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NEUROPROTECTIVE ACTIVITY OF SEEDS OF *CAESALPINIA BONDUCELLA* (ROXB) IN OXIDATIVE STRESS INDUCED RATS

Shamabai D. Hosamani[*1], Dr. Shivakumar S. Inamdar[1], Ravi Ranjan Kumar Pandey[2], Pallavi P. Kulkarni[3] and Harika Vatikuti[4]

Department of Pharmacology, H.K.E. Society's Matoshree Taradevi Rampure Institute of Pharmaceutical Sciences, Kalaburagi-585 105.

Rajiv Gandhi University of Health Sciences, Bengaluru, Karnataka.

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***Corresponding Author**
Shamabai D. Hosamani
Department of Pharmacology, H.K.E. Society's Matoshree Taradevi Rampure Institute of Pharmaceutical Sciences, Kalaburagi-585 105.

ABSTRACT

Stroke is one of the important causes of mortality and morbidity in the world. The treatment of stroke is out most important. Herbal drugs have gained lot of acceptance in the recent years because they have a relatively higher therapeutic window, less serious side effects, and are economical. Hence we evaluate the neuroprotective activity of seeds of *Caesalpinia bonducella (Roxb)* in induced rats. **Objectives:** Neuroprotective activity of *Caesalpinia bonducella (Roxb)* against ischemia reperfusion induced brain injury in rats. **Methods:** We studied the effect of extracts *of Caesalpinia bonducella (Roxb)* 200 mg/kg oral doses. Albino Wister rats of either sex weighing between 150-200 g were divided into six groups of six animals of each. Evaluated the neuroprotective effect of *Caesalpinia bonducella (Roxb)* was carried out by using the global cerebral ischemia/reperfusion model by bilateral carotid artery occlusion for 30 min followed by 24 h reperfusion. The biochemical parameters, which were measured in animals brain were lipid peroxidation(LPO), superoxide dismutase(SOD), catalase, glutathione, total thiols and total proteins of induced and treated groups. **Result:** The interpretation of results were done using statistical analysis by One-Way Analysis of variance (ANOVA) followed by Dunnet's't' test. This model showed reduced in the level of lipid peroxidation, an increase in the level of superoxide dismutase, catalase, glutathione, total thiols and total proteins. **Conclusion:** The methanolic and aqueous extract of *Caesalpinia bonducella (Roxb)* has shown significantly neuroprotective activity compare to the standard

drug (Vitamin E) but Petroleum ether has shown less significantly neuroprotective activity compared to the standard drug (Vitamin E).

KEYWORDS: Neuroprotective activity; *Caesalpinia bonducella (Roxb)*; Stroke.

INTRODUCTION

Stroke, also known as cerebrovascular accident (CVA), cerebrovascular insult (CVI), or brain attack, is when poor blood flow to the brain results in cell death. Stroke is one of the important causes of mortality and morbidity in the world, and Stroke is also a major cause of death and disability worldwide. The resulting burden on the society continues to grow with increase in the incidence of stroke. Stroke is the third largest cause of mortality after cancer and coronary heart disease and is the second largest cause of disability in adults. The incidence of stroke is 1 per 1000 people. However, this incidence varies according to age and sex. In the age group of 80 and more years, the incidence of stroke reaches values of 20 per 1000 people. The incidence rate is higher among makes of all age groups. A community based survey from different regions of India showed a crude prevalence rate of 200 per 100,000 people. Overall 9400 strokes (first ever and recurrent) were estimated to be hospitalized in 1999, with an attack rate of 208 per 100,000[1]. There are two main types of stroke: ischemic due to lack of blood flow and hemorrhagic due to bleeding. They result in part of the brain not functioning properly. Reactive oxygen species (ROS) are highly reactive ions and "free radicals" (chemical containing atoms with an unpaired electron in its outer orbit) involving oxygen molecules. Excessive free radical production has been involved in occurrence in several disease processes like Drug toxicities, Inflammation, Aging, and Carcinogenesis, Lipid peroxidation cellular membrane implicated in several specific diseases like artherosclerosis, degenerative neurological disease, reperfusion injury and oxygen toxicity. Although the restoration of blood flow to an ischemic organ is essential to prevent irreversible cellular injury, reperfusion may augment tissue in injury in excess of that produced by alone.

Caesalpinia bonducella (Roxb), is a wild highly thomy shrub belonging to the family Casealpiniaceae. It is commonly called as the gray Nicker Bean. It is a free flowering and free-fruiting plant without periodicit [2]. In this study we investigate the neuroprotective active of *Caesalpinia bonducella (Roxb)* against ischemia reperfusion induced brain injury by using brain parameters.

Objectives

1. To find the Neuroprotective activity of extract against transient global ischemia/reperfusion model in rats.
2. To find the effect of extracts on antioxidant enzyme levels against transient global ischemia/reperfusion model.

MATERIALS AND METHODS

1. Materials

1.1 Collection of the Plant Material

The dried fruits of *Caesalpinia bonducella (Roxb)* were collected in the month of July from the Tadakala village, Dist - Kalaburagi. Plant and seeds were identified and authenticated by Taxonomist Rajasamarsen K Modi, asst. Professor, Department of Botany, Govt. College Kalaburagi, with ref. no. – GCK/Bot/Herbarium/2015-16 and HGCG No. 209. The weight of the fruits was 2kg. The seeds were collected from the fruits by removing upper layer of the fruits. Weight of the seeds was 1kg which is used for our study. The seeds are powdered to fine powder; the weight of the powder is 1.025kg.

1.2 Animals

Albino rats (Wister) weighing between 150-200 g of either sex were procured from Central Animal House, MRMC Kalaburagi. The animals were acclimatised for one week under standard laboratory conditions. They were housed in polypropylenes cages and maintained at $27^0C \pm 7^0C$ under 12 hour dark/light cycle. They were fed with standard rat feed and water *ad libitum.* Ethical clearances for conducting animals experiments were obtained from the institutional Animal Ethics Committee (IAEC) HKES's MTRIPS Kalaburagi. IAEC NO.HKECOP/IAEC/73/2014-2015 and CPCSEA No. - 142/1999 CPCSEA. 5th JULY 1999.

1.3 Extraction

Preparation of extracts

The obtained powder was subjected to successive soxhlet extraction with the solvents with increasing order of polarity that is pet. ether (60^0-80^0), methanol (64.5-65.5^0). The extracts was concentrated under reduced pressure and stored separately in desiccators until further use.

Preparation of aqueous extracts

Fifty grams of air-dried powder of seeds was taken in 200 ml of water in conical flask, plugged with cotton wool and they were kept at room temperature for 48 hrs with occasionally shaking, the supernatant was collected and the solvent was evaporated to make the final volume one fourth of the original volume and stored at 4^0 C in airtight bottle.

2. Methods

2.1 Determination of acute toxicity

The acute oral toxicity was performed according to OECD guideline No. 425 method of committee for the purpose of control and supervision on experimental animals (CPCSEA). Guideline No. 425 was adopted. The screening doses selected extracts of *Caesalpinia bonducella (Roxb)* were 200 mg/kg dose.The acute toxicity studies, test extracts of *Caesalpinia bonducella (Roxb)* did not produced any mortality of the animals at dose of 2000 mg/kg. Hence 200 mg/kg fixed as LD_{50} value.

Preparation of dose

From the acute toxicity study, it was found that extracts of *Caesalpinia bonducella (Roxb)* Seeds was safe at limit dose 200 mg/kg therefore dose $1/10^{th}$ of this dose that is 2000 mg/kg and below were used in subsequent study for all extracts of *Caesalpinia bonducella* (*Roxb*).

2.2 Global cerebral ischemia model[3]

The rats of either sex with body weight 150-200 g divided into six groups and each group contains six rats. Rats were anesthetized by ketamine Hcl (45 mg/kg, ip). An small incision were done in the neck region, and the neck muscles were retreated for isolation of the common carotid artery. The internal carotid artery was subsequently isolated, and 30 min of ischemia was given to the rat by blocking the left internal branch of the common carotid artery was occluded with micro-vascular clip. After the ischemic period, the neck muscle was stitched, and an antibiotic was applied. The test drug solution or suspension was administered to the respective group of rats described below for a period of 8 days. The ischemia reperfusion was produced in groups 2-6. After the induction of 30 min ischemia and reperfusion, group 2 rats were administered with vehicle (saline 10 ml/kg orally for 8 days). Vitamin E (10 mg/kg, i.p) was administered to 6 groups of animals. Group 3, 4 and 5 were administered with petroleum ether extract, methnolic extract and aqueous extract of *Caesalpinia bonducella (Roxb).*

The different groups of rats were treated with different types of extracts like petroleum ether extract, methanolic extract and aqueous extract according to the body weight.

Group 1: Recived 2% of gum accasia, no ischemia (n=6)

Group 2: Normal saline, bilateral carotid artery occlusion (BCA) for 30 min, (n=6) or Induced group

Group 3: Petroleum ether extract (200 mg/kg), bilateral carotid artery occlusion (BCA) for 30 min, followed by reperfusion 24 h (n=6)

Group 4: Methanolic extract (200 mg/kg), bilateral carotid artery occlusion (BCA) for 30 min, followed by reperfusion 24 h (n=6)

Group 5: Aqueous extract (200 mg/kg), bilateral carotid artery occlusion (BCA) for 30 min, followed by reperfusion 24 h (n=6)

Group 6: Vitamin E (10 mg/kg) bilateral carotid artery occlusion (BCA) for 30 min, followed by reperfusion 24 h (n=6).

On the 7^{th} and 8^{th} days after 24 h of reperfusion the rats were decapitation, the brain removed and washed in cooled 0.9% of saline, and kept on ice and subsequently blotted on filter paper, then weighed and homogenized as 10 % w/v in cold formalin solution. The homogenates were centrifuged at 10,000 rpm for 20 min. at 4^0 C and the supernatant was used for lipid peroxidation assay, total thiols, total protein estimation, glutathione estimation, superoxide dismutase and catalase were analysed using spectrophotometric method.

Biochemical parameters of brain

Estimation of Lipidperoxidation (LPO)

Thiobarbituric acid reactive substances (TBARS) in the homogenate were estimated by using standard protocol. Briefly, the 0.5 ml of 10 % homogenate was incubated with 15% 2, 4, 6-trichloroanisole, 0.375% 2, 4, 6-tribromoanisole and 5 N HCl at 95°C for 15 min, the mixture was cooled, centrifuged and absorbance of the supernatant measured at 512 nm against appropriate blank. The amount of LPO was determined by using $\varepsilon = 1.56 \times 10^5$ /M/cm and expressed as TBARS n moles/mg of protein[4].

Estimation of Superoxide Dismutase (SOD)

SOD activity was determined based on its ability to inhibit the auto-oxidation of epinephrine to adrenochrome at alkaline pH[5]. Briefly, 25 µl of the supernatant obtained from the centrifuged brain homogenate was added to a mixture of 0.1 mM epinephrine in carbonate

buffer (pH 10.2) in a total volume of 1 ml and the formation of adrenochrome was measured at 295 nm. The SOD activity (U/mg of protein) was calculated by using the standard plot.

Estimation of Catalase (CAT)

CAT activity was assayed by the method of Calibore[6]. Briefly, the assay mixture consisted of 1.95 ml phosphate buffer (0.05 M, pH 7.0), 1 ml hydrogen peroxide (0.019 M) and 0.05 ml homogenate (10 %, w/v) in a total volume of 3 ml. Changes in absorbance were recorded at 240 nm. CAT activity was calculated in terms of nM, H_2O_2 consumed/min/mg protein.

Estimation of Glutathione (GSH)

GSH was estimated in various tissues by the method of Sedlak and Lindsay. Briefly, 5 % tissue homogenate was prepared in 20 mM ethylenediaminetetraacetic acid (EDTA), pH 4.7 and 100 µl of the homogenate or pure GSH was added to 0.2 M tris-EDTA buffer (1.0 ml, pH 8.2) and 20 mM EDTA, pH 4.7 (0.9 ml) followed by 20 µl of Ellman's reagent (10 mmol/l DTNB in methanol). After 30 min of incubation at room temperature, absorbance was read at 412 nm. Samples were centrifuged before the absorbance of the supernatants was measured[7,8].

Estimation of Total Thiols

This assay is based on the principle of formation of relatively stable yellow colour by sulfhydryl groups with DTNB. Briefly, 0.2 ml of brain homogenate were mixed with phosphate buffer (pH 8), 40 µl of 10 mM DTNB and 3.16 ml of methanol. This mixture was incubated for 10 min and the absorbance was measured at 412 nm against appropriate blanks. The total thiols content was calculated by using $\varepsilon = 13.6 \times 10^3/M/cm$ [9].

Estimation of Total Proteins

The total protein contents of 10 % brain homogenates were determined by using the modified Lowry's method.

Statistical Analysis

The results of the study were expressed as mean ± SEM (n=6) data was analyzed using One-Way Analysis Variance (ANOVA) followed by Dunnet's 't' test by using graph pad software and $p<0.05$*, and 0.001*** were considered as significant.

RESULTS

Acute Toxicity

LD_{50} studies were conducted in wistar rats by using OECD guidelines No. 425 for *Caesalpinia bonducella (Roxb)* extracts. It was found that even extracts 2000 mg/kg dose did not show any mortality. Hence 200 mg/kg dose was considered as safe drug.

Lipid peroxidation

Normal rat show basal TBARS levels of about 113.7±4.96 n moles/mg of proteins of brain homogenated respectively. Different extracts show a significant difference in lipid per oxidation activity as compared to induced group. Rat treated global ischemic and reperfused induced rat show significant increase in the TBARS level to about 333.82±29.55 n moles/mg of proteins of brain tissue homogenate in comparison to the normal control. Different extract like pet. ether, methanolic, aqueous and standard vitamin E in Bilateral carotid artery occlusion (BCAO) model rats show reduced levels of TBRAS significantly to about 295.7±11.75, 139.3±2.940, 161.2±1.797 and 142.2±2.081 n moles/mg of proteins in comparison to bilateral carotid artery occlusion (BCAO)induced group.(Fig.1), Table No.1.

Superoxide dismutase (SOD)

Normal basal level of SOD activity was found to be 539.2±14.04 u/mg of proteins of brain homogenate. Different extract did not shows a significant activity compared to the control group. Rats with Bilateral carotid artery occlusion (BCAO) induced model decrease level of about 324.3±4.364 in brain tissue homogenate respectively as comparison to normal control. Different extracts like pet. ether, methanolic, aqueous and standard vitamin E increases the SOD level of about 384.0±5.335, 438.8±4.833, 381.0±8.434 and 464.0±11.40 of brain tissue homogenated as comparison with Bilateral carotid artery occlusion (BCAO) induced model(Fig.2), Table No.1.

Catalase

Normal basal level of catalase activity was found to be 0.5617±0.0132 u/mg of proteins of brain homogenate, different extracts and standard did not show a significant difference in catalase activity compare to the control. Bilateral carotid artery occlusion (BCAO) induced rats decrease the level of about 0.263±0.011 of brain tissue homogenate respectively as comparison to normal control. Different extracts like pet. ether, methanolic, aqueous and standard vitamin E increase the catalase level significantly of about 0.3183±0.01447, 0.4333±0.01256, 0.3917±0.011 and 0.4733±0.012 u/mg of brain tissue homogenated

respectively as comparison with Bilateral carotid artery occlusion (BCAO) induced model(Fig.3), Table No.1.

Glutathione (GSH)

Normal basal GSH level was found to be 57.87±0.592 n moles/mg of protein of brain homogenate. Different extracts did not show a significant difference activity compare to normal. Bilateral carotid artery occlusion (BCAO) induced rat decreases the level 29.20±0.976 n moles/mg of proteins of brain homogenated as compare to normal control. The GSH levels were significantly increased to normal levels. The rats treated with different extract pet. ether, methanolic, aqueous and standard vitamin E increases GSH levels of about 27.85±0.954, 48.57±0.1513, 44.98±1.034 and 50.42±0.6123 of brain tissue homogenated respectively as compare to the bilateral carotid artery occlusion (BCAO) induced rats (Fig.4), Table No.1.

Total thiols

Normal basal total thiols were found to be 69.27±1.683 m moles/g of proteins of brain tissue homogenate. Different extracts and standard did not a significant difference activity compared to control. Bilateral carotid artery occlusion (BCAO) induced rats decrease the level 11.86±1.540 m moles/mg of proteins of brain tissue homogenated respectively as comparison to control. Total thiols were show significantly increase and reversed to normal level, different extracts like pet .ether, methanolic, aqueous and standard vit E treated groups of about in the brain homogenate 15.76±0.9986, 47.58±1.211, 43.42±1.821 and 56.99±0.9948 m moles/mg of proteins respectively as compared with the induced group(Fig.5), Table No.1.

Total proteins

Normal total proteins were found to be 7.043±0.2484 g/dL of proteins of brain tissue homogenated. Different extracts and standard did not show a significant difference activity compared to control. Bilateral carotid artery occlusion (BCAO) induced rats decrease the level 2.437±0.1039 g/dL of proteins of brain tissues as compared to control. The total proteins were shows significantly increased with different extracts like pet. ether, methanolic, aqueous and standard vitamin E treated groups of about in the brain homogenated 2.805±0.07623, 5.433±0.1460, 4.860±0.11404 and 5.792±0.2376 g/Dl of proteins as compared with Bilateral carotid artery occlusion (BCAO) induced group(Fig.6),Table No.1.

Table 1: Effect of *caesalpinia bonducella (Roxb)* of different extracts on BCA Occlusion induced global cerebral ischemia in rats.

Treatment Groups	Lipid peroxidation (nmoles/mg of proteins)	Superoxide Dismutase (u/mg of proteins)	Caatalase (u/mg of proteins)	Glutathione (nmoles/mg of proteins)	Total thiols (Mmoles/g of proteins)	Total proteins (g/dl of proteins)
Control	113.7±4.96	539.2±14.04	0.561±0.013	57.87±0.592	69.27±1.683	7.043±0.248
Induced	338.2±29.55	324.3±4.364	0.263±0.011	29.20±0.976	11.86±1.540	2.437±0.103
Pet. ether	295.7±11.75^{ns}	384.0±5.335***	0.318±0.014*	27.85±0.954^{ns}	15.76±0.998^{ns}	2.805±0.076^{ns}
Methanol	139.3±2.94***	438.8±4.833***	0.433±0.012***	48.57±1.513***	47.58±1.211***	5.433±0.146***
Aqueous	161.2±1.797***	381.0±8.434***	0.391±0.011***	44.98±1.034***	43.42±1.821***	4.860±0.110***
Vitamin E	142.2±2.081***	464.0±11.40***	0.473±0.012***	50.42±0.612***	56.99±0.994***	5.792±0.237***

Each value are expressed as mean ± standard error mean (n=6) **p<0.001,*p<0.05 when compared to induced group along treated rats; one-way ANOVA followed by Dunnett's post test.

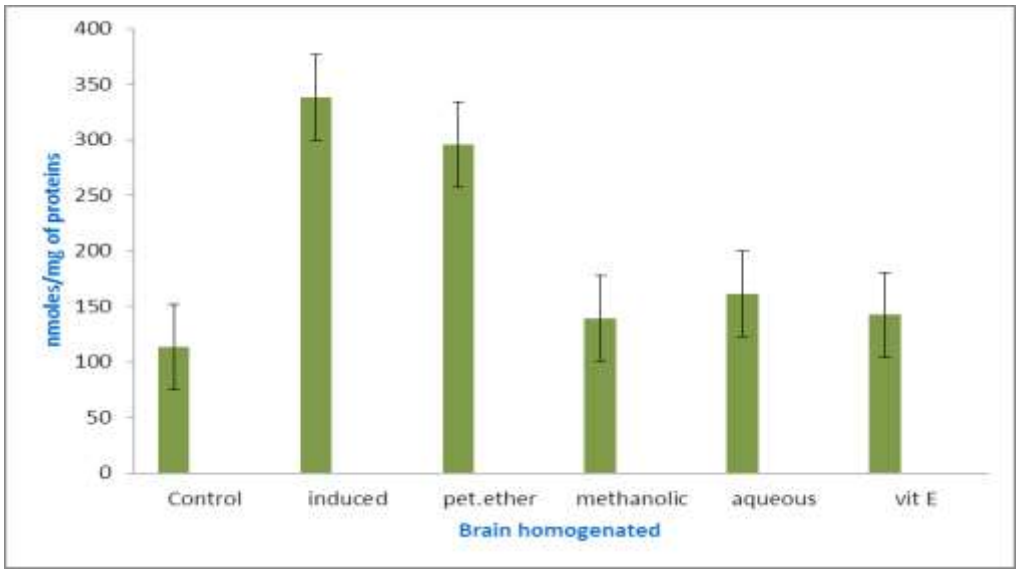

Fig. 1: Estimation of lipid peroxidation in BCA Occlusion induced global cerebral ischemia

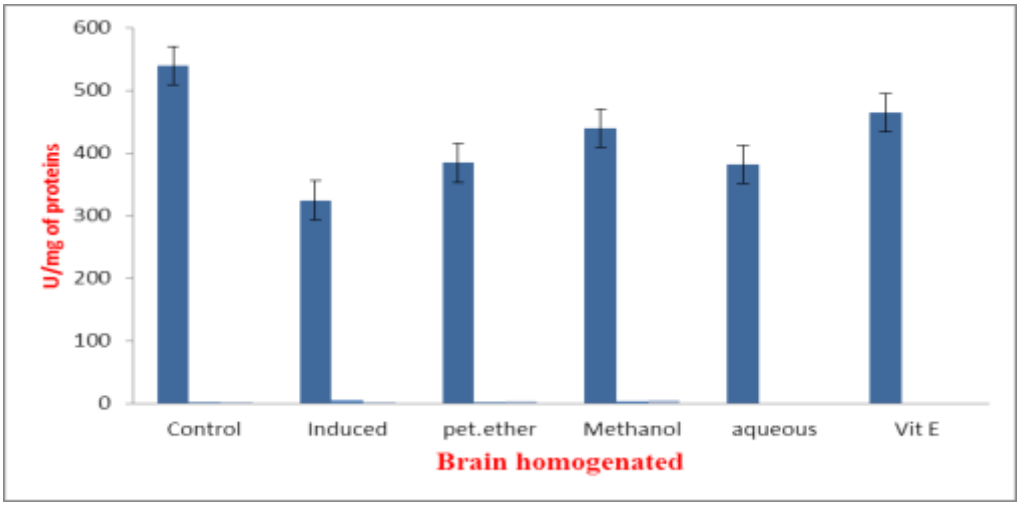

Fig. 2: Estimation of superoxide dismutase in BCA Occlusion induced global cerebral ischemia

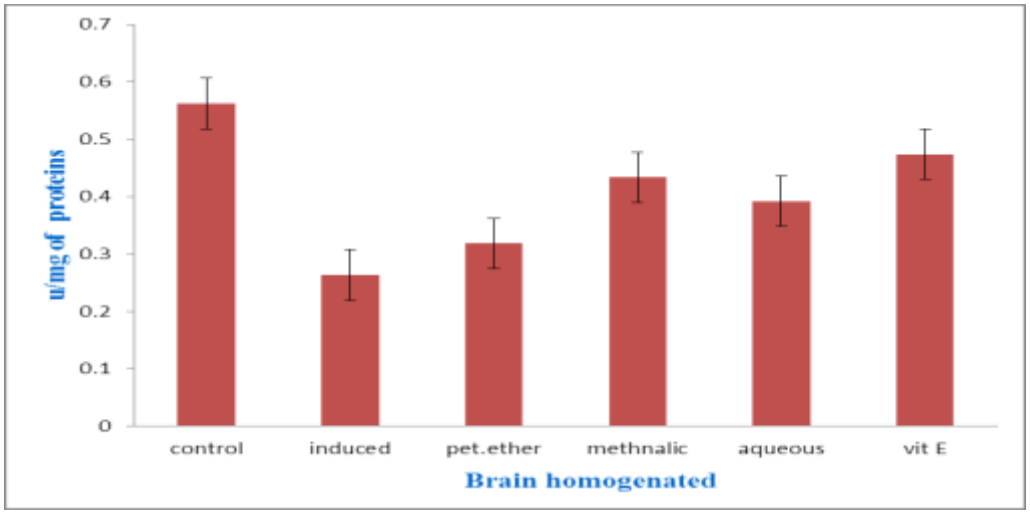

Fig.3: Estimation of catalase in BCA Occlusion induced global cerebral ischemia

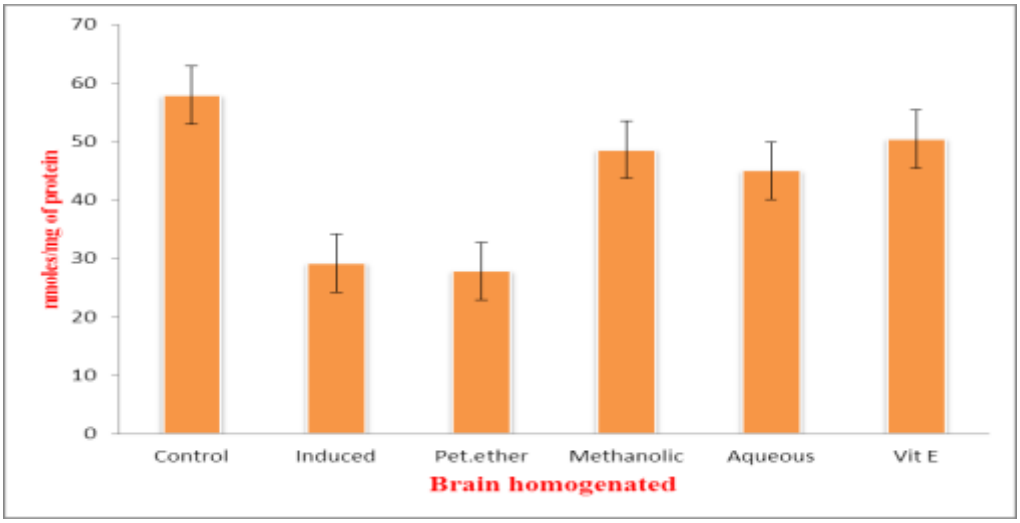

Fig. 4: Estimation of Glutathione in BCA Occlusion induced global cerebral ischemia

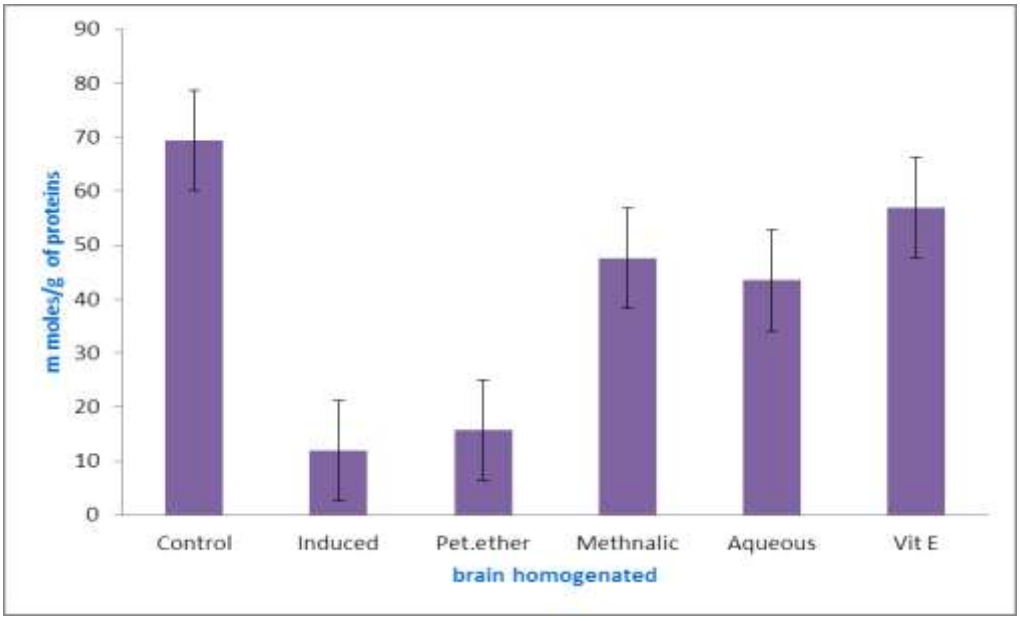

Fig. 5: Estimation of total thiols in BCA Occlusion induced global cerebral ischemia

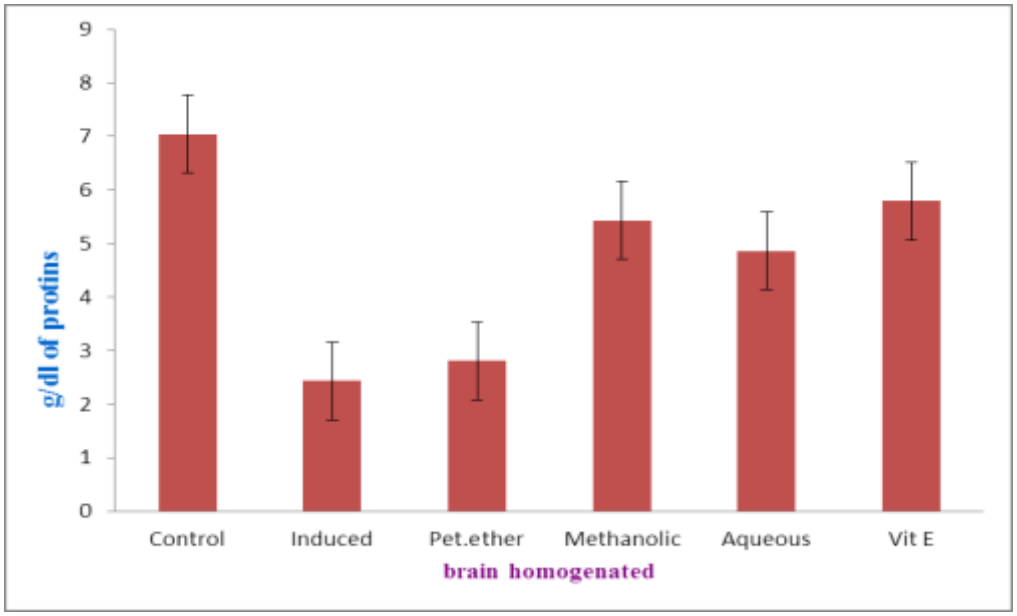

Fig.6: Estimation of Total proteins in BCA Occlusion induced global cerebral ischemia.

DISCUSSION

The neurotoxicity was produced by blocking internal carotid artery with the help of micro vascular clip followed by reperfusion. The present study was undertaken to evaluate the neuroprotective effect of *Caesalpinia bonducella (Roxb)* on ischemia reperfusion brain injury. Reperfusion of ischemic tissue results in the formation of toxic ROS including, O_2^-, OH^-, HOCl, H_2O_2 and nitric oxide-derived peroxynitrite radicals. These toxic ROS induces oxidative stress [10]. Chemically, oxidative stress is associated with increased production of

oxidizing species or a significant decrease in the capability of antioxidant defenses, such as catalase and SOD enzymes. Severe oxidative stress can cause cell death and even moderate oxidation can trigger apoptosis, while more intense stresses may cause necrosis. ROS can cause cellular damage by oxidizing membrane lipids, essential cellular proteins and DNA [11].

In the present study, antioxidant enzyme parameters such as SOD, CAT, GSH, total thiol levels and LPO were estimated in brain homogenate. *Caesalpinia bonducella (Roxb)* of different extracts like petroleum ether extract, methnolic extract, and aqueous extract treated groups showed significantly higher extent of protection ($P < 0.001$) against I/R induced oxidative stress as compared with the induced group. Enhanced LPO expressed in terms of TBARS and significantly reduced activity of antioxidant enzymes such as SOD and CAT observed in induced group confirms the brain damage due to oxidative stress. A superoxide anion radical contribute to post-ischemic/reperfusion tissue damage demonstrated in several organs and is associated with pathology of diseases and conditions such as neurodegenerative diseases, ischemia/reperfusion injury and inflammation[12]. The SOD and CAT levels were increased significantly and even comparable to induced group after treatment with *Caesalpinia bonducella (Roxb)* of different extracts like petroleum ether extract, methanol extract, and aqueous extract in ischemia/reperfusion induced rats.

Similarly, significantly decreased levels of GSH and total thiols were observed in ischemia/reperfusion treated animals or induced group. GSH is considered to be a central component in the antioxidant defenses of cells, acts both to directly detoxify ROS and as a substrate for various peroxidases. Dysfunction of the GSH system has been implicated in a number of neurodegenerative diseases [13] and is a potential contributor to oxidative damage following temporary ischemia. In present studies, there is significant ($P < 0.001$) increased levels of GSH and total thiols in the groups treated with *Caesalpinia bonducella (Roxb)* of different extracts. The level of LPO is a measure of membrane damage as well as alteration in structure and function of cellular membrane. The LPO level was significantly decreased in the *Caesalpinia bonducella (Roxb)* of different extracts like petroleum ether extract, methnolic extract, and aqueous extract treated groups, which suggest a free radical scavenging activity. In the present study, we observed that direct free radical scavenging activity was responsible for the antioxidant and neuroprotective action of the *Caesalpinia bonducella (Roxb)*.

Further, molecular level studies are required to exploit the full therapeutic potential of natural product *Caesalpinia bonducella (Roxb).*

CONCLUSION

The result of the present study indicates that, all the tested of different extracts of the *Caesalpinia bonducella(Roxb)* exerts remarkable antioxidant activity due to possible multiple effects involving significant protection against the oxidative damage, which may be attributed to its protection action on lipid peroxidation and defense contributing to the protection against oxidative damage.

This studies as given rise to new dimension in the treatment of neuronal disorder. Further the work could be intended to evaluate it effectiveness of the compounds for the treatment of neuronal disorder at its cellular level to elucidate its exact mechanism of action for the traditional claim.

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