Pharmacogn J. 2018; 10(1): 39-44
A Multifaceted Journal in the field of Natural Products and Pharmacognosy
www.phcogj.com | www.journalonweb.com/pj | www.phcog.net

Original Article

Effect of *Dalechampia indica* Wight Extract Fractions on Behavioral and Biochemical Abnormalities following Ischemia-Reperfusion Insult in Rats

Sindhura Srinivasula[1]*, Chinna Eswaraiah Maram[2]

ABSTRACT

Objective: To evaluate the cerebro protective effect of *Dalechampia indica* (DI) ethanolic (EDI) and chloroform (CDI) extract fractions against cerebral ischemia- reperfusion insult in rats. **Method:** The rats were treated with different doses (200 and 400 mg/kg) of EDI and CDI orally once daily for 30 days. After completion of pre-treatment, ischemia was induced by blocking bilateral common carotid arteries (BCCAO) for 30 mins followed by reperfusion and ferric chloride induced thrombosis. The post treatment was continued for another week after surgery. The behavioral parameters were assessed and finally, rats were sacrificed to isolate the brains to estimate biochemical parameters. **Results:** Combination of global (BCCAO) and focal (Thrombosis) ischemic model along with reperfusion has induced prominent ischemic insult indicated by antioxidant enzymes depletion and elevation of lipid peroxidation. Pre-treatment with different doses of EDI and CDI significantly improved the levels of superoxide dismutase (SOD), glutathione enzymes (GPx, GSH, GST, GR), catalase (CAT) and decreased the lipid peroxidation. **Conclusion:** Results established Cerebro protective role of Ethanolic extract fraction of DI in cerebrovascular insufficiency states and cerebral ischemic-reperfusion injury produced by bilateral common carotid artery ligation along with reperfusion and thrombosis which may attribute to presence of alkaloids,flavonoids, and phenolic compounds.

Key words: BCCAO, *Dalechampia indica*, Ferric chloride, Reperfusion, Thrombosis.

Sindhura Srinivasula[1]*, Chinna Eswaraiah Maram[2]

[1]Research scholar, Department of Pharmaceutical Science, JNTUH, Hyderabad, Telangana, INDIA.
[2]Department of Pharmacognosy, Anurag Pharmacy College, Kodad, Telangana, INDIA.

Correspondence

Sindhura Srinivasula

Department of Pharmacology, Anurag Pharmacy College, Kodad, Telangana, INDIA.

Phone no: 919640330593

E-mail: sindhura.1304@gmail.com

History

- Submission Date: 22-08-2017;
- Review completed: 11-09-2017;
- Accepted Date: 02-11-2017

DOI : 10.5530/pj.2018.1.8

Article Available online
http://www.phcogj.com/v10/i1

Copyright
© 2018 Phcog.Net. This is an open-access article distributed under the terms of the Creative Commons Attribution 4.0 International license.

INTRODUCTION

Cerebral ischemia is one of the neurodegenerative disease characterized by progressive loss of neuronal function which seriously depends on the amount of glucose and oxygen supplied by blood and the ATP produced from it. As the brain is not able to store energy like other organs it continuously requires the supply of energy for its function. So, any disturbance in this results in neuronal dysfunction and damage.[1] Among strokes, ischemic stroke accounts 85% of all cases and caused by atherothrombotic or embolic occlusion of cerebral arteries either affect part of the brain (focal) or broad area of the brain (global). The severity of the disease depends on the area affected, cause, pre-existing conditions, and risk factors.[2]

The pathophysiological processes in ischemia are very complex and interrelated which includes majorly bio energetic failure, oxidative stress, excitotoxicity, blood brain barrier (BBB) dysfunction, inflammation, and apoptosis.[3-7] Unfortunately, even after understanding the cellular and molecular mechanisms of ischemia and targeting those with various drugs such as NMDA and calcium antagonists has failed in human populations. Till date, the IV t-PA administration within first 3h of brain attack is the most effective drug to treat stroke.[8]

Though there are various promising ways of approach for treatment of stroke, our most efficacious therapy remain neuroprotective effect of natural compounds from herbs with multifactorial function such as anti-oxidation, anti-inflammation, calcium antagonization, anti-apoptosis, and neuro-functional regulation.[9] Preventive strategies such as Lifestyle modifications, control of hypertension, hyperglycemia, hyperlipidemia, smoking and treatment of cardiac condition has also produced encouraging results.[10]

Dalechampia indica Wight (Euphorbiaceae), commonly called as aliparnika found in dry hills of Andhra Pradesh and Tamil Nadu. This shrub is finely pubescent with stinging hairs. It has folio late leaves and sessile leaflets with a short petiole. Flowers are surrounded by fleshy scales formed out of deformed flowers. Female ciliate flowers with stalked glands, pubescent ovary, long style, copular stigma, depressed fruits, glandular hairs, fruiting calyx and mottled dark gray seeds are present.[11] In the present study, we evaluated Cerebro protective

Cite this article: Sindhura S, Eswaraiah MC. Effect of *Dalechampia indica* Wight Extract Fractions on Behavioral and Biochemical Abnormalities following Ischemia-Reperfusion Insult in Rats. Pharmacog J. 2018;10(1):39-44.

effect of *Dalechampia indica* extract fractions following ischemic insult in rats.

MATERIALS AND METHODS

Plant material Collection and Authentication

The whole plant of *Dalechampia indica* was collected in the month of April 2014 from Chittoor dist. The plant material was identified and authenticated by Prof. K. Madhav Shetty, Dept. of Botany, Taxonomist, SV University, Tirupati. A voucher was kept in the department of pharmacognosy for reference (ANU/COG/14/02).

Experimental Animals

The experiments were carried out in albino Wistar rats of either sex weighing 180-200 g and were procured from Anurag Pharmacy College, Kodad, India. They were kept under standard conditions at 23-25^{0}C 12h light/dark cycle and fed with standard diet and water *ad libitum*. Before the experiment, the animals were acclimatized for 1 week under laboratory conditions. The study was conducted in accordance with CPCSEA (Committee for Control and Supervision of Experiment on Animals) guidelines and approved by the Institutional Animal Ethical Committee (Registration no -1712/PO/a/13/CPCSEA).

Chemicals

All chemical agents used were of analytical grade were purchased from Sigma Chemicals Co. (St. Louis, USA) or Himedia, Mumbai.

Preparation and Fractionation of crude extract of plant material

Plant material was cut into pieces and washed properly. It was shade-dried for several days, pulverized into coarse powder and stored at room temperature (RT) for future use. The dried coarse powder (500g) of plant extract was macerated with absolute Ethanol at room temperature for 10 days in a clean and sterilized glass container with frequent agitation. Later it was subjected to filtration. The filtrate obtained was concentrated on a water bath maintaining 40°C to dryness and designated as a crude extract of DI. The crude extract obtained subjected to fractionation by suspending in hydro alcoholic (7:3% v/v) solution and extracted successively with petroleum Ether and chloroform using separating funnel. Dried plant extracts of different solvents were weighed and stored for further use. PET Ether used as defatting agent.[12] In the present study, the Ethanolic (EDI) and chloroform fractions (CDI) of crude extract were screened for phytoconstituents and Cerebro protective effect against cerebral ischemia.

Preliminary Phytochemical Analysis

The extract fractions of *D.indica* was subjected to preliminary phytochemical screening for the presence or absence of phytoconstituents using standard methods.[13] The extract fractions showed the presence of reducing sugars, anthraquinones, glycosides, tannins, phenols, and flavonoids.

Extract administration

The Ethanolic and chloroform extract fractions of *D.indica* were prepared as a uniform suspension using 1% tween 80 for oral administration in experimental animals.

Acute toxicity studies

Acute oral toxicity test of extract fractions EDI and CDI was carried out as per Acute Toxic Class Method described in OECD 423. Prior to the dosing, the animals fasted overnight. The incidence of mortality was checked for first 24h and daily thereafter for 14 days.[14] two arbitrary doses of 200 mg/kg and 400 mg/kg were selected for the study, as the extract fractions were found safe up to 2000 mg/kg without any sign of toxicity or mortality.

Experiment schedule

The animals were divided into six groups each containing 8 animals. The test group animals received different doses (200 and 400 mg/kg) of EDI and CDI orally once daily for 30 days whereas sham operated and control groups received vehicle (1% Tween 80). The treatment schedule is mentioned below.[15]

Group I (SHAM)

Sham operated rats treated with 1% tween 80 without occlusion/reperfusion/ferric chloride application.

Group II (CONTROL)

Rats treated with 1% tween 80 and BCCAO for 30 mins followed by reperfusion for 45 mins and topical application of ferric chloride on carotid artery.

Group III (EDI-1)

Rats treated with low dose (200 mg/kg p.o) of EDI and BCCAO for 30 mins followed by reperfusion for 45 mins and topical application of ferric chloride.

Group IV (EDI-2)

Rats treated with high dose (400 mg/kg p.o) of EDI and BCCAO for 30 mins followed by reperfusion for 45 mins and ferric chloride application.

Group V (CDI-1)

Rats treated with low dose (200 mg/kg p.o) of CDI and BCCAO for 30 mins followed by reperfusion for 45 mins and ferric chloride application.

Group VI (CDI-2)

Rats treated with high dose (400 mg/kg p.o) of CDI and BCCAO for 30 mins followed by reperfusion for 45 mins and topical application of ferric chloride.

Induction of stroke

After 30 days of treatment, stroke was induced by the combination of global (BCCAO) and focal model (ferric chloride induced thrombosis) followed by reperfusion. The rats were anesthetized with ketamine (80-90 mg/kg i.p.) and xylazine (5-10 mg/kg i.m.).[16] By ventral midline incision on the neck area, the left and right common carotid arteries of the rat were exposed and accompanying vagus nerves were carefully separated from them. Ischemic stroke was induced by occluding both arteries with vascular clips for 30 mins. Then, in the process of reperfusion vascular clamps were clamped and removed alternatively over a period of 45 mins.[17] Finally, the filter paper (1×1mm) saturated with 25% $FeCl_3$ was applied proximal to the surface of the left common carotid artery for 15 min.[18] During the application, care must be taken such that it should not come in contact surrounding tissue or other blood vessels. The temperature was maintained at 37 ± 0.5 °C throughout the surgery. The animals were sutured and allowed to recover by housing them in individual cages. Sham operated rats received the same surgical incision without ischemic induction.

Behavioral Parameters

Animals were trained for behavioral studies before surgery and were evaluated at 36 h after the surgery and given a score as per Garcia modified the neurological deficit scoring system.[19] This includes various sub tests such as spontaneous activity, movement, proprioception and sensory function which were described by Garcia. The maximum score is 18 represents the normal activity. Lower the score more is the deficit.

Post treatment

After inducing a stroke, the treatment schedule was continued for another week. By the end of 7th day, the animals were sacrificed by decapitation and brains were removed for neurobiochemical estimations.

Biochemical Estimations

Preparation of brain homogenate

The separated brains were washed with ice cold saline (0.9%) and quickly blotted with filter paper. After that, they were weighed, minced and homogenized in an ice cold 10 mM Tris HCL buffer (pH 7.4) at a concentration of 10% (w/v) with 25 strokes of tight Teflon pestle of glass homogenizer at a speed of 2500 rpm. Then, it was centrifuged at 5000 rpm for 20 mins to get clear supernatant which was separated and maintained at 4^0C in freezer until it is assayed for various biochemical parameters.[20]

Super oxide dismutase (SOD)

The SOD activity was determined by Misra and Fridovich method. An aliquot (0.1 ml) of the sample, 0.1ml of 0.4 Mm EDTA, and 0.5ml of carbonate buffer (pH 9.7) were mixed. To this mixture, 1ml of freshly prepared 3mM epinephrine was added to initiate the reaction and absorbance was read at 480 nm for 3 mins at 30 secs intervals. One unit of superoxide dismutase (SOD) activity was defined as the amount of enzyme necessary to cause 50% inhibition of the oxidation of adrenaline to adrenochrome during 1 min.[21]

Catalase Activity

Claiborne method was used in which 3 ml of reaction mixture consists of 1.95 ml of 0.05 M phosphate buffer (pH 7.0), 1ml of 0.019 M hydrogen peroxide and 0.05 ml brain homogenate. Absorbance was read at 240 nm. It was calculated in terms of nM Hydrogen peroxide consumed/min/mg protein.[22]

Reduced glutathione assay (GSH)

It was estimated by Jollow *et al* method with DTNB (5,5-dithiobis-2-nitrobenzoic acid) as a substrate. 1.0 ml of brain homogenate was precipitated with 1.0 ml of 4% sulfosalicylic acid and kept at 4^oC for 1h. The sample was centrifuged for 20 min. The final assay mixture was prepared by mixing 0.1 ml of the filtered aliquot, 2.7 ml 0.1M phosphate buffer (pH 7.4) and 0.2 ml of 100 mM DTNB. The mixture developed yellow color which was read at 412 nm using spectrophotometer.[23]

Glutathione-S-transferase assay (GST)

Activity was assayed by Habig *et al* method. The 2ml of reaction mixture consists of 0.3 ml of brain homogenate sample, 1.475 ml of 0.1 M phosphate buffer (pH 6.5), 0.2 ml of 1 mM reduced glutathione and 0.025 ml of 1 mM CDNB (1-Chloro 2,4-dinitrobenzene). The absorbance of different samples was recorded at 340 nm. The enzyme activity was calculated as nM CDNB conjugate formed/min/mg protein.[24]

Glutathione reductase assay (GSR)

Carlberg and Mannervik method was used for this. The reaction solution (2ml) composed of 0.1 ml homogenate, 1.65 ml of 0.1 M phosphate buffer (pH 7.6), 0.1 ml 0.1 mM NADPH, 0.1 ml 0.5 mM EDTA and 0.05 ml 1 mM oxidized glutathione the activity of enzyme was estimated by measuring disappearance of NADPH at 340 nm and was calculated as nM NADPH oxidized/min/mg protein.[25]

Glutathione peroxidase assay (GPx)

This activity was assayed by Mohandas *et al* method. The 2ml reaction mixture consists of 0.1 ml homogenate of brain, 1.49 ml of 0.1 M (pH 7.4) phosphate buffer, 0.1 ml 1 mM sodium azide, 0.05 ml (1 IU/ml) glutathione reductase, 0.05 ml 1 mM GSH, 0.1 ml (1 mM) EDTA, 0.1 ml 0.2 mM NADPH (nicotinamide adenine dinucleotide) and 0.01 ml 0.25 mM H_2O_2. The disappearance of NADPH at 340 nm was recorded.[26]

Lipid peroxidation Estimation

MDA is used to indicate the level of lipid peroxidation. This was assayed by heat induced acid reaction following Slater and Sawyer method in which 0.1 ml brain homogenate mixed 1.0 ml 10% trichloro acetic acid (TCA) and 1.0 ml 0.67% thiobarbituric acid (TBA) to form the reaction mixture. This was placed in a water bath for 30 min and after that, it was kept on crushed ice for 10 mins to develop pink color. The ice-cold solution was centrifuged and supernatant absorbance was read at 532 nm using spectrophotometer against blank. The results were expressed as nM MDA/mg protein.[27]

Statistical analysis

All the data are presented as mean ± SEM. The significance of the difference in means between sham operated, control, standard and treated animals for different parameters were determined by using One-way Analysis of Variance (ANOVA) followed by Dunnett's multiple comparison tests. Data were considered statistically significant at $P < 0.05$ and highly significant at $P<0.0001$. Statistical analysis was performed using Graph pad prism 7.03 software, San Diego, CA, USA.

RESULTS

Effect of EDI and CDI on neurological deficits

Garcia modified neurological deficit score includes various subtests that cover the areas of spontaneous activity, mobility, motor coordination and sensory aspects. By this, we evaluated the stroke induced functional deficits in control group and effect of EDI and CDI on that impairments. In this system, maximum the score of normal activity is 18. Less the score more is the deficit. As shown in Figure 1, the control group significantly ($p<0.0001$) decreased the maximum score from 14.7± 0.57 to 8.93±0.50. The EDI-2 showed significant ($p<0.01$) improvement in the neurological scores compared to control group. EDI-1, CDI-1 and CDI-2 groups exhibited non-significant ($p>0.05$) effect on neurological scores.

Effect of EDI and CDI on LPO levels

Lipid peroxidation levels in the brain homogenate of control animals were found to be significantly ($p<0.0001$) high compared to sham animals, while treatment with an ethanolic extract of DI significantly ($p<0.0001$) decreased these elevated levels compared to ischemic control group. The chloroform extract slightly reduced LPO levels with no significant effect. The results were given in Table 1.

Effect of EDI and CDI on enzymatic and non-enzymatic glutathione (GSH, GPx, GR, and GST) Levels

Because of an ischemic effect, a significant reduction in GSH, GPx, GR and GST levels in brain homogenate of control rats was seen compared to sham rats. These defensive glutathione enzyme levels were significantly ($p\leq 0.0001$) improved by treatment with high dose of EDI compared to ischemic control group. The EDI-1, CDI-1, and CDI-2 had not significantly increased the GSH, GPx, GR and GST levels. This indicates the antioxidant potential of the extract fractions seen in Table 2.

Effect of EDI and CDI on SOD and CAT

Ischemia significantly ($p\leq 0.0001$) reduced the SOD and CAT levels in control groups compared to sham operated group. Both EDI-1 and EDI-2 significantly ($p\leq 0.001$ and $p\leq 0.0001$) improved enzyme levels depleted in control group respectively whereas CDI showed the slight effect which was insignificant Table 1.

Table 1: Effect of various doses of EDI and CDI on biochemical parameters of rat ischemic brain.

Groups	Dose	SOD	CAT	LPO	GSH
		(U/mg protein)	(nmol H_2O_2/min/mg protein)	(nmol MDA/mg protein)	(nmol DTNB oxidized/ min/mg protein)
SHAM	% tween 80	8.69±0.35	122.00±1.54	4.10±0.35	1.99±0.09
CONTROL	% tween 80	4.63±0.47[a]	75.45±1.35[a]	9.65±0.12[a]	0.82±0.05[a]
EDI-1	200 mg/kg	6.65±0.38[b***]	94.47±4.16[b***]	6.37±0.31[b]	0.93±0.03[c]
EDI-2	400 mg/kg	7.59±0.21[b]	118.34±6.71[b]	4.13±0.63[b]	1.38±0.13[b]
CDI-1	200 mg/kg	4.49±0.15[c]	77.52±2.47[c]	7.11±0.75[b***]	0.82±0.06[c]
CDI-2	400 mg/kg	5.19±0.67[c]	87.38±1.62[b**]	6.89±0.39[b**]	0.95±0.11[c]

Values are reported as Mean ± SD (n=8), [a] $p<0.0001$ compared to sham, [b]$p<0.0001$, [b***]$p<0.001$, [b**]p, 0.01, [b*]$p<0.05$ and [c]$p>0.05$ compared to control. P-mg/protein.

Table 2: Effect of different doses of EDI and CDI on Glutathione related enzyme levels in rat brain.

Groups	Dose	GPx	GST	GR
		(nmol NADPH oxidized/ min/mg protein)	(nmol CDNB conjugate/ min/mg protein)	(nmol NADPH oxidized/ min/mg protein)
SHAM	% tween 80	93.90±3.36	80.20±0.89	64.60±1.32
CONTROL	% tween 80	48.20±1.84[a]	25.10±0.80[a]	23.54±2.62[a]
EDI-1	200 mg/kg	52.70±2.81[C]	40.67±9.32[b**]	36.73±2.75[b**]
EDI-2	400 mg/kg	69.84±2.63[b**]	56.39±2.36[b]	47.97±1.83[b]
CDI-1	200 mg/kg	50.36±2.31[C]	26.71±3.77[C]	27.86±1.45[C]
CDI-2	400 mg/kg	51.95.20±4.29[C]	32.54±0.29[b**]	34.82±7.01[b**]

Values are reported as Mean ± SD (n=8), [a] $p<0.0001$ compared to sham, [b]$p<0.0001$, [b***]$p<0.001$, [b**]p, 0.01, [b*]$p<0.05$ and [C]$p>0.05$ compared to control.
Gpx –Glutathione peroxidase, GST-Glutathione S transferase and GR- Glutathione Reductase

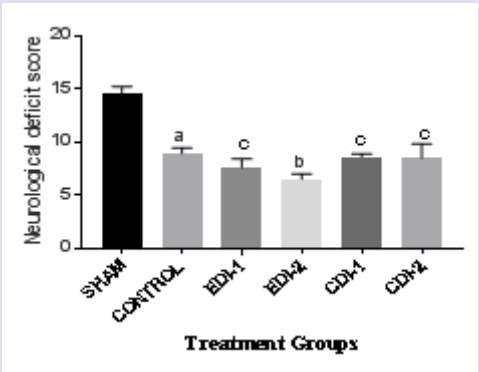


Figure 1: Effect of extract fractions on Garcia modified neurological deficit score system.

DISCUSSION

Ischemic stroke is the major cause of death and severe physical disability in industrialized countries and accounts for 80% of all strokes.[28] Multiple pathological processes that take place in ischemia are an overproduction of oxidants, inactivation of detoxification systems, excitotoxicity, inflammation and disruption of membranes.[29] The imbalance between the production of free radicals and body's counter mechanisms to eliminate those results in oxidative stress.[30] Various experimental studies strongly support the association between oxidative stress and ischemic damage. In the present study, the neurochemical and behavioral alterations in ischemic reperfusion rats and effect of *Dalechampia indica* extract fractions on them was evaluated. For this, cerebral ischemia in rats was induced by two vessel occlusion (2VO) along with intermittent reperfusion along with the ferric chloride induced thrombosis in right common carotid artery. After inducing stroke, the behavioral and antioxidant status of brain tissue of all animal groups were assessed.

Neurological deficit scores were measured to know, to what extent the model was successful in inducing brain damage and extract fractions (EDI and CDI) effect on it. As these behavioral deficits reflect the ischemic damage, the severity of scores is directly related to extent of cerebral damage.[31] In the present study, Low scores of controls group indicates a high deficit in neurological function which has been improved by different doses of EDI and CDI which might be due to the neuroprotectant phytoconstituents present in it.

Ischemic reperfusion leads to enhanced production of reactive oxygen species such as superoxide anions, nitric oxide, free radicals and hydrogen peroxide that directly reacts with various cellular components such as nucleic acid, lipids and proteins resulting in cellular death.[32] Reactive species that cause damage have a very short half-life, which makes them very difficult to measure directly.so, we measure the antioxidant ability of the brain tissue as biomarker of oxidative stress and lipid peroxidation as biomarker of oxidative product formed during ischemic reperfusion.[33] SOD, CAT, and glutathione (GSH) related enzymes (GPx, GST and GR) are the important ones that play key role in cleaning up the free radicals from our body.

SOD in the cells is the first soldier at command to defend against superoxide radical attack and protects the cells from its deleterious effect. Change in its concentration during stroke was strongly disputed. Glutathione (GSH) along with its army of enzymes such as glutathione peroxidase

(GPx), glutathione S transferase (GST) and glutathione reductase (GR) plays a major role in antioxidant defense system by scavenging free radicals and reactive species, prevents the oxidative damage. Next, comes the enzyme catalase (CAT) which is specific hydrogen peroxide scavenger. MDA, stable derivative of thiobarbituric acid is used as reliable one to measure the extent of lipid peroxidation.[34,35] In the present study, the brain tissue of control rats after 10 days of inducing stroke showed the decreased levels of enzymes (SOD, CAT, GPx, GST, GR, and GSH) and increased levels of lipid peroxidation compared to sham operated rats. This clearly indicates severe oxidative damage in the brain of control rats which has been minimised by preventive treatment with different doses of ethanolic and chloroform extract fractions of *Dalechampia indica* by improving the antioxidant status of the brain tissue and decreasing the lipid peroxidation. Even the behavioral deficits scores estimated in all group of animals were also correlated with ischemic damage. This significant reversal of neurochemicals and neurological deficits by extract fractions might be due to the antioxidant nature of phytochemicals such as poly phenolic compounds, alkaloids, tannins etc. present in them.[36-38]

CONCLUSION

The results of the present study suggest the potential role of an ethanolic fraction of *Dalechampia indica* in ischemic stroke over Chloroform fraction. EDI contains many active constituents with potent antioxidant effects and offered significant neuroprotection than CDI against ischemic reperfusion injury which may be attributed to decreased lipid peroxidation and increased endogenous antioxidant defense enzymes. However, further research is required for isolation of compounds responsible for protection.

ACKNOWLEDGEMENT

The authors thank Management, Anurag Pharmacy College for providing Lab facility.

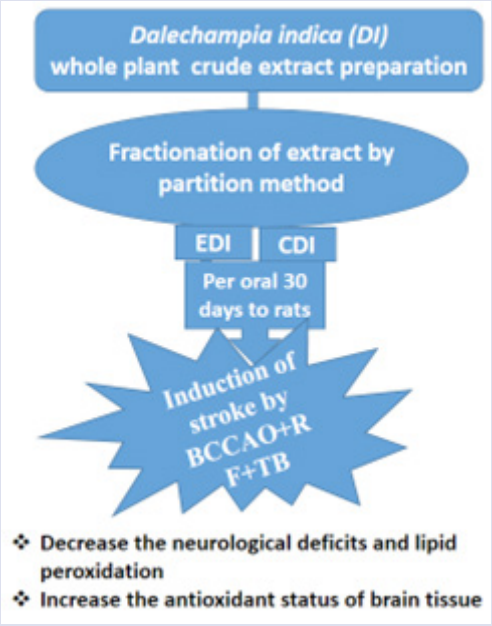
CONFLICT OF INTEREST

Authors declare that there is no conflict of interests regarding the publication of this paper.

REFERENCE

1. Philip Bath MW. Acute stroke. West J Med. 2000;173(3):209-12.
2. Donnan GA, Fisher M, Macleod M, Davis SM. Stroke. Lancet. 2008;371:1612-23.
3. Sims NR, Muyderman H. Mitochondria, oxidative metabolism and cell death in stroke. Biochemical Biophysical Acta 2010;1802(1):80-91.
4. Allen CL, Bayraktutan U. Oxidative stress and its role in the pathogenesis of ischaemic stroke. Inter J Stroke. 2009;4(6):461-70.
5. Hazell AS. Excitotoxic mechanisms in stroke: an update of concepts and treatment strategies. Neurochemistry Inter. 2007;50(7-8):941-53.
6. Jin R, Yang G, Li G. Inflammatory mechanisms in ischemic stroke: the role of inflammatory cells. J Leukocyte Biol. 2010;87(5):779-89.
7. Bramlett HM, Dietrich WD. Pathophysiology of cerebral ischemia and brain trauma: similarities and differences. J Cerebral Blood Flow Metabolism. 2004;24(2):133-50.
8. Natalie T, Cheng, Anthony S. Kim. Intravenous Thrombolysis for Acute Ischemic Stroke within 3 H versus Between 3 and 4.5 H of Symptom Onset. Neurohospitalists. 2015;5(3):101-9.
9. Ghosh N, Ghosh R, Bhat ZA, Mandal V, Bachar SC, Nima ND, *et al*. Advances in herbal medicine for treatment of ischemic brain injury. Nat Prod Communication. 2014;9(7):1045-55.
10. Jose R, Romero, Jane M, Aleksandra P. Stroke prevention: modifying risk factors. There Adv Cardiovasc Dis. 2008;2(4):287-303.
11. Matthew KM. An Excursion Flora of Central Tamil Nadu, India. New Delhi: Oxford and IBH Publishing Company.1995: pp.452.
12. Sindhura S, Dr. Eswaraiah MC. Comparative Evaluation of Crude Extract Fractions of the Whole Plant of Taxillus heyneanus and *Dalechampia indica* for Antioxidant Activity, Total Phenolic and Flavonoid Content. IOSR Journal of Pharmacy. 2017;7(5):53-60.
13. Harborne JB. 2nd ed. London: Chapmann and Hall; 1984. Phytochemical Methods. A Guide to Modern Techniques of Plant Analysis; p.192.
14. Organization for Economic Cooperation and Development. OECD guideline 423. Oral Toxicity Study in Rodents. OECD guideline for the testing of chemicals. 2001;423:1-8.
15. Shah ZA, Vohora SB. Antioxidant/restorative effects of calcined gold preparations used in Indian systems of medicine against global and focal models of ischaemia. Pharmacology Toxicology. 2002;90(5):254-9.
16. Huaien Z, Jerome L, Siegfried H. Life-long protection from global cerebral ischemia and reperfusion in long-lived Mclk1+/– mutants. Experimental Neurology. 2010;223(2):557-65.
17. Lukas JS, Matthias E, Alexander K. Bilateral Common Carotid Artery Occlusion as an Adequate Preconditioning Stimulus to Induce Early Ischemic Tolerance to Focal Cerebral Ischemia. J Vis Exp. 2013;(75):4387.
18. Bonnard T, Hagemeyer CE. Ferric Chloride-induced Thrombosis Mouse Model on Carotid Artery and Mesentery Vessel. J Vis Exp. 2015;29(100): 1-6(2015;29(100):e52838-)
19. Garcia JH, Wagner S, Liu KF, Hu XJ. Neurological deficit and extent of neuronal necrosis attributable to middle cerebral artery occlusion in rats. Statistical validation. Stroke. 1995;26(4):627-34.
20. Chunlin Yan, Ji Zhang, Shu Wang, Guiping Xue, Yong Hou. Neuroprotective effects of rutaecarpine on cerebral ischemia reperfusion injury. Neural Regen Res. 2013;8(22):2030-8.
21. Misra HP, Fridovich I. The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. Journal of Biological chemistry. 1972;247(10):3170-5.
22. Claiborne A. Catalase activity. In: Handbook of Methods for Oxygen Radical Research. Greenwald Ra (ed), CRC Press, Boca Raton, FL. 1985: pp.283-4.
23. Jollow DJ, Mitchell JR, Zampaglione N, Gillette JR. Bromobenzene-induced liver necrosis. Protective role of glutathione and evidence for 3,4-bromobenzene oxide as the hepatotoxic metabolite. Pharmacology. 1974;11(3):151-69.
24. Habig WH, Pabst MJ, Jakoby WB. Glutathione S-transferases. The first enzymatic step in mercapturic acid formation. J Biological Chem. 1974;249(22):7130-9.
25. Carlberg I, Mannervik EB. Glutathione level in rat brain. J Biological Chem. 1975;250:4475-80.
26. Mohandas J, Marshal JJ, Duggin GG, Horvath JS, Tiller DJ. Differential distribution of glutathione and glutathione-related enzymes in rabbit kidney. Possible implications in analgesic nephropathy. Biochemical Pharmacology. 1984;33(11):1801-7.
27. Slater TF, Sawyer BC. The stimulatory effects of carbon tetrachloride and other halogenoalkanes or peroxidative reactions in rat liver fractions *in vitro*. Biochemistry J. 1971;123(5):805-14.
28. Towfighi A, Saver JL. Stroke declines from third to fourth leading cause of death in the United States: historical perspectives and challenges ahead. Stroke. 2011;42(8):2351-5.
29. Chamorro A, Dirnagl U, Urra X, Planas AM. Neuroprotection in acute stroke: targeting excitotoxicity, oxidative and nitrosative stress, and inflammation. Lancet Neurol. 2016;15(8):869-81.
30. Siesjo BK, Agardh CD, Bengtsson F. Free radicals and brain damage. Cerebrovascular Brain Metabolism Rev. 1989 Fall;1(3):165-211.
31. Ma SW, Zhang XT, He SJ. Protective effects of ginkgolide B on cerebral ischemia reperfusion injury in rats. Zhongguo Yaoxue Zazhi. 2011;46(13):993-7
32. Warner DS, Sheng H, Batinic-Haberle I. Oxidants, antioxidants and the ischemic brain. J Experimental Biol. 2004;207(18):3221-31.
33. Rodrigo R, Fernandez-Gajardo R, Gutierrez R, Matamala JM, Carrasco R, Miranda-Merchak A, *et al*. Oxidative stress and pathophysiology of ischemic stroke: novel therapeutic opportunities. CNS Neurological Disorders Drug Targets. 2013;12(5):698-714.
34. Maral J, Puget K, Michelson AM. Comparative study of superoxide dismutase, catalase and glutathione peroxidase levels in erythrocytes of different animals. Biochemistry Biophysical Res Communication. 1977;77(4):1525-35.
35. Zaidi SM, Al-Qirim TM, Banu N. Effects of antioxidant vitamins on glutathione depletion and lipid peroxidation induced by restraint stress in the rat liver. Drugs R,D. 2005;6(3):157-65.
36. Kim HK, Kim M, Kim S, Kim M, Chung JH. Effects of green tea polyphenol on cognitive and acetylcholinesterase activities. Bioscience Biotechnology Biochemistry. 2004;68(9):1977-9.
37. Kiran PS, Richard AA. Effect of Polyphenols on Oxidative Stress and Mitochondrial Dysfunction in Neuronal Death and Brain Edema in Cerebral Ischemia. Inter J Mole Sci. 2011;12(11):8181-207.
38. Peng-Fei WU, Zui Z, Fang W, Jian-Guo C. Natural compounds from traditional medicinal herbs in the treatment of cerebral ischemia/reperfusion injury. Acta Pharmacologica Sinica. 2010;31(12):1523-31.

GRAPHICAL ABSTRACT



SUMMARY

- Pre-treatment with EDI (Ethanol) & CDI (Chloroform) fraction of extract decreased the neurological damage by improving antioxidant status (SOD, CAT, GPx, GSH, GR, GST) and decreasing the lipid peroxidation of brain tissue.
- Results showed EDI has better cerebroprotective effect than CDI.

ABOUT AUTHORS

Mrs. Sindhura Srinivasula: Research Scholar of JNTUH, Department of Pharmaceutical sciences, Hyderabad, India under the supervision of Dr. ChinnaEswaraiah M. Her research is focused on the effects of medicinal plants and natural products on the animal models of cerebral stroke and also their toxicities and other biological activities.

Dr. Chinna Eswaraiah Maram: Professor & principal in Anurag Pharmacy College,Kodad (M), Telangana,India guided several Ph.Dstudents and published 77 Research papers in National & International professional journals. He is having life memberships from various professional bodies like Fellow Indian chemistry (FIC), Association of Pharmaceutical Teachers of India (APTI) and Society of Pharmacognosy. He is Executive and Associate Editor of few International and National Journals in Pharmacy, Biotechnology and Applied Sciences.

Cite this article: Sindhura S, Eswaraiah MC. Effect of *Dalechampia indica* Wight Extract Fractions on Behavioral and Biochemical Abnormalities following Ischemia-Reperfusion Insult in Rats. Pharmacog J. 2018;10(1):39-44.