankar, B. Neuroprotective functions of Alpinia galanga in forebrain ischemia induced neuronal damage and oxidative insults in rat hippocampus. *Indian J. Pharm. Educ. Res, 52*:s77-s85, 2018.

Ojo, O. B.; Amoo, Z. A.; Olaleye, M. T.; Jha, S. K. & Akinmoladun, A. C. Time and brain region-dependent excitatory neurochemical alterations in bilateral common carotid artery occlusion global ischemia model. *Neurochem. Res., 48(1)*:96-116, 2023.

Qin, C.; Yang, S.; Chu, Y.-H.; Zhang, H.; Pang, X.-W.; Chen, L.; Zhou, L. Q.; Chen, M.; Tian, D. S. & Wang, W. Signaling pathways involved in ischemic stroke: molecular mechanisms and therapeutic interventions. *Signal Transduct. Target. Ther., 7(1)*:215, 2022.

Qu, D.; Li, W.; Zhang, S.; Li, R.; Wang, H. & Chen, B. Traumatic brain injury is associated with both hemorrhagic stroke and ischemic stroke: a systematic review and meta-analysis. *Front. Neurosci., 16*:814684, 2022.

Raghavendra, M.; Trigunayat, A.; Singh, R.; Mitra, S.; Goel, R. & Acharyaa, S. Effect of ethanolic extract of root of *Pongamia pinnata* (L) pierre on oxidative stress, behavioral and histopathological alterations induced by cerebral ischemia–reperfusion and long-term hypoperfusion in rats. *Indian J. Exp. Biol., 45(10)*:868-76, 2007.

Roger, V. L.; Go, A. S.; Lloyd-Jones, D. M.; Adams, R. J.; Berry, J. D.; Brown, T. M.; Carnethon, M. R.; Dai, S.; de Simone, G. & Ford, E. S. Heart disease and stroke statistics--2011 update: a report from the American Heart Association. *Circulation, 123(4)*:e18-e209, 2011.

Saini, P.; Lakshmayya, L. & Bisht, V. S. Anti-Alzheimer activity of isolated karanjin from *Pongamia pinnata* (L.) pierre and embelin from Embelia ribes Burm. *Ayu, 38(1-2)*:76, 2017.

Sarshoori, J. R.; Asadi, M. H. & Mohammadi, M. T. Neuroprotective effects of crocin on the histopathological alterations following brain ischemia-reperfusion injury in rat. *Iran. J. Basic Med. Sci., 17(11)*:895-902, 2014.

Shirwaikar, A.; Malini, S. & Kumari, S. C. Protective effect of *Pongamia pinnata* flowers against cisplatin and gentamicin induced nephrotoxicity in rats. *Indian J. Exp. Biol., 41(1)*:58-62, 2003.

Singh, V.; Krishan, P. & Shri, R. Antioxidant-mediated neuroprotection by Allium schoenoprasum L. leaf extract against ischemia reperfusion-induced cerebral injury in mice. *J. Basic Clin Physiol. Pharmacol., 29(4)*:403-10, 2018.

Speetzen, L. J.; Endres, M. & Kunz, A. Bilateral common carotid artery occlusion as an adequate preconditioning stimulus to induce early ischemic tolerance to focal cerebral ischemia. *J. Vis. Exp., (75)*:e4387, 2013.

Wahul, A. B.; Joshi, P. C.; Kumar, A. & Chakravarty, S. Transient global cerebral ischemia differentially affects cortex, striatum and hippocampus in Bilateral Common Carotid Arterial occlusion (BCCAo) mouse model. *J. Chem. Neuroanat., 92*:1-15, 2018.

Wong, M. L. & Medrano, J. F. Real-time PCR for mRNA quantitation. *Biotechniques, 39(1)*:75-85, 2005.

Xu, B.; Bai, L.; Chen, L.; Tong, R.; Feng, Y. & Shi, J. Terpenoid natural products exert neuroprotection via the PI3K/Akt pathway. *Front. Pharmacol., 13*:1036506, 2022.

Ya, B. L.; Li, H. F.; Wang, H. Y.; Wu, F.; Xin, Q.; Cheng, H. J.; Li, W. J.; Lin, N.; Ba, Z. H.; Zhang, R. J.; *et al*. 5-HMF attenuates striatum oxidative damage via Nrf2/ARE signaling pathway following transient global cerebral ischemia. *Cell Stress Chaperones, 22(1)*:55-65, 2017.

Yadav, R. D.; Jain, S.; Alok, S.; Prajapati, S. K. & Verma, A. *Pongamia pinnata*: an overview. *Int. J. Pharm. Sci. Res., 2(3)*:494, 2011.

Yousefzadeh, N.; Jeddi, S. & Ghasemi, A. Induction of euthanasia using carbon dioxide in rat: an overview of the available practical guidelines. *EBNESINA, 23(2)*:81-91, 2021.

Zhang, R.; Yang, N.; Ji, C.; Zheng, J.; Liang, Z.; Hou, C. Y.; Liu, Y. Y. & Zuo, P. P. Neuroprotective effects of Aceglutamide on motor function in a rat model of cerebral ischemia and reperfusion. *Restor. Neurol. Neurosci., 33(5)*:741-59, 2015.

Zhang, Z.; Zhang, N. & Ding, S. Glial cell line-derived neurotrophic factor in brain repair after focal ischemic stroke. *Neural Regen. Res., 17(8)*:1735-6, 2022.

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