ISSN: 0976-4607
Research Article

International Journal of Innovative Pharmaceutical Research

Journal homepage: www.ijipr.com

Evaluation of Neuroprotective Activity of *Aegle Marmelos(Fruit)* Against Sodium Nitrite Induced Neurotoxicity in Albino Wistar Rats

Vengal Rao P*, Ashok Kumar CK, Jeevan Kumar V, Nathasha Jacksom D and Prasanth Kumar M

Sree Vidyanikethan College of Pharmacy, A.Rangampet, Tirupati, Andhra Pradesh, India-517102.

ABSTRACT

Neurodegenerative diseases result in the loss of functional neurons and synapses. Current treatments for most of these diseases are less than adequate and our best hope is to prevent these devastating diseases. Neuroprotective approaches work best prior to the initiation of damage, suggesting that some safe and effective prophylaxis would be highly desirable. Oxidative stress is implicated as one of the primary factor that contributes to the neurodegenerative diseases,brain damage, stroke, hypoxia etc. *Aegle marmelos* is one of the herbal drug traditionally used as Nervine tonic, Antidiuretic, Antioxidant, Antihyperlipidemic etc. The aim and objective of the study is to investigate the neuroprotective effect of hydroalcoholic seed extract of *Aegle marmelos* on hypoxic neurotoxicity induced in wistar rats. The animals were divided in to five groups of 8 animals each. Hypoxic neuronal damage was induced by the administration of sodium nitrite 30mg/kg p.o for 14 days. The hydroalcoholic extract of was administered at doses 200mg/kg, 400mg/kg b.w, p.o for 14 days. Alteplase 0.9mg/kg i.v was used as a standard. Alteration in various biochemical and antioxidant levels was estimated. The drug treated groups showed normal neurological behavior comparable with that of normal control group. As the model is clinically relevant it will further enhance the mechanistic understanding of neuronal damage and help in developing newer and better therapeutic strategies to manage oxidative stress.

Keywords: Oxidative stress, *Aegle marmelos*, Hypoxia, Neurotoxicity.

INTRODUCTION

Cerebral Stroke (Harsh Mohan, 2005; Robbin and Cotran, 2010) is a sudden and dramatic development of focal neurologic deficit, varying from trivial Neurologic disorder to hemiplegia and coma.

Major Mechanisms causing brain damage are,

- Hypoxia, Ischemia and Infarction resulting from impairment of blood supply and oxygenation of CNS tissue.
- Haemorrhage resulting from rupture of CNS vessels.

The metabolism of the brain depends exclusively on oxygen and glucose and adds up to the consumption of molecular oxygen and 120 g glucose per day. In comparison to other organs this situation is unique. Albeit the weight of the brain accounts for only 2% of the total body weight, the brain claims 20% of total body perfusion and 20% of total oxygen consumption. Even short durations of reduced brain perfusion leading to lack of oxygen and energy metabolites may lead to irreversible structural damage, ultimately leading to cell death (Kobayashi T *et al.*, 1998).

- The incidence of stroke is approximately 250-400/100,000. An estimated total of 200,000 strokes occur per year.
- Stroke mortality amounts—irrespective of all therapeutic efforts—still are 25 to 30%.
- Stroke is the third leading cause of death in the world.

Aegle marmelos (Linn.) belongs to family Rutaceae, commonly known as bael (Hindi) and golden apple (English). It is found throughout India and is known from pre-historic time. Aegle marmelos has been used from time immemorial in traditional systems of medicine for relieving constipation, diarrhoea, dysentery, peptic ulcer and respiratory infections. Several studies on different parts of Aegle marmelos are known to have some therapeutic properties, such as anti diarrheal, anti-hyperglycemic, anticancer, radioprotective,brain tonic, antidepressant .and analgesic activities (Astrup J *et al.*, 1981; Hossmann KA, 1994).

***Corrosponding author**

Vengal Rao P
Email id: pvengalrao.pvr@gmail.com

The present study was aimed to evaluate the Neuroprotective potential of *Aegle marmelos* (Fruit) against sodium nitrite induced neurotoxicity.

MATERIALS AND METHODS

Plant Material

The *Aegle marmelos* (Fruit) used in the present study was collected from natural habitat in and around Tirupathi, Andhra Pradesh. The plant is authenticated by Asst Prof. K. Madhav chetty, Department of Botony, Sri Venkateswara University.

Preparation of Plant Extract

Dried fruits of *Aegle marmelos* were reduced to fine powder (# 40 Size mesh) and around 200g of powder is subjected to Successive hot continuous extraction (soxhlet) with hydro alcohol (1:1) (Gupta YK *et al*., 2004; Xuejiang W *et al*., 1999). After the effective extraction, The solvents were distilled off the extract was subjected to concentrated on water bath and then extract obtained with each solvent was preserved for further studying.

Phytochemical Analysis

The Hydroalcoholic extract was subjected to the phytochemical analysis using conventional protocol like alkaloids, flavonoids, carbohydrates, glycosides, saponins, proteins aminoacids, fixed oils, mucilage etc (Ichikawa H *et al*., 2002; Jingyi W *et al*., 1997; Chaudhary G *et al*., 2003).

Animals

Inbreed strains of *Wista*r rats of either sex weighing 150-200g were taken for the study. The animals were maintained in propylene cages at room temperature and standard 12h day/night cycle. The animals were fed with standard rodent pellet diet and water ad *libitum* (Hemayet Hossain *et al*., 2012). The experimental protocol was approved by Institutional Animal Ethical Committee (IAEC) of Sree Vidyanikethan College of Pharmacy with CPCSEA Registration No 930/Po/a/2006/CPCSEA.

Experimental Procedures

Grouping of animals

Animals were divided in to five groups (n=6), Group-I Normal Control, Group-II –Treated with Sodium nitrite (30mg/kg p.o for 14 days) Group-III – Treated with Sodium nitrite (30mg/kg p.o for 14 days)+Hyroalcoholic extract of *Aegle marmelos* (200mg/kg p.o for 14 days) Group-IV –Treated with Sodium nitrite (400mg/kg p.o for 14days)+Hyroalcoholic extract of *Aegle marmelos* (400mg/kg p.o for 14days) Group-V –Treated with Alteplase (0.9mg/kg i.v for 14 days)+Sodium nitrite (30mg/kg p.o for 14days) (Pankaj Nainwal *et al*., 2011; Saha P et al., 2011; Upaganlawar A *et al*., 2011).

Blood (2ml) was collected 24 hours from all animals after the last dose of the drug from retro- orbital sinus plexus under mild ether anesthesia and allowed to clot for 30 minutes. Serum was separated by centrifugation at 2500 rpm for 15 minutes and used for analyses of liver function test (BVS Lakshmi *et al*., 2011). The rats were then sacrificed by cervical dislocation and the liver was dissected out. Liver was quickly excised and perfused with chilled normal saline to completely remove all the blood cells. A part of the liver was stored in formalin for histopathological examination. One gram of liver in 10 ml of 0.1M phosphate buffer (pH 7.4) homogenized using remi homogenizer to obtain 10% homogenate. The homogenate was centrifuged at 3000 rpm for 15 min. Supernatant was collected and transferred to Eppendrof tube and was centrifuged at 12000 rpm for 30 minutes. The supernatant was used for the estimation of total thiols in the tissue (Sankari M *et al*., 2010; Rahul V et al., 2010).

Bio chemical parameters

The blood samples collected and the tissue homogenate prepared is subjected for the estimation of Glutathione, TBARS, Dopamine, Nitrates (Badmanaban *et al*., 2010).

Statistical analysis

The results were reported as Mean ± SEM of different observations. Experimental data were analyzed using oneway analysis of variance (ANOVA) to compare the difference between the control and treated values. Different value of P was considered significantly. Graph Pad Prism Version was used for statistical calculations (Mahamuni SS *et al*., 2012).

RESULTS

Effect of Hydro alcoholic Extract of *Aegle marmelos* on sodium nitrite induced Neurotoxicity

Sodium nitrite treated animal showed significant elevation of serum biochemical parameter such as Nitrates, TBARS and decreased levels of Glutathione and Dopamine shown in Table.1,2. Pre- treatment with Alteplase-0.9mg/kg p.o. and hydroalcoholic extract (HAE) at 200 mg/kg and 400 mg/kg p.o. for 14 days had produced significant protective effect on sodium nitrite - induced neuronal damage by maintaining the morphological changes and normalizing the elevation of serum biochemical parameter and therefore inhibited the Histopathological abnormalities caused by Sodium nitrite. *Aegle marmelos* showed dose dependent protection against Sodium nitrite induced neuronal damage.

DISCUSSION

Oxidative stress is implicated as one of the primary factor that contributes to the neurodegenerative diseases, brain damage, stroke, hypoxia etc. Earlier reports revealed that animal treated with sodium nitrite was resulted in hypoxic condition .It is well documented that hypoxic condition may lead to an intracellular calcium over load which correlates to neuronal injury and degeneration (Sayyed Nadeem *et al*., 2012; Vivek Srivastava *et al*., 2011).

Table.1 Effect of Hydroalcoholic extract of *Aegle marmelos* on Dopamine, Nitrate levels

Group	Treatment	Dopamine µg/mg of Tissue	Nitrates µg/ml
Group-I	Control(Normal Saline)	64.3±14.56	12.250±0.559
Group-II	Sodium Nitrite	40.1±10.6[a**]	47.875±1.630[a**]
Group-III	HAE of *Aegle marmelos* Sodium Nitrite	43.0±11.92[b]*	38.625±1.362[b]*
Group-IV	HAE of *Aegle marmelos* +Sodium Nitrite	51.2±12.24[b]**	22.875±1.246[b]**
Group-V	Alteplase+Sodium Nitrite	61.0±0.756[b]**	14.500±0.567[b]**

Values are given as Mean ±SEM for n=8in each group,comparision were made between a)Group-I and Group-II b)Group-II with Group-III, GroupIV, GroupV * symbol statistical significance done by one way ANOVA followed by Dunnett"s test P<0.01

Table.2 Effect of Hydroalcoholic extract of *Aegle marmelos* on Glutathione, TBARS levels

Group	Treatment	Glutathione µg/mg of Tissue	TBARS µM/mg
Group-I	Control(Normal Saline)	32.875±0.350	0.157±0.004
Group-II	Sodium Nitrite	15.0±1.180[a**]	0.359±0.006[a**]
Group-III	HAE of *Aegle marmelos* Sodium Nitrite	20.375±0.778[b]*	0.322±0.005[b]*
Group-IV	HAE of *Aegle marmelos* +Sodium Nitrite	24.00±0.625[b]**	0.306±0.006[b]**
Group-V	Alteplase+Sodium Nitrite	31.0±0.756[b]**	0.166±0.005[b]**

Values are given as Mean ±SEM for n=8in each group,comparision were made between a)Group-I and Group-II b)Group-II with Group-III, GroupIV, GroupV * symbol statistical significance done by one way ANOVA followed by Dunnett"s test P<0.01

The present study revaled that the animals treated with *Aegle marmelos* at dose 200mg/kg and 400mg/kg b.w (p.o) was observed for significant antioxidant property. Sodium nitrite treatment in rats lead to alterations in biochemical parameters.i.e elevation in nitrates, TBARS level with decreased Dopamine and glutathione levels (Saha P *et al*., 2011; Dirnagl U *et al*., 1999).

The level of Dopamine and glutathione is significantly reduced in negative control when compared with that of normal control group.upon treatment with HAE of *Aegle marmelos* dose 200mg/kg and 400mg/kg b.w (p.o) significantly increased the decreased levels in a dose dependant manner($p<0.01$).

The level of Nitrates and TBARS is significantly increased in negative control when compared with that of normal control group. Upon treatment with HAE of *Aegle marmelos* dose 200mg/kg and 400mg/kg b.w (p.o) significantly reduced the increased levels in a dose dependent manner ($p<0.01$) (Martin RL et al., 1994).

Aegle marmelos significantly alters Neurological and biochemical parameters in plasma and brain, presence of flavonoids may contribute for the significant neuroprotective effect.

CONCLUSION

The role of oxidative stress in genesis of Neurodegenerative diseases has been widely studied.The high oxygen consumption rate coupled with low antioxidant potential of the brain is the main triggering factor for the enhanced release of free radical. The present study concluded that *Aegle marmelos* may be effective in the therapy of various neurodegenerative diseases, which may be due to effective free radical scavenging property of the plant might be one of the reason (Chen H et al., 1992).

ACKNOWLEDGEMENT

The authors sincerely thank to Dr. C.K. Ashok Kumar, Principal, Sree Vidyanikethan College of Pharmacy, A.Rangampet, Tirupati, India for providing the necessary facilities to carry out this research work.

REFERENCES

Astrup J, Siesjo BK, Symon L. Thresholds in cerebral ischemia-theischemic penumbra. *Stroke*. 1981;12:723-725.

Badmanaban et al. Studies On Anthelmintic And Antimicrobial Activity Of The Leaf Extracts Of *Lagenaria Siceraria* Mol. *Journal of Global Pharma Technology*. 2010;2(4):66-70.

BVS Lakshmi, et al. Hepatoprotective activity of *Lagenaria siceraria* fruit extracts against carbontetrachloride-induced hepatic damage in rats. *Research Journal of Pharmaceutical, Biological and Chemical Sciences*. 2011;2(1):130-137.

Chaudhary G, Sharma U, Jagannathan NR, GuptaYK. Evaluation of Withania somnifera in a middle cerebral artery occlusion model of stroke in rats. *Clin Exp Pharmacol Physiol*. 2003;30(5-6):399–404.

Chen H, Chopp MN, Vande Linde MQV, et al. The effects of post. ischemic hypothermia on the neuronal injury and brain metabolism after forebrain ischemia in the rat. *J Neurol Sci*. 1992;107:191–198.

Dirnagl U, Iadecola C, Moskowitz MA. Pathobiology of ischaemic stroke. An integrated view. *Trends Neurosci*. 1999;22:391–397.

Gupta YK, et al. Animal model for cerebral ischemia for evaluation of drugs. *Indian J Physiol Pharmacol*. 2004;48(4):379-394.

Harsh Mohan. Text Book Of Pathology, 5th edition, Jaypee publishers, 2005; 904-921.

Hemayet Hossain, et al. Evaluation of Anti-Inflammatory Activity and Determination of Total Flavonoids and Tannin Contents of *Lagenaria Siceraria* Root. *Indian journal of pharmacological research*. 2012;3(8):2679-2685.

Hossmann KA. Viability thresholds and the penumbra of focal ischemia. *Ann Neurology*. 1994;36:557–565.

Ichikawa H, Konishi T. In vitro antioxidant potentials of traditional Chinese medicine, Shengmai San and their relation to in vivo protective effect on cerebral oxidative damage in rats. *Biol Pharm Bull*. 2002;25(7):898–903.

Jingyi W, Yasuhiro M, Naoya H, Seok RC,Yoshiharu Y, Nagara T, Fumiko T, Shigeru M, Junji K. Observation on the effects of Chinese medicine zhenxuanyin for improving cerebral blood flow in rats with cerebral ischemia. *J Tradit Chin Med*. 1997;17(4):299–303.

Kobayashi T, Mori Y. Ca2+ channel antagonists and neuroprotection from cerebral ischemia. *Eur J Pharmacol*. 1998;363:1–15.

Mahamuni SS, et al. Evaluation Of Phytochemical And Antimitotic Potential Of *Lagenaria Siceraria* Fruit Using Onion Root Model. *Indian journal of pharmacological research*. 2012;3(8).

Martin RL, Lloyd HG, Cowan AI. The early events of oxygen and glucose deprivation: Setting the ecence for neuronal death. *Trends Neurosci*. 1994;17:251–2578.

Pankaj Nainwal, et al. Study of Antihyperlipidemic Effect on the Juice of The Fresh Fruits of *Lagenaria Siceraria*. *International Journal of Pharmacy and Pharmaceutical Sciences*. 2011;3(1).

Rahul V, et al. Effect of *Lagenaria siceraria* fruit powder on sodium oxalate induced urolithiasis in Wistar rats. *J Ayurveda Integr Med*. 2012;3(2):75–79.

Robbin and Cotran. Pathologic basis of Disease. Elsevier.Inc, 2010; 1290-1310.

Saha P, et al. Evaluation Of Acute And Subchronic Toxicity Of *Lagenaria Siceraria* Aerial Parts. *International journal of pharmaceutical sciences and research*. 2011;2(6):1507-1512.

Saha P, et al. Evaluation of Anticancer activity of Lagenaria siceraria Aerial parts. *International Journal of Cancer Research*. 2011: 7(3): 244-253.

Sankari M, et al. Immunosuppressive activity of aqueous extract of*Lagenaria siceraria (standley)* in mice. *Der Pharmacia Lettre*. 2010:2(1):291-296.

Sayyed Nadeem, et al. Lagenaria siceraria fruit extract ameliorate fat amassment and serum TNF-α in high-fat diet-induced obese rats. *Asian Pacific Journal of Tropical Medicine*. 2012;698-702.

Upaganlawar A, et al. Cardioprotective Effects of *Lagenaria siceraria* Fruit Juice on Isoproterenol-induced Myocardial Infarction in Wistar Rats: A Biochemical and Histoarchitecture Study. *J Young Pharm*. 2011;3(4):297-303.

Vivek Srivastava, et al. Antiulcer activity of methanolic fruit extract of Lagenaria Siceraria. *International journal of pharmaceutical research and development*. 2011;3:187-192.

Xuejiang W, Magara T, Konishi T. Prevention and repair of cerebral ischemia-reperfusion injury by Chinese herbal medicine, shengmai san, in rats. *Free Radic Res*. 1999;31(5):449–455.