The Journal of Phytopharmacology 2015; 4(6): 276-281
Online at: www.phytopharmajournal.com

Research Article

ISSN 2230-480X
JPHYTO 2015; 4(6): 276-281
November- December

Desai Yogesh Hareshchandra
PG Scholar; Department of Dravyaguna Vijnanam, Sri Dharmasthala Manjunatheshwara College of Ayurveda, Udupi-574118, India

Ravi Mundugaru
Research Officer; Department of Pharmacology and Toxicology, SDM Centre for Research in Ayurveda and Allied Sciences, Udupi-574118, India

Shridhara Bairy T
Professor and Head; Department of Dravyaguna Vijnanam, Sri Dharmasthala Manjunatheshwara College of Ayurveda, Udupi-574118, India

Ravikrishna S
Assistant Professor; Department of Dravyaguna Vijnanam, Sri Dharmasthala Manjunatheshwara College of Ayurveda, Udupi-574118, India

Ravi Shankar B
Professor of Experimental Medicine and Director; Department of Department of Pharmacology and Toxicology, SDM Centre for Research in Ayurveda and Allied Sciences, Udupi-574118, India

Correspondence:
Dr. Ravi Shankar B
Professor of Experimental Medicine and Director; Department of Department of Pharmacology and Toxicology, SDM Centre for Research in Ayurveda and Allied Sciences, Udupi-574118, India

Neuro-protective role of seeds of *Mucuna pruriens* BEK and *Mucuna monosperma* DC in wistar albino rats

Desai Yogesh Hareshchandra, Ravi Mundugaru, Shridhara Bairy T, Ravikrishna S, Ravi Shankar B*

ABSTRACT

Mucuna pruriens Bek. and *Mucuna monosperma* DC. seeds were extensively used in Ayurveda for neuromuscular disorders. The objective of present study was to screen the neuroprotective activity of the test drugs in wistar albino rats. The cerebral ischemia was induced by bilateral common carotid artery occlusion for 60 minutes followed by reperfusion. At the end of the experiments under anaesthesia animals were sacrificed and brain was removed. Anti oxidant and histopathological examination was carried out of the brain tissue. Both test drugs have been shown considerable anti oxidant activity in comparison to BCCAO control group. Histopathological examination revealed there is a decreased cellularity and predominance of immature neurons in the granular layer was observed in hypothalamus in control rats while the test drug has shown normal cytoarchitecture. The sections of hippocampus from *Mucuna monosperma* DC group exhibited decreased cellularity of the granular layer and vacuolization was observed and *Mucuna pruriens* Bek treated group exhibited almost normal cytoarchitecture. In conclusion the test drugs possess moderate anti oxidant and cellular integrity maintaining potential in different brain regions and hence it supports its therapeutic claim in neuromuscular disorders.

Keywords: Neuroprotective, Cerebral ischemia, Anti-oxidants, Hippocampus, Lipid peroxidation.

INTRODUCTION

Brain tissue needs continuous supply of oxygen and glucose in order to maintain structural and functional integrity. It has been reported that the cerebral ischemia is the third leading causes for death in many developing countries behind other cerebrovascular disorders such as ischemic heart disease [1, 2]. Cerebral ischemia is a major factor and cause of mortality and morbidity followed by traumatic brain injury resulting from head injury[3-5].

Mucuna pruriens Bek and *Mucuna monosperma* DC both species are explained under same genus in Ayurveda and it is commonly known as Kapikacchu. These are important drugs used by many *Ayurvedic* physicians in day to day practice. These drugs mainly useful in *Vrusya karma* (spermatogenesis) and neurological disorders. Acharyas have explained this drug under *balya* means it nourish and strengthen neuromuscular system [6].

Mucuna pruriens Bek contains the following chemical composition such as amino acids –L-dopa (L 3, 4 dihydroxy phenyl alanine) about 1.5%, Licithin 12.5%, gallic acid, glucoside, glutathione Alkaloids such as nicotine, mucunine, mucunadine, prurienine, prurieninine and fat. It has the following folklore uses such as aphrodisiac, tonic, anthelmintic, inflammations reducing, to improve the blood; juice given for headache.- the seeds are alexipharmic and cure scorpion-sting; laxative, aphrodisiac, tonic; useful in gonorrhea.

Mucuna pruriens Bek extracts have been long used in tribal communities as a toxin antagonist for various snakebites. Research on its effects against *Naja* spp. (cobra), *Echis* (Saw scaled viper), *Calloselasma* (Malayan Pit viper) and *Bangarus* (Krait) have shown it has potential use in the prophylactic treatment of snakebites [7,8].

There is a great demand and intensive search for effective neuro protective agents in ischemic brain diseases. Thus the present study was aimed to evaluate the neuro-protective activity of Mucuna species against bilateral common carotid artery occlusion induced cerebral ischemia in Wistar albino rats.

MATERIALS AND METHODS

Test Drug

The seed powder of *Mucuna monosperma* DC. was prepared in *Bhaishjyakalpana* practical lab attached

with SDM College of Ayurveda and Allied Sciences Udupi and *Mucuna pruriens* Bek seed powder was purchased from S.D.M. Ayurvedic Pharmacy, Udupi. These Two species of *Mucuna* genus has been used throughout study.

Dose Selection:

The dose selection was done on the basis of body surface area ratio using the table of Paget and Barnes (1964- quoted by Ghosh 1984) [9]. It was done as follows:

Rat dose = Human dose X surface area ratio convertibility factor for rat (0.0185) X 5/ Kg body weight of rat.

Rat dose = Human dose X 0.018 X 5
i.e. 250mg X 0.018 X 5 = 22.5 mg/kg

Route of Drug Administration:

The test drugs were administered in the form of suspension made in 0.5% carboxy methyl cellulose. The test drugs and vehicle control were administered according to the body weight of the animals by oral route with the help of oral catheter.

Experimental animal

Healthy Wistar albino rats of either sex weighing between 200- 250g were used for the experimentation. The animals were procured from the animal house facility attached to the Pharmacology Laboratory of SDM Centre for Research in Ayurveda and Allied Sciences, Udupi. Rats were maintained at standard laboratory conditions such as exposing to natural day and night cycles with ideal laboratory condition of 25±2 ^{0} C temperature and 55% humidity. They were fed with rat pellet feed supplied by Sri Durga feed and normal tap water given *ad libitum* throughout the experimental study. The study protocol was approved by Institutional ethical committee (SDMCRA/IAEC-2012-13, DG 02) and principles of laboratory animal care guidelines were followed throughout the experimentation.

Experimental design

The selected rats were grouped into four groups with six animals in each. Group I administered with 0.5% CMC at a dose of 5ml/kg body weight served as control group. Group II administered with 0.5% CMC at a dose of 5ml/kg body weight and in which bilateral carotid occlusion was carried out served as BCCAO control group. Group III administered with Brahmi gritha (1.08ml/kg) and served as reference standard. Group-IV & V administered with aqueous extract of *Mucuna pruriens* Bek (MPB) & *Mucuna monosperma* DC (MMD) seeds suspended in 0.5% CMC and served as test I and II (22.5mg/kg). Group specific drugs were administered for seven consecutive days prior to bilateral common carotid artery occlusion (BCCAO). The ischemic neuronal damage was induced by the following method. The Rats were anaesthetized by administering combination of Ketamine (100mg/kg) and xylazine (3mg/kg). Using 70% ethanol, the surgical area was sterilized at the ventral regions of neck. A midline incision was made and soft tissues were pulled apart and both bilateral common carotid arteries were exposed. The cerebral ischemia were induced by simultaneous occlusion of both common carotid artery for 60 min, followed by acute ischemic reperfusion injury which was produced by untying the temporary ligature and releasing the thread. 48h after cerebral ischemic reperfusion all animals were sacrificed under anaesthesia. The brain tissue was excised, a part of brain was used for anti oxidant activity and a part of brain was preserved in 10% formalin for histopathological investigation [10, 11].

Anti oxidant activity in brain homogenate

Preparation of brain homogenate

Excised brain was cleaned with ice cold saline and stored at-20^{0}C. Before subjecting the brain tissue to antioxidant activity, the brain tissue samples were thawed and homogenized with 10 times (w/v) ice-cold 0.1M Phosphate Buffer (pH 7.4). Aliquots of homogenates from rat brain were used to determine catalase and glutathione peroxidise activity along with lipid peroxidation employing standard procedures. Catalase activity in tissue was measured according to the procedures of Sinha *et al.*, 1972 [12]. 1ml of homogenate solution was taken in 5 ml of phosphate buffer. To this 4 ml of 0.2 MH_2O_2 in phosphate buffer was added and time was noted. Exactly after 180 seconds of adding H_2O_2, a set of 1ml of reaction mixture from the above was taken in 2 ml dichromate acetic acid. Then it was kept in boiling water bath for 10 minutes. Cooled all the tubes under running tap water and finally reading was taken at 570 nm against reagent blank. Catalase activity in the tissue was expressed as µmoles H_2O_2 consumed /mg protein /min. Lipid peroxidation activity was determined by measuring the content of the Thiobarbituric acid reactive substances (TBARs) following the procedure of Ohkawa *et al.*, 1979 [13]. A standard stock solution of malondialdehyde was prepared in distilled water using 1,1,3,3 tetraethoxypropane. The solution was stored at 4ºC and diluted just before use as the working standard contained 50 nmoles/ml. Pipette 0.1ml of homogenate, 0.2 ml of 8.1% sodium dodecylsulfate (SDS), 1.5 ml of 20% acetic acid solution and 1.5 ml of 0.8% aqueous solution of TBA into suitably labeled test tubes. Reaction mixture was made up to 4.0 ml with distilled water and heated at 95°C for 60 min in water bath. After cooling the test tubes under tap water, 1.0 ml of distilled water and 5.0 ml of the mixture of n-butanol and pyridine (15: 1, v/v) was added and the mixture was shaken vigorously. Centrifuged the test tubes at 4000 rpm for 10 minutes and the absorbance of the upper layer was measured at 532 nm. Standard malondialdehyde was processed in similar fashion. Level of lipid peroxide was expressed as µmoles of MDA formed /g wet tissue. Glutathione peroxidase was estimated by taking 0.2 ml of EDTA, sodium azide, reduced glutathione and H_2O_2 in a test tube. Added 0.4 ml of buffer and 0.2 ml of homogenate mix and incubated at 37 ^{0}C for 10 minutes. The reaction was arrested by the addition of 0.5 ml of TCA and tubes were centrifuged. Taking 0.5 ml of supernatant added 4 ml of disodium hydrogen phosphate and 0.5 ml of DTNB. The colour developed was read at 420 nm immediately. Standard was also treated in the similar way. The glutathione peroxidase activity is expressed as µM of glutathione utilized per mg protein per minute at 37 ºC [13]. Histopathological study was carried out by transferring brain into 10% formalin immediately after excision from rats. Sections of less than 5µm thickness of fore brain were prepared and stained with haematoxyline and eosin for microscopical observations [14]. In addition serum level of AChE activity and C-reactive protein were estimated using commercial kits.

RESULT

In the present study moderate decrease was observed in serum AChE activity in BCCAO group in comparison to the normal control. This decrease was found to be reversed moderately in *M. pruriens* Bek. group and significantly in *M. monosperma* DC treated groups.). C-reactive protein was not affected to significant extent and hence was not of much usefulness in determining the neuroprotective activity of the test plants (Table 1).

Anti - oxidant

The Brahmi gritha and *Mucuna pruriens* Bek [MPB] group have shown moderate increase in catalase activity in the brain homogenate. However, the increase was found to be statistically non-significant. In *Mucuna monosperma* DC [MMD] group there is considerable but statistically non-significant decrease in comparison to BCCAO control group. In Brahmi gritha administered group there

is a moderate decrease in the glutathione peroxidase activity in brain homogenate in comparison to BCCAO group. MPB and MMD groups have shown mild elevation in the glutathione peroxidase activity in comparison to the BCCAO control group. Lipid peroxidation was moderately decreased in Brahmi gritha administered group while in MPB and MMD treated group non significant mild increase was observed in comparison to BCCAO control group (Table 2).

Table 1: Effect of test drug on antioxidant activity in the brain homogenate

Groups	Acetyl Choline esterase (IU/L)	C-reactive protein mg/100ml
Positive control	528.53±50.73	0.706±0.042
Standard (Bhrahmi ghruta)	406.7±36.23	0.663±0.140
Test 1 (*Mucuna monosperma*)	883.81±29.40**	0.573±0.039
Test 2 (*Mucuna pruriens*)	702.96±100.05	0.762±0.068

Data expressed in Mean ± SEM, ** p<0.01, compared with BCCAO control.

Table 2: Effect of test drug on antioxidant activity in the brain homogenate

Group	Catalase activity µmoles H_2O_2 consumed µmoles/mg /min	Glutathione Peroxidase activity (µmoles/g /min at 37°C)	Lipid Peroxidation µmoles of MDA formed /g wet tissue
Positive control	0.13 ± 0.021	26.23 ±4.75	1.22±0.54
Standard (Bhrahmi ghruta)	015±0.011	18.98±2.06	0.311±0.049
Test 1 (*Mucuna monosperma*)	0.05±.0047	25.03±2.88	3.04±0.47*
Test 2 (*Mucuna pruriens*)	0.21±0.043	34.52±8.19	1.55±0.30

Data expressed in Mean ± SEM, * p<0.05, compared with BCCAO control

Histopathology

Hippocampus:

Microscopic examination of the midbrain sections containing hippocampus especially the dentate gyrus from BCCAO control rats revealed cellular organization with distinct granular layer, hilar region, and molecular layers. The cellularity of the granular layer was found to be less in sections from three rats. In this layer the proportion of mature cells was found to be higher in comparison to the immature cells. Vacuolization was also observed in few sections in the neutrophils. Brahmi gritha group exhibited almost normal cytoarchitecture. The molecular layer, the granular layer and the hilus region exhibited normal profile. However, decreased cellularity was observed in section from one rat.

The sections of hippocampus from *Mucuna monosperma* DC group exhibited decreased cellularity of the granular layer in sections from three rats. Vacuolization was also observed in few sections. In *Mucuna pruriens* Bek treated group almost normal cytoarchitecture was observed. However, decreased cellularity was observed in section from one rat in the granular layer. (Figure 1)

Fore brain

The fore brain sections from BCCAO group exhibited almost normal cytoarchitecture in majority of the rats except for section from one rat in which cellular organization was found to be disturbed and in another rat oedema was observed. The sections from Brahmi gritha, *Mucuna monosperma* DC and *Mucuna pruriens Bek* groups also exhibited normal cytoarchitecture. (Figure 2)

Mid brain

In the BCCAO control rats cellular organization was found disturbed. There were areas of cell infiltration and vacuolization of the neutrofils was found in almost all the sections. Examination of sections from Brahmi gritha revealed almost normal cytoarchitecture in most of the sections studied. Examination of the sections of midbrain from *Mucuna monosperma* DC group has shown mild to moderate edematous changes in sections from two rats. Cell infiltration was found to be less in comparison to the BCCAO rats. Vacuolization of the neurofil was extensive. In sections from *Mucuna pruriens* Bek group almost normal cytoarchitecture was observed. (Figure 3)

Cerebellum

The sections of cerebellum from BCCAO group exhibited normal cytoarchitecture. The sections from Brahmi gritha, *Mucuna monosperma* DC and *Mucuna pruriens Bek* groups also exhibited normal cytoarchitecture. (Figure 4)

DISCUSSION

Serum Cholinesterase

At present there are no diagnostic biomarkers identifying mild stroke. However, recently it has been shown that circulating Acetyl choline esterase [AChE] activity reflects inflammatory response, since acetylcholine suppresses inflammation [15]. In the present study moderate decrease was observed in serum AChE activity in BCCAO group in comparison to the normal control. This decrease was found to be reversed moderately in *M. pruriens* Bek. group and significantly in *M. monosperma* DC treated groups. Surprisingly, a moderate decrease which is statistically non-significant was observed in Brahmi gritha. The exact reason for the non-conformity of activity in this parameter in the context of overall activity is not clear. *M. monosperma* DC produced better effect in this parameter.

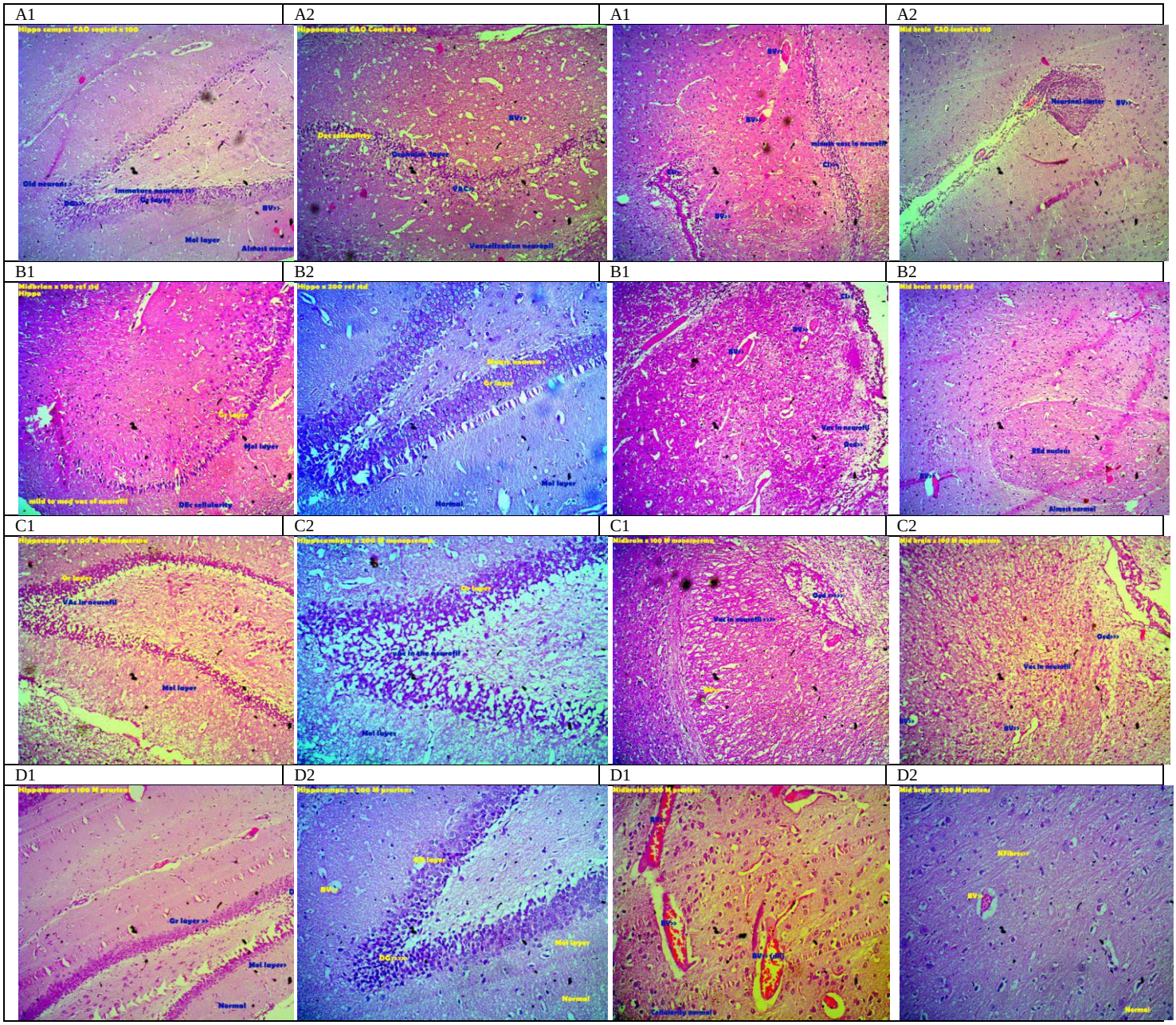

Figure 1: Photomicrograph of representative brain sections focused on hippocampus region of BCCAO positive control group (A 1& 2) , Reference standard treated with Brahmi gritha (B1& 2), Test groups MMD and MPB represented in (C1& 2, D1& 2).

BCCAO control rats cellularity was found to be decreased & predominance of immature neurons in the granular layer was observed where as standard and test drug administered group exhibited almost normal cytoarchitecture of molecular layer, granular layer and the hilus region.

Figure 2: Photomicrograph of representative brain sections focused on midbrain region of BCCAO positive control group (A 1& 2) , Reference standard treated with Brahmi gritha (B1& 2), Test groups MPB and MMD represented in (C1& 2, D1& 2).

BCCAO control group rats have showed disturbed cellular organization, cell infiltration and vacuolization of the neutrophils was found in almost all the sections. Examination of sections from Brahmi gritha revealed almost normal cytoarchitecture in most of the sections studied. Examination of the sections of midbrain from *Mucuna monosperma* DC group has shown mild to moderate edematous changes CI- Cell infiltration, Oed- Oedema

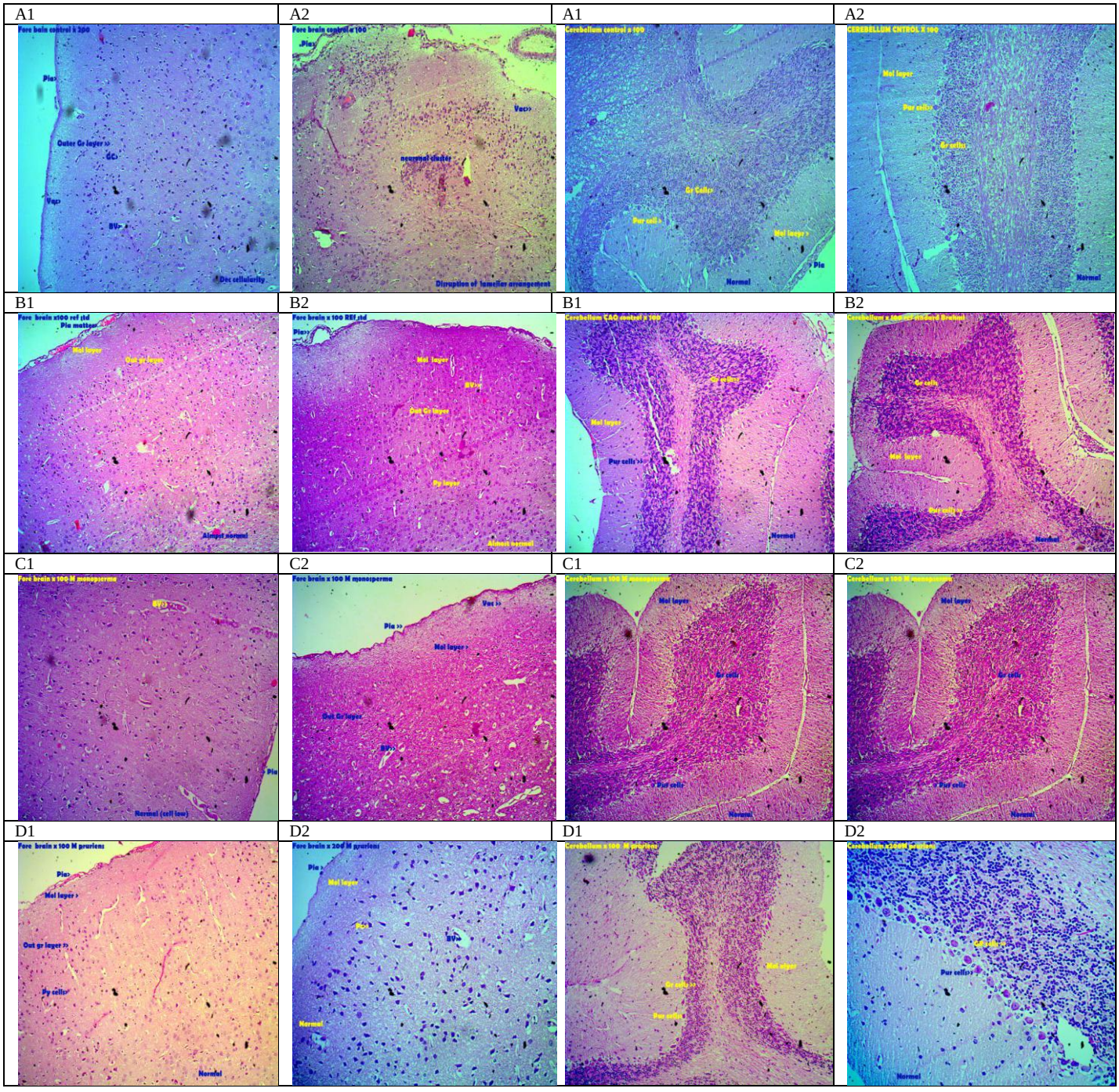

Figure 3: Photomicrograph of representative brain sections focused on fore brain region of BCCAO positive control group (A 1& 2) , Reference standard treated with Brahmi gritha (B1& 2), Test groups MMD and MPB represented in (C1& 2, D1& 2).
The fore brain sections from BCCAO group exhibited almost normal cytoarchitecture. The sections from reference standard, test-1 and test-2 groups also exhibited normal cytoarchitecture.

Figure 4: Photomicrograph of representative brain sections focused on cerebellum region of BCCAO positive control group (A 1& 2), Reference standard treated with Brahmi gritha (B1& 2), Test groups MMD and MPB represented in (C1& 2, D1& 2).
The fore brain sections from BCCAO group exhibited almost normal cytoarchitecture. The sections from reference standard, test-1 and test-2 groups also exhibited normal cytoarchitecture.

Serum C Reactive Protein:

After arterial occlusion, ischemic brain injury is accompanied by acute local inflammation and a dramatic plasma level rise of inflammatory cytokines [16]. The elevated plasma level of the acute phase reactant C-reactive protein (CRP) is an outcome-predicting factor after stroke or myocardial infarction. CRP is an acute phase reactant produced by the liver in response to acute inflammatory stimuli and has been demonstrated to have direct effects on cerebral brain micro vascular endothelial cells (EC). In the present study C-reactive protein was not affected to significant extent and hence was not of much usefulness in determining the neuroprotective activity of the test plants.

Effect of *Mucuna* species on anti-oxidant activity:

Catalase is part of the body's endogenous anti-oxidant system – increased level of this enzyme is indicative of enhanced anti-oxidant system. Lipid peroxidation is the primary process which is responsible for the generation major part of free radicals. Deceased lipid

peroxidation is indicative of decreased generation of free radicals. Glutathione peroxidase is involved in the metabolism of glutathione. Increased activity of this enzyme may lead to decreased anti-oxidant system through decreased availability of glutathione. In the present study Catalase activity was found to be moderately but non-significantly increased in *M. pruriens* Bek. treated group whereas moderate but statistically non-significant decrease was observed in *M. monosperma* DC. treated group. In reference standard marginal increase was observed.

Glutathione peroxidase activity was found to be moderately decreased in standard group and moderately increased in *M. pruriens* Bek. treated group whereas *M. monosperma* DC. did not affect this parameter.

Lipid peroxidation was found to be remarkably decreased in reference standard and moderately decreased in *M. pruriens* Bek. treated group. In *M. monosperma* DC. treated group significant increase was observed.

Analysis of the data related to all the three parameters of the anti-oxidant activity study indicates presence of moderate anti-oxidant activity in *M. pruriens* Bek. treated group and pro-oxidant activity in *M. monosperma* DC. Thus the former has better anti-oxidant activity profile in comparison to the latter and may be the reason for the observed difference in the activity profile. However, lack of anti-oxidant activity did not result in total lack of neuroprotection indicating that other mechanism is also operative.

Effect of *Mucuna* species on histopathological profile

Histopathological examination of brain region revealed that BCCAO did not affect the cytoarchitecture of cerebellum while changes in some rats were observed in midbrain and midbrain with hippocampus. In one rat the cellular organization in the forebrain was found to be disturbed. In mid brain cell infiltration especially near the areas of meninges attachment was observed after BCCAO. In addition decreased cellularity was observed. In *M. pruriens* Bek. treated group cell infiltration in mid brain was less however, mild edematous changes were observed. The BCCAO induced changes in the hippocampus were much less in this group. In *M. monosperma* DC. group moderate changes were observed. *M. pruriens* Bek. produced better neuroprotection in comparison to *M. monospernma* DC.

CONCLUSSION

Based on the data generated and careful analysis it can be inferred that both the *M. pruriens* Bek. and *M. monosperma* DC. exhibit different degree of neuroprotection. The overall profile indicates better protective ability in the *M. pruriens. Bek.*

REFERENCES

1. Dimagl U, Iadecola C, Moskowitz M A: Pathobiology of ischemic stroke: an integrated view. Trends Neurosci. 1999;22:391-97
2. Terent A, Marke L A, Asplund K, Norrving B, Jonsson E, 0 WP: Costs of stroke in Sweden. A national perspective Stroke. 1994; 25:2363-69
3. Wolfe C D: The impact of stroke. Dr Med Dull 2000; 56:275-286
4. Baethmann A, Eriskat J, Stoffel M, Chapuis D, Wirth A, Plesnila N: Special aspects of severe head injury: recent developments. Curr Opin An aesth esiol. 1998; 11:193-200
5. Graham D I, Adams J H, Doyle D: Ischaemic brain damage in fatal non-missile head injuries. J Neurol Sci. 1978;39:213-34
6. Kirtikar K R, Basu B D, Indian Medicinal Plants, Vol 1st , Shiva offset press, Dehra Dun. 1991: Pp838.p779
7. Sri Chakrapanidatta, Chakradatta with Vaidyaprabha Hindi commentary by Dr. Indradev Tripathi edited by Prof. Ramanath Dwivedy, Chaukhambha Sanskrit Bhavan, Varanasi, Edition: reprint.2010;Pp542,p135
8. Vaidya Bapalal G, Nigantu Adarsha vol 1, Varanasi; Chaukhambha Bharati Academy,Edition 2 .1999; Pp 919,p.455.
9. Paget G E, Barnes J M. Evaluation of drug activities. In: Lawrence DR, Bacharach AL, editors. Pharmacometrics.Vol. 1. New York: Academic press; 1964. p. 161.
10. Nakashima M, Niwa M, Iwai T, Uemastu T. Involvement of Free radicals in cerebral vascular reperfusion injury evaluated in a transient focal cerebral ischemic model of rats. Free Radic Biol Med. 1999; 26:722.
11. Yanpallewar S U, Hota D, Rai S, Kumar M, Acharya S B. Nimodipine attenuates biochemical, behavioural and histopathological alteration induced by acute transient and long term bilateral carotid artery occlusion in rats. Pharmacol. Res. 2004;49:143.
12. Sinha K A. Colorimetric assay of catalase. Anal Biochem 1972; 47: 389-94.
13. Ohkawa H, Ohishi N, Yagi K: Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem. 1997; 95: 351-58.
14. Bancroft J D, Gamble M. Theory and practice of histological techniques. 5th ed. Queen's Medical Centre, Nottingham, UK; Churchill Livingstone, 2002.
15. Dong – Jie, Roger J Evans,Zong –Wei Yang. ά7nAChR and Hypertensive organ damage. Hyper.ahajournals.org. 2011;57:298-307
16. Fassbender K, Rossol S, Kammer T, Daffertshofer M, Wirth S, Dollman M, Hennerici M. Proinflammatory cytokines in serum of patients with acute cerebral ischemia: kinetics of secretion and relation to the extent of brain damage and outcome of disease. J Neurol Sci. 1994; 122: 135–39

HOW TO CITE THIS ARTICLE
Hareshchandra DY, Mundugaru R, Bairy TS, Ravikrishna S, Shankar RB. Neuro-protective role of seeds of *Mucuna pruriens* BEK and *Mucuna monosperma* DC in wistar albino rats. The Journal of Phytopharmacology 2015;4(6):276-281.