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Original Research Article

Effect of *Mucuna pruriens* (Linn.) on Global Cerebral Ischemia-induced Motor Incoordination

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Abstract

Purpose: *To evaluate the effect of Mucuna pruriens on ischemia-induced motor incoordination.*
Methods: *Male Wistar rats were used in this study were divided into eight groups of six rats each. The bilateral common carotid artery (BCA) was occluded to induce global cerebral ischemia. Carboxymethylcellulose (CMC) was used as a vehicle for this while quercetin was used as the standard. The rats were fed with the methanol plant extract of Mucuna pruriens (MP) seeds for ten days. The treatment groups include treatment plus BCA occlusion. Various behavioral parameters such as locomotor activity, motor coordination, spatial learning, and cerebral infarction area were evaluated.*
Results: *A decline in locomotor activity, motor coordination, and spatial learning was observed in the ischemia-induced Wistar rats. The group treated with Mucuna pruriens showed significant protection against brain damage when compared with negative control group.*
Conclusion: *Mucuna pruriens provides effective neuroprotective activity in BCA occlusion-induced cerebral ischemia in rats, and thus a potential therapeutic agent for the clinical management of cerebral ischemia.*

Keywords: *Global ischemia, Mucuna pruriens, Oxidative stress, Brain damage, Locomotor activity, Motor coordination*

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INTRODUCTION

A decreased blood supply to the cerebral hemisphere leads to a decline in the level of oxygen and metabolic substances of the brain which leads to brain injury. Reactive oxygen species (ROS) induced oxidative stress is a major cause of brain injury [1]. Several components of ROS are generated after ischemic injury which plays a major role in post-ischemic nerve cell loss [2].

Ischemic brain injury also leads to behavioral problems such as decreased memory level and motor disorder [3]. The reaction between free radicals and biomolecules of the cerebrum can result in a decreased level of neurons leading to memory loss [4]. The hippocampal region of the

brain which controls learning and memory is affected by ischemic insults. The CA1 region of the hippocampus is easily damaged as compared to the CA3 region and dentate gyrus. A period of 24 h of ischemia results in cell death in the CAI region [5]. Mitochondrial dysfunction, an increase in glutamate release, and ROS lead to neuronal death using the autophagic pathway or apoptosis [6]. The reason for brain damage in ischemia might be due to an increase in the rate of oxidative metabolic activity, increased levels of ROS metabolites, presence of more amount of polyunsaturated fatty acids, and non-duplicating property of neurons [7].

Many plant products are widely studied for alleviating these apoptotic and necrotic changes in neuronal cells due to their antioxidant nature. *Mucuna pruriens Linn*, (MP) commonly called *Kapikacchu* or velvet bean is a popular medicinal plant in India due to its multiple medicinal values. It has been used as an aphrodisiac, nervous disorders, atherosclerosis, and Parkinson's disease [8]. The plant's seed comprises high levels of phenolic compounds which is responsible for its antioxidant property. The present research aims to study the neuroprotective potential of *Mucuna pruriens* extracts on global cerebral ischemia-induced motor incoordination.

EXPERIMENTAL

Preparation of plant extract

The seeds of the *Mucuna pruriens* plant were dried and a fine pure powder was obtained from these seeds. They were first extracted with -80 °C petroleum ether for de-fatting and the remaining residue was extracted with 95 % methanol for 72 h. The product was then transferred and filtered, and used for the present study.

Animals

The male Wistar rats taken for the study were housed in polypropylene cages at a humidity of 60 – 67 % and room temperature of 24 ± 2˚C and was provided food and water and even intervals. Permission for the animal study was obtained from the Institutional Ethical Committee and the study followed international guidelines for handling animals used in laboratory studies.

Animal grouping and treatments

The Wistar rats were grouped into eight with 6 rats in each group. 0.25% w/v carboxymethylcellulose (CMC,) was used as a vehicle. Quercetin was used as the standard drug. Group I was used as a normal control group [10ml/kg of oral CMC (0.25 % w/v)] and no occlusion of BCA. In Group II, 200mg/kg oral alcoholic extract was given with no occlusion of BCA. Group III subjects were fed with 25 mg/kg oral Quercetin with no occlusion of BCA. In Group IV subjects (Negative control), occlusion of BCA was done for 30 min and was followed by reperfusion. Group V subjects were fed with 50 mg/kg of oral alcoholic extract along with occlusion of BCA. 100mg/kg oral alcoholic extract was fed to Group VI rats, along with occlusion of BCA. A 200 mg/kg oral alcoholic extract was fed to Group VII subjects along with occlusion of BCA. In Group VIII subjects, 25 mg/kg Quercetin was given orally and BCA is occluded.

Induction of ischemia is done by carotid arterial occlusion (BCA) of both sides [9]. 45 mg/kg intraperitoneal ketamine was used to anaesthetize the rats. Both carotid arteries were separated from the surrounding structures by an incision in the neck and were clamped for about 30 minutes to induce ischemia. Later, both clamps were removed and the incision was sutured.

Evaluation of locomotor activity

A digital actophotometer was used to assess the locomotor activity of the rats. This activity indicates the alertness of the mind. After weighing and numbering the animals, each one was kept individually in an actophotometer and the basal activity score was recorded. A decrease in the activity of animal was considered as an index of CNS depression.

Determination of motor coordination

Motor coordination and balancing capacity were measured using a rotarod test, where the rats have to balance themselves on a rotating rod. The animal was placed on a rotating rod where rod velocity was kept constant at 15 rpm. The time when each animal falls from the rotating rod was recorded automatically.

Determination of spatial learning

The T-maze apparatus was used in the evaluation of spetial learning as described earlier [10]. This apparatus has two arms on opposite side. In this experiment, we observe the ability of the animal in differentiating the side of the apparatus in order to get food. The percentage bias, number of alternations, and percentage correct response were recorded.

Assessment of cerebral infarction area

The 2,3,5-triphenyl tetrazolium chloride (TTC) stain was used to identify the infarcted area of the cerebrum [11]. Following ischemia, the rats were decapitated, and the brain was kept in saline for 30 min, brain was coronally cut (1mm thick) and kept in saline that contained 2 % TTC. The viable part stains red and the non-viable part remains in its original (white) color.

Statistical analysis

The result was represented as means ± S.E.M. with one-way ANOVA, following Tukey's multiple comparisons post hoc test with the help of a Graph Pad Prism version 5. A value of $p < 0.05$ was considered statistically significant.

RESULTS

Locomotor activity

A significant reduction ($P<0.05$) in the locomotor action of rats in the group with BCA occlusion was observed, in comparison with the control group. Increased locomotor action was observed in groups V, VI, and VII in comparison with the negative control Group IV. The quercetin pre-treated ischemic group (Group VIII) showed an increase in locomotor activity when compared to the negative control group IV (Figure 1).

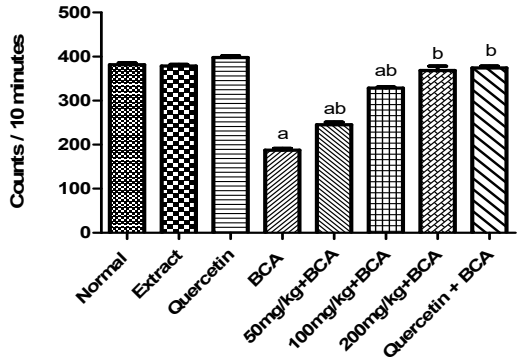

Figure 1: Effect of plant extrac (MP) on ischemic model. The bar diagram shows the locomotor activity measured using actophotometer. Methanolic extract of *Mucuna pruriens* with the doses: 50, 100, and 200 mg/kg. $^{a}P < 0.05$ when in comparison to normal group, and $^{b}P < 0.05$ in comparison with ischemic group

Motor co-ordination using the Rotarod test

The motor coordination of the Wistar rats in the groups with BCA occlusion were found to be significantly low in comparison to the control group. These changes were also observed in BCA occlusion with 50 and 100 mg/kg of *MP* extract pre-treated group. In the group of Wistar rats with BCA occlusion who received 200 mg/kg of *Mucuna pruriens* extract, the motor coordination showed a significant rise. A similar increase was observed in the group where BCA occlusion was done with intake of Quercetin (Figure 2).

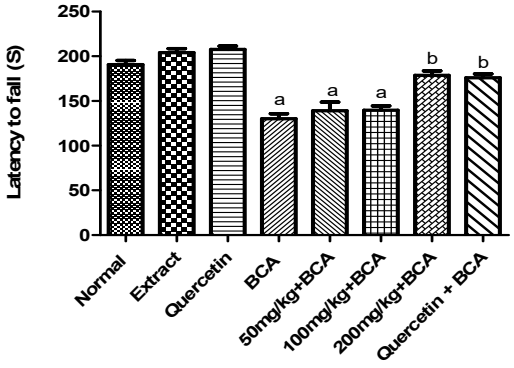

Figure 2: Effect of plant extrac (MP) on ischemic model. The bar diagram represents the Rotarod test. Methanolic extract of *Mucuna pruriens* with the doses: 50, 100 and 200 mg/kg. $^{a}p < 0.05$ in comparison with normal group; $^{b}p < 0.05$ in comparison with ischemic group

Spatial learning

The Wistar rats of the BCA occlusion group exhibited impairment in spatial learning when compared to those in the normal group, which was statistically significant *($p < 0.05$)*. It was seen that a dose-dependent rise in spatial learning in the groups pre-treated with 50, 100, and 200 mg/kg doses of *Mucuna pruriens* with occlusion of BCA when compared to the BCA occlusion (negative control) group. The protection in spatial learning is also observed in the Quercetin pre-treated BCA occlusion group (Figure 3 and Figure 4).

Rewarded alternation

In this test, the animals of the BCA occlusion group exhibited impairment in spatial learning when compared to the normal group and was found to be statistically significant ($p < 0.05$). An increase in spatial learning was noted in the *Mucuna pruriens* pre-treated BCA occlusion group (50, 100, and 200 mg/kg) when compared to that of the BCA occlusion group. Increased spatial learning was also observed in the BCA occlusion group pre-treated with quercetin (Figure 5).

Assessment of cerebral infarction area:

The cerebral infarction area of BCA occlusion-induced ischemic model was measured using TTC staining method. As shown in the figure 6, the normal brain tissues were stained red whereas the infarction areas were stained in white. There was no significant difference in infarction area was observed in Quercetin and extract control when compared to the normal control group. This shows the safety of extract/quercetin treated groups indicating absence of cell death. In Group IV (Figure 6 D), significant changes ($p < 0.05$) were seen with an increased rate of infarction. A significant ($p < 0.05$) rise in protective function is noted in groups V, VI, and VII (Figure 6 E, F and G). The Group VIII subjects, treated with Quercetin also showed a significant increase (< 0.05) in protection (Figure 6 H).

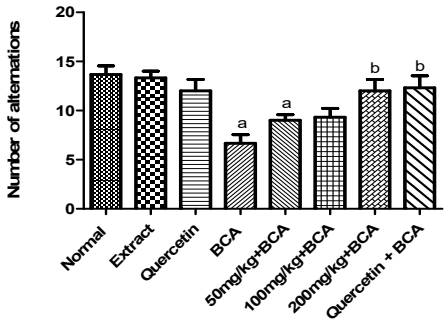

Figure 3: Effect of plant extract (MP) on ischemic model. Bar diagram shows number of alternations in spontaneous alternation test. Methanolic extract of *Mucuna pruriens* at doses of 50, 100, and 200 mg/kg. $^{a}P < 0.05$ in comparison to normal group, $^{b}P < 0.05$ in comparison to ischemic group.

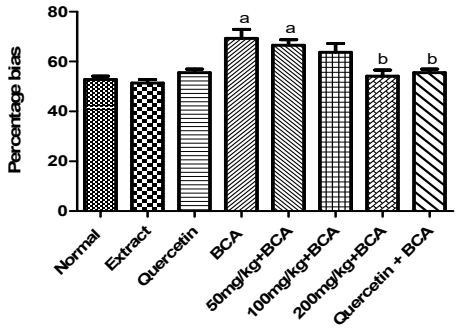

Figure 4: Effect of plant extract (MP) on ischemic rats. Bar diagram shows percentage bias in spontaneous alternation test. Methanolic extract of *Mucuna pruriens* at doses: 50, 100, and 200 mg/kg. $^{a}p < 0.05$ in comparison to normal group; $^{b}p < 0.05$ in comparison with ischemic group

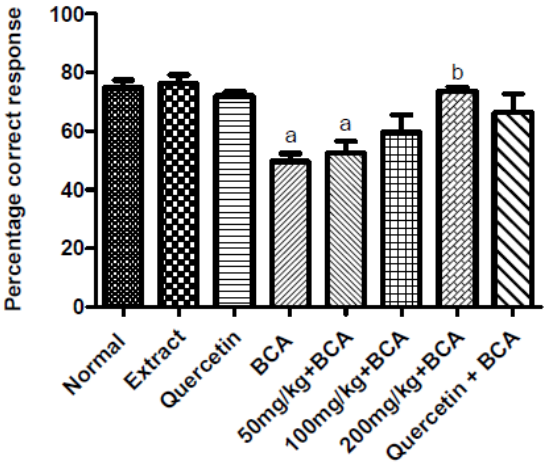

Figure 5: Effect of plant extract (MP) on ischemic model. Bar diagram showing percentage correct response in rewarded alternation test. Methanolic extract of *Mucuna pruriens* at doses: 50, 100, and 200 mg/kg. $^{a}P < 0.05$ in comparison to normal group, $^{b}P < 0.05$ in comparison to ischemic group

Figure 6: Effect of plant extrac (MP) on ischemic model showing TTC stained sections of various groups. Pic: (A) CMC; (B) 200 mg/kg of extract; (C) 25mg/kg of quercetin; (D) CMC + BCA occlusion; (E, F, and G) 50, 100, and 200 mg/kg doses of extract respectively + 30 min of BCA occlusion; (H) 25 mg/kg of quercetin +30 min of BCA occlusion

DISCUSSION

It is well-known that ischemic brain injury results in the generation of free radicals which damage proteins, nucleic acids as well as lipids. This damage leads to increased levels of lipid peroxidation and membrane damage. To protect from this the antioxidant defense system of the body tries to neutralize the free radicals. *Mucuna Pruriens* decreases the amount of hydrogen peroxide by increasing the antioxidant defense enzymes and thus prevents neuronal injury.

There is a great need for lifestyle modifications after the occurrence of ischemia [12]. The spatial

learning and memory function of rats was significantly impaired by global ischemia [13]. The T-Maze test was performed to evaluate memory retention ability. Ischemic animals showed a significant reduction in learning and memory activity. This was supported by a previous study which reports that cerebral ischemia causes decreased hippocampal CA1 neurons and cognitive impairment in the Y-maze test [14]. In the T-maze test, the memory deficit caused by ischemia was significantly reversed by treatment with *Mucuna pruriens*, suggesting the beneficial effect of *Mucuna pruriens* in ischemic induced cognitive dysfunction.

Previous studies have reported that occlusion of the middle cerebral artery results in the suppression of locomotor activity. Improvement of this locomotor function can be possible by a reduction in cerebral infarction after successful neuroprotective treatments [15]. In the present study, ischemia resulted in the suppression of locomotor activity, and the activity is increased in the extract-treated group suggesting its protective effect on infarction.

The Rotarod test was performed to observe the motor coordination of the rats. Decreased balance and motor coordination was noticed in the BCA occlusion group, whereas *Mucuna pruriens* extract-treated groups showed improvement in these activities. A similar result was observed in a previous study where ischemia resulted in motor impairment and exercise improved motor activity [16]. This motor impairment may be due to the striatal damage caused by oxidative stress as well as neuronal loss [17]. The protective activity of *Mucuna pruriens* in response to AlF3-induced behavioral and neuronal damage was also observed in the earlier study [18].

The TTC staining method has been used in this study to check the infarction area of the brain. In this method, the viable area of the brain stains deep red. This is because the TTC is reduced to formazone by the mitochondria of the cell and ischemic tissue which has damaged mitochondria remains unstained [11]. In this study, similar unstained areas were present in ischemic animals, and protection was observed in *Mucuna pruriens* treated animals showing its neuroprotective activity.

These results of neuro-degeneration are also confirmed by the histopathological and biochemical observations of different treatment groups in our earlier study [19]. As discussed earlier, oxidative stress results in impaired motor coordination and cognitive function. *Mucuna pruriens* has shown improved sexual behavioral profile and haloperidol-induced tardive dyskinesia. These are indicative of alterations in the levels of noradrenaline, dopamine, and other neurotransmitters and the same has been reported [20].

CONCLUSION

The results show decreased learning and memory ability, and impaired motor coordination and locomotor activity in stress-induced animals, but motor coordination, locomotor activity, learning and memory ability are reversed when treated with *mucuna pruriens* extract. The neuroprotective activity of *Mucuna pruriens* against oxidative stress-induced brain damage is comparable and even superior to the standard drug, quercetin, in some instances. Esters of fatty acids and antioxidants may be responsible for the beneficial effects of *Mucuna pruriens*.

DECLARATIONS

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Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Conceiving and design of the study were done by Vanishri S. Nayak and K.S.R. Pai. The collection and analyzing of the data were done by Vanishri S. Nayak, Nitesh Kumar, K.S.R. Pai, and Sunil S Nayak. The manuscript was written by Vanishri S. Nayak, Sunil S Nayak, and Hemalatha Bangera. All authors have read and approved the manuscript for publication.

REFERENCES

1. *Kinouchi H, Epstein CJ, Mizui T, Carlson E, Chen SF, Chan PH. Attenuation of focal cerebral ischemic injury in transgenic mice over expressing CuZn superoxide dismutase. Proc Natl Acad Sci USA. 1991; 88: 11158-11162.*
2. *Oliver CN, Starke-Reed PE, Stadtman ER, Liu GJ, Carney JM, Floyd RA. Oxidative damage to brain proteins, loss of glutamine synthetase activity, and production of the free radicals during ischemia/reperfusion-induced injury to gerbil brain. Proc Natl Acad Sci USA. 1990; 87: 5144-5147.*
3. *Wattanathorn J, Jittiwat J, Tongun T, Muchimapura S, Ingkaninan K. Zingiber officinale mitigates brain damage and improves memory impairment in focal cerebral ischemic rat. Evid Based Complement Alternat Med 2011; 2011: 429505.*
4. *Rao KS. Free radical induced oxidative damage to DNA: Relation to brain aging and neurological disorders. Indian J Biochem Biophys 2009; 46: 9-15.*
5. *Kovalenko T, Osadchenko I, Nikonenko A, Lushnikova I, Voronin K, Nikonenko I, Muller D, Skibo G. Ischemia-induced modifications in hippocampal CA1 stratum radiatum excitatory synapses. Hippocampus 2006; 16: 814-825.*
6. *Alexander G. Nikonenko, Lidija Radenovic, Pavle R. Andjus, And Galyna G. Skibo. Structural Features of Ischemic Damage in the Hippocampus, The Anatomical Record 2009; 292: 1914–1921*
7. *Evans PH. Free radicals in brain metabolism and pathology. Br Med Bull 1993; 49: 577-587.*
8. *Bhaskar A, Nithya V, Vidhya VG. Phytochemical evaluation by GC-MS and antihyperglycemic activity of Mucuna pruriens on Streptozotocin induced diabetes in rats. J Chem Pharm Res 2011; 3: 689-696.*
9. *Farbiszewski R, Bielawski K, Bielawska A, Sobaniec W. Spermine protects in vivo the antioxidant enzymes in transiently hypoperfused rat brain. Acta Neurobiol Exp 1995; 55: 253-258.*
10. *Dunnett SB, Low WC, Iverseni SD, Stenvi U, Bjorklund A. Septal transplants restore maze learning in rats with fornix fimbria lesions. Brain Res 1982; 251: 335-348.*
11. *Bederson JB, Pitts LH, Germano SM, Nishimura MC, Davis RL, Bartkowski HM. Evaluation of 2,3,5-triphenyltetrazolium chloride as a stain for detection and quantification of experimental cerebral infarction in rats. Stroke 1986; 17: 1304-1308.*
12. *Tamim R. Al-Tamimi, Hafidh A. Ba-Omar, Sunil Nadar. Knowledge regarding secondary prevention lifestyle practices among patients with ischemic heart disease in Oman. Sultan Qaboos University Med J 2017; 17:88-92.*
13. *Cheng O, Li R, Zhao L, Yu L, Yang B, Wang J, Chen B, Yang J. Short-term sleep deprivation stimulates hippocampal neurogenesis in rats following global cerebral ischemia/reperfusion. PLoS ONE 2015; 10: e0125877.*
14. *Li DQ, Duan YL, Bao YM, Liu CP, Liu Y, An LJ. Neuroprotection of catalpol in transient global ischemia in gerbils. Neurosci Res 2004; 50: 169-177.*
15. *Wang Y, Chang CF, Morales M, Chiang YH, Harvey BK, Su TP, Tsao LI, Chen SY, Thiemermann C. Diadenosine tetraphosphate protects against injuries induced by ischemia and 6-hydroxydopamine in rat brain. J Neurosci 2003; 23: 7958-7965.*
16. *Sosa PM, Schimidt HL, Altermann C, Vieira AS, Cibin FWS, Carpes FP, Mello-Carpes PB. Physical exercise prevents motor disorders and striatal oxidative imbalance after cerebral ischemia-reperfusion. Braz J Med Biol Res 2015; 48: 798-804.*
17. *Lu F, Nakamura T, Toyoshima T, Liu Y, Hirooka K, Kawai N, Okabe N, Shiraga F, Tamiya T, Miyamoto O, Keep RF, Itano T. Edaravone, a free radical scavenger, attenuates behavioral deficits following transient forebrain ischemia by inhibiting oxidative damage in gerbils. Neurosci Lett 2012; 506: 28-32.*
18. *Nayak VS, Kumar N, Dsouza AS, Nayak SS, Pai KSR. Modulatory role of mucuna pruriens against aluminium fluoride induced neuronal and behavioural alterations in rats. Journal of Clin Diagnos Res 2018; 12: FC01-FC04.*
19. *Nayak VS, Kumar N, D'Souza AS, Nayak SS, Cheruku SP, Pai KSR. The effects of Mucuna pruriens extract on histopathological and biochemical features in the rat model of ischemia. Neuroreport 2017; 28: 1195-1201.*
20. *Suresh S, Prakash S. Effect of Mucuna pruriens (Linn.) on oxidative stress-induced structural alteration of corpus cavernosum in streptozotocin-induced diabetic rat. J Sex Med 2011; 8: 1943-1956.*