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Determination of Possible Mechanism of Cerebroprotective Action of flavonoid of *Dalbergia latifolia* against Cerebral Ischemia Reperfusion Induced- Cerebral Infarction in Rats.

Sudhir C Kulkarni*, Nagarathna PKM, C Vasanthkumar, and N Srisainadh

Department of Pharmacology, Karnataka College of Pharmacy, Bangalore, Karnataka - 560064, India

Research Article

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***For Correspondence**

Department of Pharmacology, Karnataka College of Pharmacy, Bangalore, Karnataka - 560064, India
Mobile: +91 9538011523.

Keywords: Cerebral ischemia, stroke, bilateral common carotid artery occlusion, cerebral infarction, oxidative stress, flavonoids, Anti-oxidants.

ABSTRACT

Dalbergia latifolia is commonly called as "Beete and sitsal". In the present study the mechanism of cerebroprotective action of methanolic extract of bark of *Dalbergia latifolia* (DL) was carried out against the global model of ischemia in rats. The animals were pre-treated with DL for a period of 1week (500 mg/kg) p.o. All the inhibitors were given 10 min prior to the occlusion i.p. The animals were anaesthetized with thiopentone sodium (45mg/kg) and infarction was induced by Bilateral Common Carotid Artery Occlusion (BCCAO) for 30 mins with the help of suture threads, the knots were released with the help of slip knots. The animals were allowed to re-perfuse for a period of 48 hrs. Then the animals were sacrificed brain was decapitated. Biochemical estimations for Super oxide dismutase, Catalase, Myeloperoxidase & Malondialdehyde and histological examinations were carried out. The results obtained showed that the *Dalbergia latifolia* extract is a potent cerebroprotective with a possible mechanism of Cyclooxygenase (COX) pathway inhibitor, as it shown the synergistic activity along with the COX inhibitor.

INTRODUCTION

Stroke is one of the leading causes of death and disability in the world. WHO has estimated that between 1990 to 2020 the world will witness an increase in stroke mortality of 78% in woman and 106% in man [1]. Stroke is the world's second leading cause of mortality, resulting around 6,000,000 deaths annually [2]. It is estimated that the lifetime risk for stroke is between 8% and 10% [3]. Pathogenically, stroke involves a heterogeneous group of processes. Vessel occlusions (ischemic stroke) account for 85% of all strokes, while primary intracerebral bleeding (hemorrhagic stroke) accounts for the remainder [4]. Ischemic stroke accounts for 87% of all strokes. Among persons aged 45 to 64 years, 8% to 12% of ischemic strokes result in death within 30 days [5].

Flavonoids possess a highly reactive hydroxyl group that gets oxidized by electron-donation, thus stabilizing the radical to a less reactive molecule. One way of reaction is the direct scavenging of free radicals, for example superoxide anions, singulet oxygen and lipid peroxyl radicals. Structurally important features defining the reducing potential of flavonoids are the hydroxylation pattern, α 3,4'-dihydroxy catechol structure in the B-ring, the planarity of the molecule and the presence of 2,3-unsaturation in conjugation with a 4-oxo function in the C-ring. There is considerable evidence that flavonoids efficiently attenuate the deleterious effect of free radicals and reactive oxygen and reactive nitrogen species (ROS/RNS). Quercetin and some structurally related flavonoids, for example, showed a marked cytoprotective effect in PC12 cells exposed to H_2O_2 [6]. Also in PC12 cells, the addition of flavonoids or flavonoid-rich extracts suppressed the hydroperoxideinduced increase in ROS, thus improving cell survival [7, 8].

Suzuki et al. [9] administered catechin extract in drinking water to rats for five days before and during middle cerebral artery occlusion (MCAO) and reperfusion to examine their protective effects on various deteriorative processes following stroke. Catechins significantly ameliorated neurological deficits observed after reperfusion by the inhibition of inducible Nitric Oxide synthase (iNOS) expression and infiltration of neutrophiles. In addition, the

formation of peroxynitrite was found to be decreased due to the potent radical scavenging properties of catechins. Hong et al. [10] used green tea extract in the drinking water ad libitum for 3 weeks before ischemia in gerbils. This treatment reduced the infarct volume, the number of apoptotic cells, and lipid peroxidation, and inhibited the ischemia-induced hyperactivity.

Dalbergia latifolia is a large glabrous tree with a single stem and having a characteristic smell [11]. It is distributed in Bihar, Bundelkhand and Central India [12]. It contain dalbinol a new 12α-hydroxyrotenoid [13], sisafolin coumarin from seeds, β- sitosterol, also contain dalbergichromene, lupeol, latifolin and dalbergin from bark of the tree, heartwood contains latinone, neoflavonoid dalcriodon [14] and latinone, a substituted phenanthrene-1, 4-quinone was isolated from *Dalbergia latifolia* [15]. Ethanomedicinally, the stem barks contain tannin is used for treatment of leprosy, obesity and worm [12]. EtOH (50%) extract of the bark exhibits spasmogenic, and anthelmintic activity against *Ascaridia galli* [16]. Ethanomedicinally, the stem bark contain tannin is used for treatment of leprosy and worm [17]. Many species of Dalbergia are important timber trees, valued for their decorative and often fragrant wood, rich in aromatic oils [18, 19]. Traditionally various species are reported to be used as aphrodisiac, abortifacient, expectorant, anthelmentic, antipyretic, appetizer, allays thirst, vomiting, burning sensation, cures skin diseases, ulcers, diseases of the blood, reduces obesity, used in leucoderma, dyspepsia, dysentery, for diseases of the eye and nose, syphilis, stomach troubles, leprosy, leucoderma, scabies and ringworm [20].

MATERIALS AND METHODS

Collection of plant material

The bark part of *Dalbergia latifolia* was collected in the month of june-2012 from forest of Tirupati and it was identified and authenticated by K.Madhava chetty, and the voucher specimen was deposited in Department of Pharmacology, Karnataka College of Pharmacy, Bangalore. The plant material was thoroughly cleaned to remove the adhering soil, mud and debris. The bark was dried in the shade at room temperature to a constant mass. The plant material was coarsely powdered using blender. The powder was stored in a airtight container protected from light.

Preparation of extract

The plant material obtained was subjected to grinding using a mechanical blender to get fine powder of uniform size. About 250gm of the powder was treated with methanol in a soxhelet apparatus for 2hrs. The liquid extract thus obtained was subjected for evaporation to remove the excess of solvent under shade, which will give a solid mass of the drug extract. The yield of the extract was calculated and stored for further use. The solid methanolic drug extract was further subjected to column chromatography for isolation of flavonoid, the flavonoid presence in the drug was confirmed with the help of chemical tests for flavonoids, adsorption column chromatography and TLC were used to purify the flavonoid. Spectral data was used to predict the possible structure of the compound.

Experimental animals

Albino male wistar rats (8-10 weeks old) weighing 250-280g, were used for the experiment. They were acclimatized for one week prior to experiment. Animals were caged in fully ventilated room, were maintained in 12:12 h light and dark cycle and were housed at temperature of 25 ± 2°C. They had free access to a standard chow diet and water *ad libitum*. All the experiments conducted on the animals were in accordance with the standards set for the use of the laboratory animal use and the experimental protocols were duly approved by the IAEC (Institutional Animal Ethical Committee).

Experimental design

Rats were randomly divided into 10 groups, 6 animals per group.

- Group 1: Ischemic reperfusion (I/R)
- Group 2: Sham(Surgical incision)
- Group 3: Vehicle
- Group 4: *Dalbergia latifolia* (DL)
- Group 5: L-NAME
- Group 6: L-NAME + DL
- Group 7: Nimesulide
- Group 8: Nimesulide +DL
- Group 9: Allopurinol
- Group 10: Allopurinol + DL

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Induction of cerebral ischemia

Animals were anesthetized by giving thiopentone sodium (45 mg/kg) i.p. Occlusion: Surgical technique for the induction of cerebral ischemia was adopted from the earlier published method of Anshuman Trigunayat 2009. Under anesthesia midline incision was given. Common carotid arteries were identified and isolated carefully from Vago-sympathetic nerve. Ischemia was induced by occluding bicommon carotid arteries (BCCAO) with thread for 30 min and reperfusion was allowed for 48 hr by removing the thread. Neck incision area was closed with thread using suture. The DL group animals were given pre-treatment for 7 days p.o (500mg/kg), all the inhibitors were given (Allopurinol-10mg/kg, L-NAME-10mg/kg and Nimesulide- 20mg/kg) 10 min before occlusion.

After the period of re-perfusion the animals were sacrificed and the brain was decapitated, bio chemical estimations were carried out. SOD activity was determined by the method developed by Kakkar *et al*, [21]., CAT activity was measured by the method of Aebi *et al*, [22]., MDA activity was measured by the method of Okhawa *et al*, [23] and MPO activity was measured by the method of Mullane *et al,* [24]. Brain tissues were further subjected histopathological studies and the results obtained were compared.

OBSERVATIONS AND RESULTS

In I/R group: The cerebral levels of MDA and MPO were significantly increased and decreased levels of SOD and Catalase found (Figure 1-4). The histopathological studies also showed there were neuronal degeneration, glial cell proliferation, congestion, hemorrhage, lymphocyte infiltration and vacuolation which indicated damage to the brain (Figure-5).

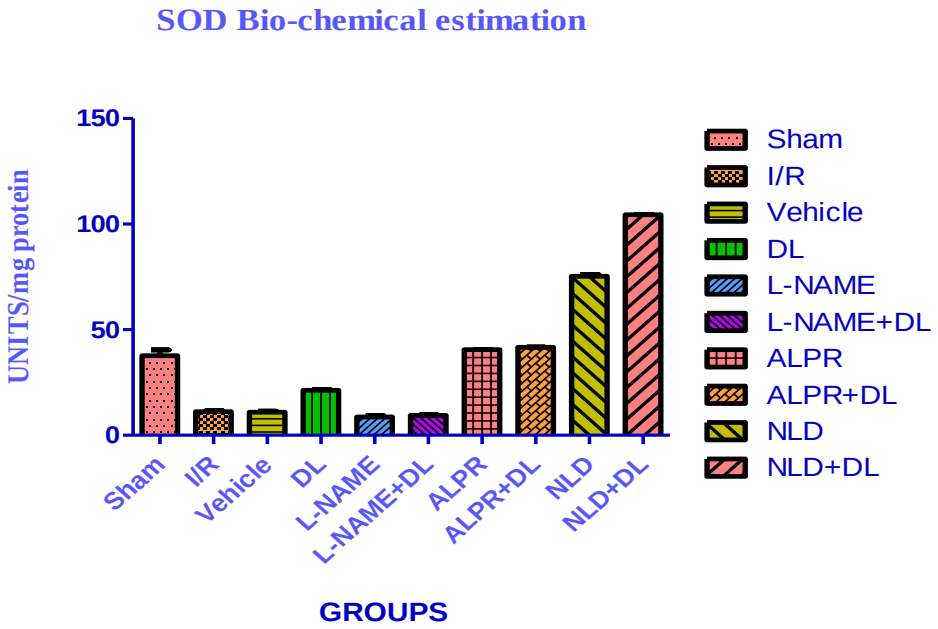

Figure 1: Histogram representing effect on cerebral SOD levels after treatment protocol.

In sham group: The cerebral levels of the SOD, CAT, MPO and MDA showed no neurological deficit (Figure 1-4). The histopathological studies also showed there was no neuronal degeneration in the tissue (Figure-6).

In vehicle group: The cerebral levels of SOD, CAT, MDA and MPO were nearer to the values of the I/R group (Figure 1-4). The histopathological studies also showed neuronal degeneration, congestion and mild vacuolation (Figure-7).

In Allopurinol group: The cerebral levels of SOD and CAT increased significantly where as the levels of MDA and MPO significantly decreased when compared to the I/R group (Figure 1-4). The histopathological studies also supported the biochemical estimations showing minimum damage to the brain, mild vacuolations were seen (Figure-11).

In Allopurinol+DL group: The cerebral levels of the SOD and CAT increased significantly where as the levels of MDA and MPO significantly decreased similar to that off the Allopurinol group when compared with the I/R group (Figure 1-4). The histopathological reports also showed that there was little damage to the brain and along with that mild congestion was observed (Figure-12).

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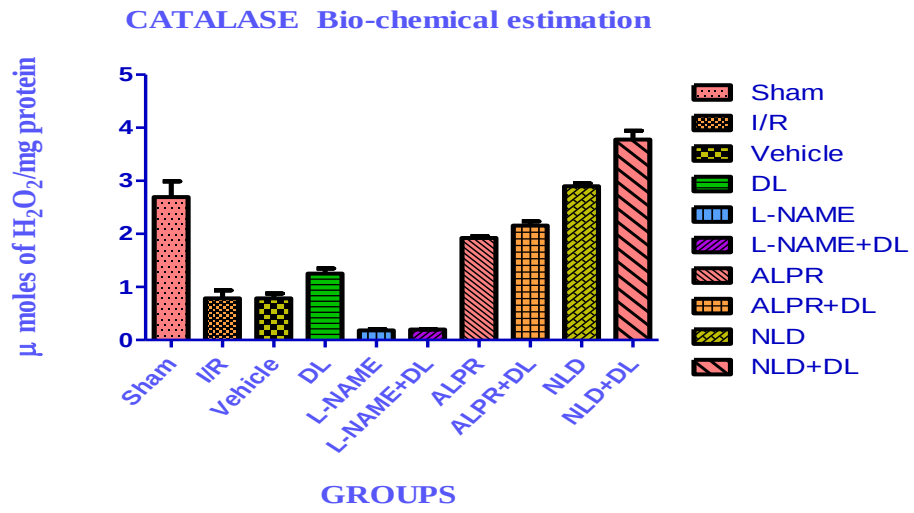

Figure 2: Histogram representing effect on cerebral catalase levels after treatment protocol.

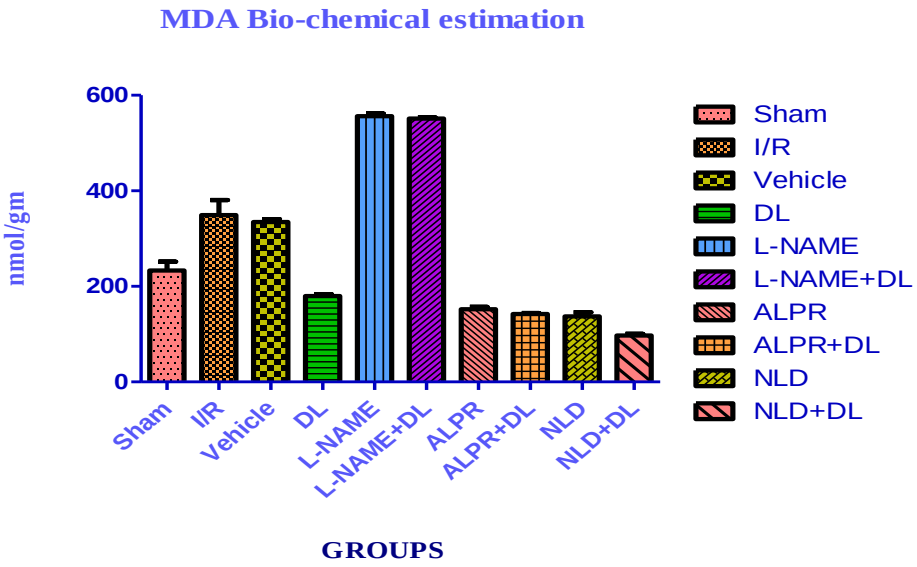

Figure 3: Histogram representing effect on cerebral MDA levels after treatment protocol.

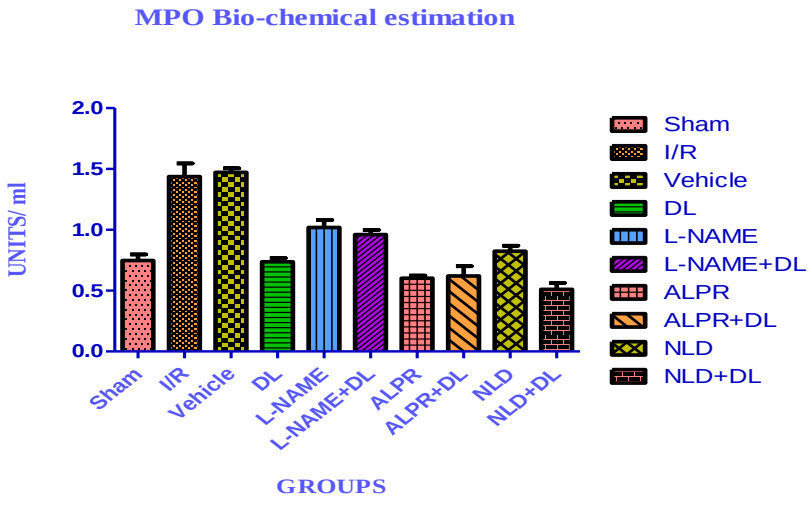

Figure 4: Histogram representing effect on cerebral MPO levels after treatment protocol.

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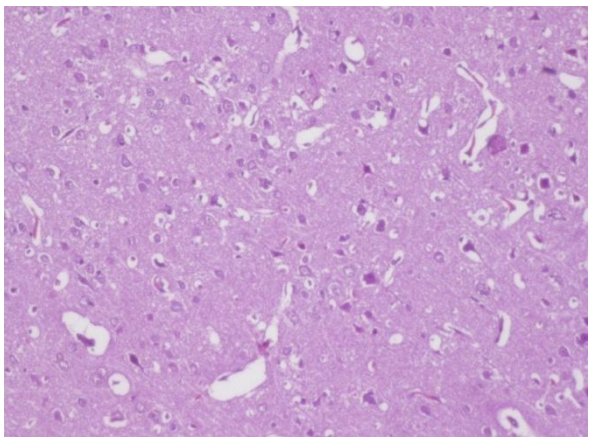

Figure 5: Histopathological slide of I/R group.

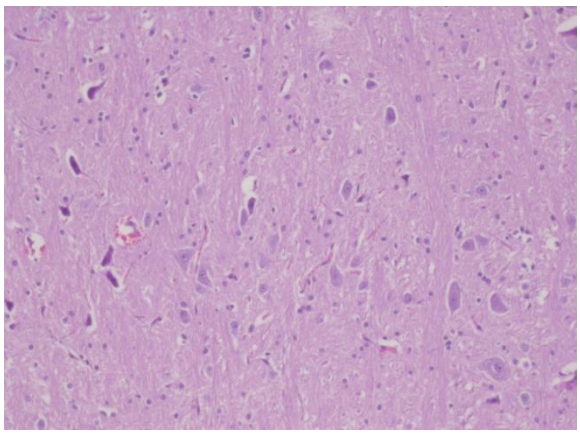

Figure 6: Histopathological slide of sham group.

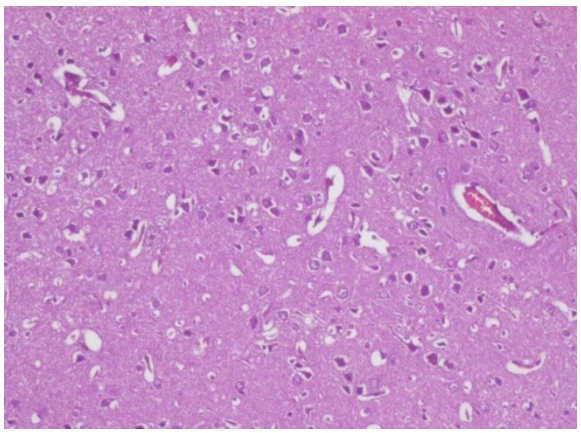

Figure 7: Histopathological slide of vehicle group.

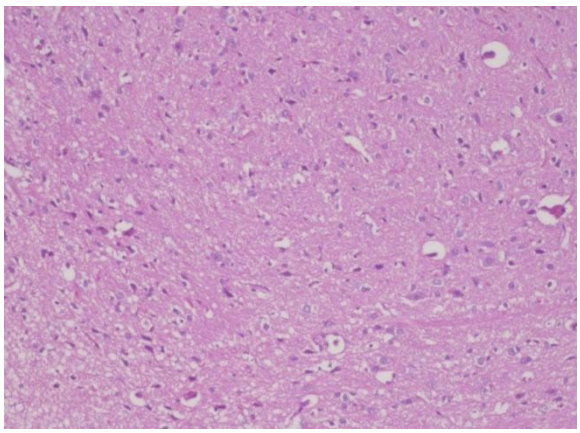

Figure 8: Histopathological slide of DL group.

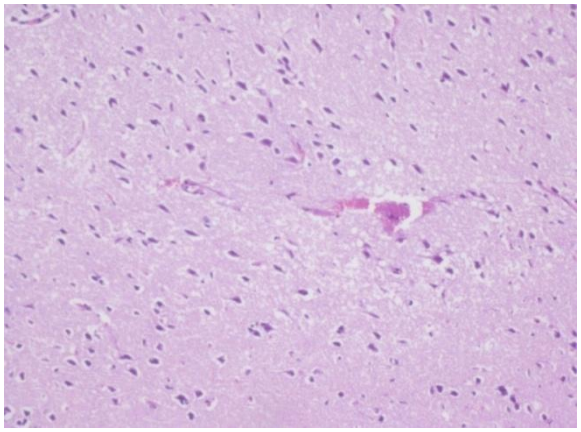

Figure 9: Histopathological slide of L-NAME group.

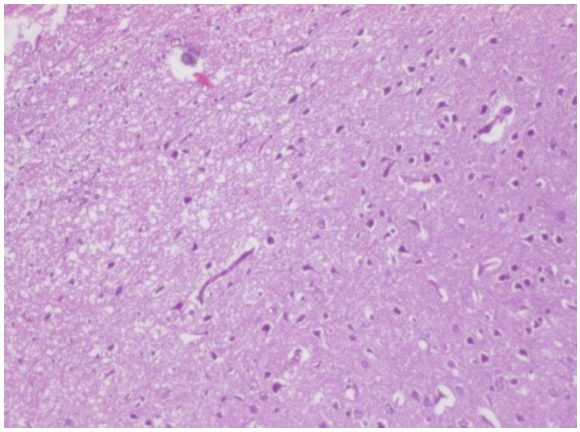

Figure 10: Histopathological slide of L-NAME+DL group.

In DL group: The cerebral levels of SOD and CAT were significantly increased and the levels of MDA, MPO significantly decreased when compared with the I/R group (Figure 1-4). The histopathological studies showed minimum of neuronal degeneration, mild vacuolation relatively lower congestion (Figure-8).

In L-NAME group: The cerebral levels of SOD and CAT decreased where as the levels of MDA and MPO significantly increased showing damage to the brain (Figure 1-4). The histopathological results showed that there was a considerable amount of neuronal damage, lymphocyte infiltration and vacuolation (Figure-9).

In L-NAME+DL group: The cerebral levels of SOD and CAT, decreased and the levels of MDA and MPO significantly increased when compared to the I/R group (Figure 1-4). The histopathological studies also showed the neuronal degeneration, congestion and vacuolation (Figure-10).

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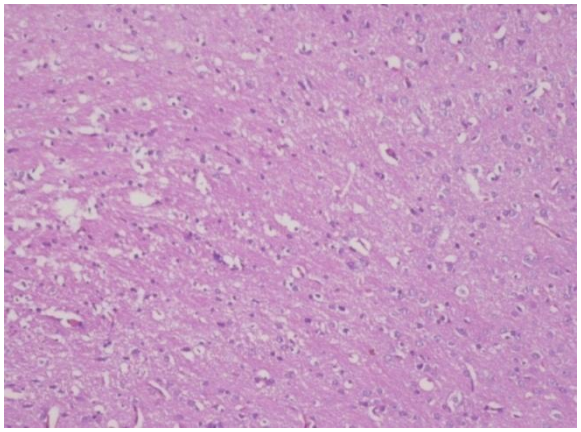

Figure 11: Histopathological slide of Allopurinol group.

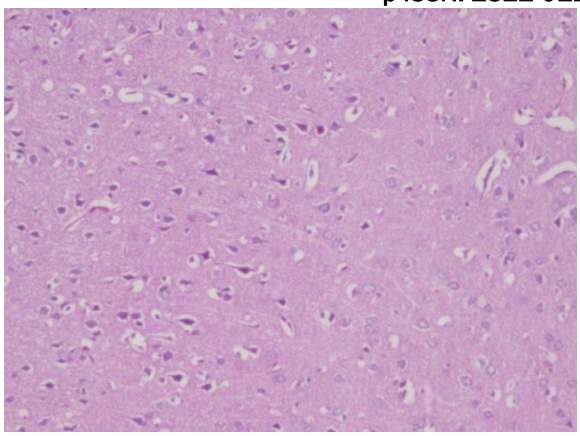

Figure 12: Histopathological slide of Allopurinol+DL group.

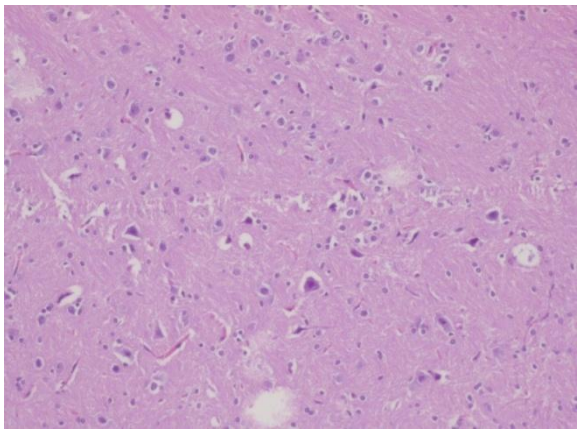

Figure.13: Histopathological slide of Nimesulide group.

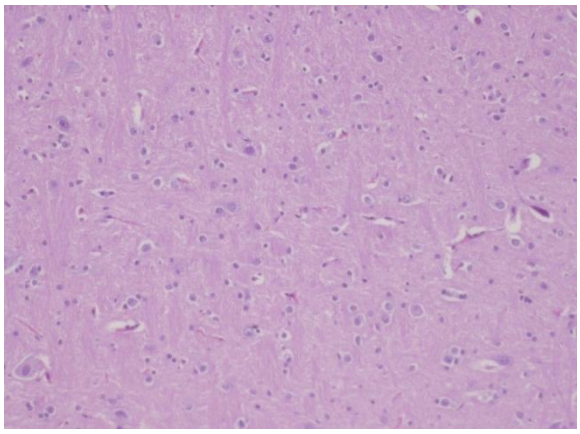

Figure.14: Histopathological slide of Nimesulide+DL group.

In Nimesulide group: The cerebral levels of the SOD, CAT were significantly increased and the levels of MDA, MPO significantly decreased when compared to that of I/R group (Figure 1-4). The histopathological studies also showed that there was no neuronal degeneration in case of this group and the cellular components seemed to be normal (Figure-13).

In the Nimesulide+DL group: The cerebral levels of the SOD, CAT were significantly increased and the levels of MDA, MPO significantly decreased when compared to that of I/R group similar as in Nimesulide group (Figure 1-4). The histopathological studies also showed not much considerable neuronal damage only mild vacuolations were seen (Figure-14).Oxidative stress is believed to be a major source for generation of post cerebral ischemic injury. Various experimental models of cerebral ischemic reperfusion injury showed significant neuroprotection when treated with antioxidants [25]. Reactive oxygen species have been denoted as one of the earliest and most important components of tissue injury after reperfusion of ischemic organ and the extent of brain injury appears to depend on the experimental pattern of ischemia/reperfusion: free radical production is continuous during ischemia, while during reperfusion it is primarily confined to the early stage when fresh oxygen is supplied to the ischemic region. The brain is very susceptible to the damage caused by oxidative stress, due to the high rate of oxidative metabolic activity, high polyunsaturated fatty acid contents, relatively low antioxidant capacity and inadequate neuronal cell repair activity. Oxidative damage includes the excessive production of reactive oxygen species, including lipid peroxidation, protein oxidation and DNA damage, which can lead to cell death. Furthermore, reactive oxygen species can activate diverse downstream signaling pathways, such as xanthine oxidase (XO) pathway, over expression of cyclooxygenase-2 (COX-2) and of inducible nitric oxide synthase (iNOS) have recently emerged as important determinants of post-ischemic inflammation, which contributes to the progression of brain damage [26]. Lipid peroxidation has been established as a major mechanism of cerebral injury. The mechanism involves a process whereby unsaturated lipids are oxidized to form additional radical species as well as toxic by-products that can be harmful to the host system [27]. Polysaturated lipids are especially susceptible to this type of damage when in an oxidizing environment and they can react to form lipid peroxides [28]. Lipid peroxides are themselves unstable and undergo additional decomposition to form a complex series of compounds including reactive carbonyl compounds [29]. In the present study we observed increase in the tissue MDA activity in ischemic

reperfused brain when compared with the sham groups and the results were in agreement with previous studies. Treatment with *Dalbergia latifolia* (500mg/kg p.o) alone provided cerebroprotection but still more significant cerebroprotection was observed when DL was administered along with the Nimesulide (20mg/kg i.p) which showed the synergistic activity of DL along with Nimesulide. Combined treatment of DL+ L-NAME (10mg/kg i.p) showed no cerebroprotective activity and DL+Allopurinol (10mg/kg i.p) also showed some cerebroprotective activity indicating involvement of some Xanthine oxidase property of the DL. Reactive species can be decreased or eliminated by a number of enzymatic and nonenzymatic antioxidant mechanisms. SOD, which catalyzes the dismutation of the superoxide anion (O^{-2}) into hydrogen peroxide and molecular oxygen, is one of the most important antioxidative enzymes [30]. In the present study, SOD and CAT activity decreased in the ischemic reperfused group compare to sham group and the results were in agreement with previous studies. [30] This may be due to an excessive formation of superoxide anions. A decrease in SOD activity can result in the decreased removal of superoxide anions, which can be harmful to the brain. The decline in the enzyme level may be explained by the fact that excessive superoxide anions may inactivate SOD, thus, resulting in an inactivation of the H_2O_2 scavenging enzyme. The reduced SOD and CAT activity were increased by administration of the DL alone but the activity was significantly increased when the DL was administered with Nimesulide (20mg/kg). Less effective when given along with the L-NAME but slightly effective when administered along with Allopurinol (10mg/kg).

Table 1: Results of Bio-Chemical Estimation of Bio-Chemical Markers- Sod, Cat, MDA And MPO.

Groups	CAT (µmoles of H_2O_2 metabolized/mg protein)	SOD (Units/mg protein)	MDA (nmol/gm wet tissue)	MPO (Units/ml)
Sham	2.69±0.2952***	37.66±6.661***	233.3±18.40***	0.75±0.0500***
I/R	0.78±0.1515	11.13±1.255	349.2±31.38	1.44±0.1094
Vehicle	0.79± 0.0909ns	10.98±1.227ns	334.6±5.973ns	1.47±0.0342ns
DL	1.25± 0.0983***	21.36±0.8244***	179.9±3.668***	0.74±0.0304***
L-NAME	0.18± 0.0246***	8.65±1.557ns	556.5±5.031***	1.02±0.0612***
L-NAME+ DL	0.20± 0.0068***	9.46±0.5040ns	551.5±2.069***	0.96±0.0370***
ALPR	1.92± 0.0320***	40.58± 0.3488***	152.3±5.210***	0.60±0.0222***
ALPR+ DL	2.16± 0.0773***	41.72± 0.2825***	142.2±2.072***	0.62±0.0791***
NLD	2.90±0.0549***	75.33± 2.083***	137.5±8.407***	0.82±0.0437***
NLD+ DL	3.78±0.1673***	104.4± 0.5260***	97.05±3.549***	0.51±0.0528***

Inflammation is an important component of the pathogenesis of cerebral ischemia. Proinflammatory molecules such as NO synthase-2 (NOS 2), cyclooxygenase-2 (COX 2), chemokines, and adhesion molecules have been implicated in the development of cerebral ischemic injury. Myeloperoxidase (MPO) is a highly cationic glycosolated heme enzyme secreted by activated phagocytes at site of inflammation. MPO is an enzyme that is involved in the production of free radicals. Indeed, MPO uses H_2O_2 and NO_2^- to generate reactive nitrogen species [31].

The activities of enzyme MPO was also increased in ischemic reperfused group compare to sham group. Administration of the DL alone shown inhibition of inflammatory mediators but they were significantly inhibited when DL was administered along with the Nimesulide (20mg/kg i.p). Whereas the L-NAME showed no cerebral activity but the Allopurinol and DL+Allopurinol groups showed some cerebroprotective activity when compared to the I/R group.

CONCLUSION

In the present study the mechanism of cerebroprotective action of methanolic extract of bark of *Dalbergia latifolia* (DL) was carried out against the global model of ischemia in rats. Biochemical estimations for Super oxide dismutase, Catalase, Myeloperoxidase & Malondialdehyde and histological examinations were carried out. The results obtained showed that the *Dalbergia latifolia* extract is a potent cerebroprotective with a possible mechanism of Cyclooxygenase (COX) pathway inhibitor, as it shown the synergistic activity along with the COX inhibitor which may due to flavanoids in the extract.

REFERENCES

1. Popkin BM. Nutrition in transition, the changing global nutrition challenge. Asian Pacific J Clin Nutrition. 2001; 10 (supplement) S: 13-S18.
2. WHO Global Infobase. [https://apps.who.int/infobase/Mortality.aspx].
3. Seshadri S, Beiser A, Kelly-Hayes M, Kase CS, Au R, Kannel WB, Wolf PA. The lifetime risk of stroke - Estimates from the Framingham Study. Stroke. 2006; 37: 345-50.

4. Beal CC: Gender and stroke symptoms: A review of the current literature. J Neurosci Nursing. 2010; 42: 80-7.
5. Rosamond W, Flegal K, Furie K, et al. Heart disease and stroke statistics 2008 update: a report from the American Heart Association Statistics Committee and Stroke Statistics Subcommittee. Circulation. 2008; 117: e25–146.
6. Dajas F, Rivera-Megret F, Blasina F et al. Neuroprotection by flavonoids. Braz J Med Biol Res. 2003; 36: 1613-1620.
7. Sasaki N, Toda T, Kaneko T, Baba N, Matsuo M. Protective effects of flavonoids on the cytotoxicity of linoleic acid hydroperoxide toward rat pheochromocytoma PC12 cells. Chem Biol Interact. 2003; 145: 101-116.
8. Horakova L, Licht A, Sandig G et al. Standardized extracts of flavonoids increase the viability of PC12 cells treated with hydrogen peroxide: effects on oxidative injury. Arch Toxicol. 2003; 77: 22-29.
9. Suzuki M, Tabuchi M, Ikeda M, Umegaki K, Tomita T. Protective effects of green tea catechins on cerebral ischemic damage. Med Sci Monit. 2004; 10: BR166-BR174
10. Hong JT, Ryu SR, Kim HJ, Lee JK, LeeSH, Yun YP, Lee BM, Kim PY. Protective effect of green tea extract on ischemia/reperfusion-induced brain injury in Mongolian gerbils. Brain Res. 2001; 888: 11–18.
11. Prasad AGD, KSJ Cahndra and ANY Reddy. Initiation of a genetic improvement program for *Dalbergia latifolia* in Karnataka, India. Paper presented at the International *Dalbergia* workshop.1993.
12. Kirtikar KR and BD Basu. Indian medicinal plants. International Book Distributor, Dehradun, India. 2005. pp: 824.
13. Shyam, S and KU Chibber. Dalbinol-a new 12a-hydroxyrotenoid from *Dalbergia latifolia* seeds. Phytochem. 1978; 17: 1442-1443.
14. Rastogi RP and BN Mehrotra. Compendium of Indian Medicinal Plants. Lucknow and New Delhi: Central Drug Research Institute, Publications and Information Directorate. 1993l; pp: 245.
15. Thurlough O, MOS Criodain, JM Mary and MXD Dervilla. Latinone, a phenanthrene-1,4- quinone from *Dalbergia latifolia*. Phytochem. 1981; 20: 1089-1092.
16. Khare CP, 2007. Indian Medicinal Plants an Illustrated dictionary, Springer Science+Business Media, LLC.New York, USA, pp: 200.
17. KR Kirtikar and BD Basu, Indian medicinal plants. International Book Distributor, Dehradun, India, 2005.
18. Wealth of Indian Raw Materials. Vol. 2 Publication and information directorate, CSIR, New Delhi, 1972.
19. RN Chopra, SL Nyer and IC Chopra, Supplement to the glossary of Indian medicinal plants, CSIR, New Delhi, 1980.
20. KM Nadkarni, Indian material Medica. third ed., Bombay, Popular book depot, 1954.
21. Tiwari M, Dwived UN, Kakkar P. Suppression of oxidative stress and pro -inflammatory mediators by cymbopogon citrates D. Stapf extract in lipopolysaccharide stimulated murine alveolar macrophages. Food Chem Toxicol. 2010; 48: 2913-9.
22. Komatsu T, Yamazaki H, Nakajima M, Yokoi T. Identification of catalase in human livers as a factor that enhances phenytoin dihydroxy metabolite formation by human liver microsomes. Biochem Pharmacol. 2002; 63: 2081-90.
23. Kristofikova Z, Klaschka J, Hana T. Effect of aging on lipid peroxide levels induced by L-glutamic acid and estimated by means of a thio barbituric acid test in rat brain tissue. Exp Gerontol. 1995; 30: 645-57.
24. Shi-Jye C, Wann-Cherng P, Chin-Mao H, Deh-Ming C, Lin SH. Effects of various body temperatures after lipopolysaccharide-induced lung injury in rats. Chest 2005; 128: 327-36.
25. Thirumurugan G et. al., Behavioral assessment studies in cerebral ischemia induced by bilateral carotid artery occlusion in Rats. Ann Bio. 2010; 1 (1): 208-23.
26. Collini M, Aragno M, Mastrocola R, Gallichio M, Rosa AC, Dianzani C, Danni O et.al., Modulation of the oxidative stress & inflammatory response by PPAR-γ agonist in the hippocampus of rats exposed to cerebral ischemia/reperfusion. Eur J Pharmacol. 2006; 530: 70-80.
27. Margaill I, Plotkine M, Lerouet D. Antioxidant strategies in the treatment of stroke. Free Rad Biol Med. 2005; 39(4): 429.
28. Adibhatla et. al., Phospholipase A2, hydroxyl radicals, and lipid peroxidation in transient terebral ischemia. Antioxid Redox Signaling. 2003; 5(5): 647-54.
29. Kinuta Y, Kikuchi H, Ishikawa M, Kimura M, Itokawa Y. Lipid peroxidation in focal cerebral ischemia. J Neurosurg. 1989; 71(3): 421-9.
30. Akondi RB, Kumar P, Annapurna A, Pujari M. Protective effect of rutin and naringin on sperm quality in streptozotocin (STZ) induced type 1 diabetic rats. IJPR. 2011; 10(3): 585-96.
31. Ansari MA, Hussain SK, Manjunath PM, Goli D. Neuroprotective effect of allopurinol and nimesulide against cerebral ischemic reperfusion injury in diabetic rats. Eur Rev Med Pharmacol Sci. 2013; 17(2): 170-8.