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Assessment of neuroprotective activity of *Spathodea campanulata*.P.Beauv flower extract on Mono Sodium Glutamate induced neurotoxicity in albino rats.

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ABSTRACT

The objective of the Present study was to evaluate neuroprotective potential of *Spathodea campanulata.P.Beauv* against Monosodium Glutamate (MSG) induced neurotoxicity in *wistar* albino rats. The albino rats received 70% ethanolic extract at a dose of 250 and 500 mg/kg and ascorbic acid 500 mg/kg (p.o) daily for 7 days. On all the 7 days, MSG (2g/kg, i.p.) was administered one hour before drug treatment. During the treatment the animals were observed for neurobehavioral performance for 50 minutes daily. Oxidative damage and histopathological analysis of tissue were also assessed. Extract and ascorbic acid administration significantly improved body weight and attenuated locomotors activity, rota rod and balance beam test performance as compared with MSG treated group. Synchronous administration of extract during monosodium glutamate treatment reversed the enzyme levels to the normal. This study shows that the Ethanolic extract of *Spathodea campanulata.P.Beauv* (EESCF) has a neuroprotective effects in rats and attenuates the oxidative damage, neurotoxicity caused by consumption of MSG.

Keywords: Neuroprotective activity, Monosodium glutamate; *Spathodea campanulata*.P.Beauv

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INTRODUCTION

Neurodegenerative diseases occur when nerve cells in the brain or peripheral nervous system lose function over time and ultimately die. Although treatments may help relieve some of the physical or mental symptoms associated with neurodegenerative diseases, there is currently no way to slow disease progression and no known cures[1].

Life expectancy is increasing as result of better health care. This invites increased incidence of age related neurodegenerative disorders causing many social challenges and imposing huge economical burden[2]. The risk of being affected by a neurodegenerative disease increases dramatically with age. As a result of increased lifespan, along with a larger generation, means more people may be affected by neurodegenerative diseases in coming decades. This needs to improve our understanding of what causes neurodegenerative diseases and develop new approaches for treatment and prevention[3]. Common neurodegerative disorders comprise of dementias like Alzheimers disease. Parkinsons disease, Huntingtons disease and cerebrovascular stroke. Available therapies for these disorders are not very satisfactory. Increased oxidative stress and inflammation are the major underlying cause for neurovascular damage. Other contributing factors for these disorders include diabetes mellitus, hypertension, hyperlipidemia, enhanced platelet aggregation and coagulation activity, ischaemic heart disease, atherosclerosis, endothelial dysfunction and hyperhomocysteinemia[2,4], when tissues get oxidized, they slowly get damaged and eventually decrease in function. Excess oxidative damage can occur in the body when there is a lack of antioxidants such as vitamins C, E, CoQ10 and other factors that can influence neurodegeneration are genetics[5] and environmental neurotoxins such as heavy metals like aluminium, mercury, cadmium, arsenic as well as pesticides can boost our risk of developing neurodegenerative disorders[6].

Natural products from plants, animals and minerals have been the basis of the treatment of human disease. Today estimate that about 80% of people in developing countries still relays on traditional medicine based largely on species of plants and animals for their primary health care. Herbal medicines are currently in demand and their popularity is increasing day by day. About 500 plants with medicinal use are mentioned in ancient literature and around 800 plants have been used in indigenous systems of medicine. India is a vast repository of medicinal plants that are used in traditional medical treatments. There has been an increase in demand for the phytopharmaceutical products of Ayurveda in Western countries, because of the fact that the allopathic drugs have more side effects. Many pharmaceutical Companies are now concentrating on manufacturing of herbal and phytopharmaceutical products[7]

Spathodea campanulata P.Beauv reported to be useful as diuretic, anti-inflammatory, antidote, enemas, antisecretolytic, antiparasitic, antimalarial, anti-HIV and anti-diabetic. It is further reported that the plant is useful in the treatment of kidney diseases, herpes, stomachache, urethral inflammations and fungal infections. The literature survey of this plant revealed that this plant possess quercetin caffeic acids, oleanolic acid, steroids, polyphones, flavonoids, tannins and cardiac glycosides[8,9]. Herbs are reported to contain phenolic compounds these phenolic components are known as antioxidants and antioxidants are reported to have organ protective properties. It is evident from the literature survey that the plant is reported to contain phenolic compounds these phenolic components are known as antioxidants and antioxidants are known for organ protective activity in nature[10]**.** So the present study was designed to screen for *in vitro* and *in vivo* antidiabetic activity of *Spathodea campanulata*.P.Beauv flower extract.

EXPERIMENTAL DESIGN

Experimental Animals

Either sex of *Swiss* albino mice and *Wister* albino rats were used for the pharmacological screening, which were procured from Sri Venkateshwara Enterprises, Bengaluru. All the animals were acclimatized and maintained as per the CPCSEA guidelines. They were provided with standard rat feed and water *ad libitium*. The husk in the cages was renewed thrice a week to ensure hygienity and maximum comfort for animals. Ethical clearance was obtained from the IAEC prior to the beginning of the research; the registered no is SCSCP/IAEC08.

Mono sodium glutamate induced Neurotoxicity [11]:

The animals were divided into five groups of six rats each and treated for 7 days as follows;

Group 1: Normal saline (i.p.) + vehicle (p.o.).
Group 2: MSG 2 g/kg (i.p.) + normal saline (p.o.).
Group 3: MSG 2g/kg (i.p.) + ascorbic acid 100 mg/kg, (i.p.)
Group 4: MSG 2 g/kg (i.p.) + 70%EESCF 250mg/kg (p.o)
Group 5: MSG 2 g/kg (i.p.) + 70%EESCF 500mg/kg (p.o)

The gap between 70%EESCF and MSG is 1 hour. MSG dose was selected as per literature. After the drug treatment, rats were kept for observation for the behavioral changes for 50 minutes daily. On 8th day the rats were evaluated for body weight change, locomotor activity, balance beam task and rota rod test. Estimation of GSH, SOD, CAT, lipid peroxidation and total protein (TP) rats were sacrificed and brains were isolated on 9th day.

RESULTS

Effect of 70%EESCF on body weight change and behavioural change in MSG treated Rats:

There was a marked decrease in body weight in MSG treated groups. 70%EESCF showed dose dependent increase in the body weight of animals, at 500 mg/kg increased the body weight upto 07.34%.

MSG treated rats showed the fall off time was 24.89±5.28s, balanced beam movement 11.80±1.072s and locomotion 42.90±2.60, where as in case of Pre-treatment with 70%EESCF (250 and 500 mg/kg) significantly improved the motor coordination to 30.29±1.36, 44.76±3.29, 11.98±1.92, 19.10±2.50 and 63.96±3.90, 114.60±1.27 in fall off time, balance beam and locomotion respectively. All these values are less than the standard group. Results are shown in table 01.

Table 01. Effect of 70%EESCF on body weight change and behavioural change in MSG treated Rats

Treatment	% Body weight change	Rotarod test(s)	Balance beam test(s)	Locomotor activity (count/5min)
Negative control	-----	75.70±5.687	28.84±4.5733	151.4±4.601
Positive control	13.04**	24.89±5.28***	11.80±1.90**	42.90±2.60***
Standard (vit.c)	08.90	53.38±3.82***	25.58±4.29***	128.00±5.06***
250mg/kg 70%EESCF	03.80	30.29±1.36*	11.98±1.92*	63.96±3.90*
500mg/kg 70%EESCF	07.34	44.76±3.29**	19.10±2.50***	114.60±1.09***

Values are expressed as mean ± SEM (n = 6), where, ***P<0.001 – highly significant; **P<0.01- very significant; *P<0.05- significant, when compared to MSG alone treated rats. One-way ANOVA followed by Dunnett's comparison test.

Effect of 70%EESCF on tissue GSH, LPO, SOD, CAT and TP levels in MSG induced Neurotoxicity:

There was a depletion of GSH, SOD, CAT, TP levels in MSG treated groups. Treatment with 70%EESCF at 500 mg/kg dose showed dose dependent increase in GSH, SOD, CAT, TP levels by 46.19%, 71.42%, 87.45% and 62.65% respectively, where as LPO has decreased by 31.95% the results are summarized in table 02.

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Table 02. Effect of 70% EESCF on tissue GSH, LPO, SOD, CAT and TP levels in MSG induced Neurotoxicity:

Treatment	GSH		LPO		SOD		CAT		TP	
	Mean±SEM	%increase	Mean±SEM	%inhibition	Mean±SEM	%increase	Mean±SEM	%increase	Mean±SEM	%increase
Negative control	0.263±0.002	----	0.111±0.007	-----	0.042±0.001	-----	0.411±0.003***	-----	0.666±0.016	-----
Positive control	0.171±0.001	-----	0.194±0.001	-----	0.021±0.008	-----	0.175±0.007	-----	0.332±0.023	-----
Standard (Vit. C)	0.263±0.001***	53.80	0.124±0.001***	36.08	0.040±0.001***	90.47	0.400±0.009***	91.50	0.603±0.031***	81.62
250mg/Kg 70%EESCF	0.210±0.005 NS	22.81	0.165±0.004NS	14.94	0.026±0.002NS	23.80	0.188±0.019 NS	7.42	0.435±0.033*	31.02
500mg/Kg 70% EESCF	0.250±0.010**	46.19	0.132±0.003**	31.95	0.036±0.003***	71.42	0.381±0.013***	87.45	0.540±0.021**	62.65

Each value is expressed as mean ± SEM (n = 6), where, NS represents non significant; ***P<0.001 – highly significant; **P<0.01- very significant; *P<0.05- significant, when compared to MSG alone treated rats. One-way ANOVA followed by Dunnett's comparison test.

Histopathological Studies in MSG induced neurotoxicity:

Negative Control:

Normal control (-ve control) section studies showed that the hippocampus shows densely packed pyramidal cells in both layers. The interconnected neuropil fibres region appear intact and it also shows intact pyramidal cells along with intact neuropil fibres.

Positive Control:

MSG treated group (+ve control) section studies showed that the hippocampus shows loosely arranged layers of pyramidal cells. The region shows most of the pyramidal cells with interconnected neuropil fibers replaced by vacuolated neurons and spongiform degeneration of neuropil fibers.

Standard (Vit.C):

Treatment done with Vit.C 100mg/kg section studies showed that the hippocampus shows packed pyramidal cells with few showing vacuolations. The interconnected neuropil fibers in region appear intact. The region shows intact pyramidal cells along with intact neuropil fibres.

250mg/kg 70%EESCF:

Treatment done with 250 mg/Kg 70%EESCF section studies showed that the hippocampus shows loosely packed pyramidal cells in both the region and in region and it also shows focal degenerated pyramidal cells along with focal loss of neuropil fibres.

500mg/kg 70%EESCF:

Treatment done with 500mg/Kg 70%EESCF showed the section studied from the hippocampus shows densely packed pyramidal cells. The marked region shows intact pyramidal cells in clusters and interconnected neuropil fibers region appear intact. Histophatology reports are shown in fig. 1 a, b, c, d and e.

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Histopathological Studies in MSG induced neurotoxicity:

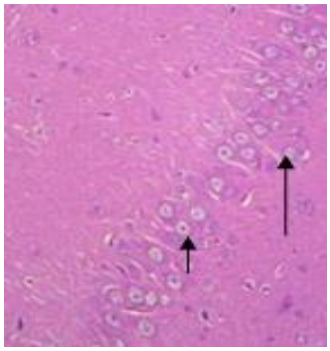

Fig. 1.a. Negative control

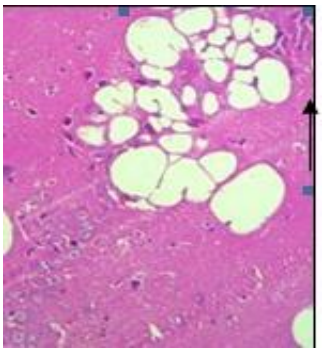

Fig. 1.b. Positive control

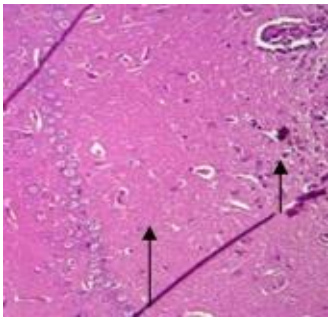

Fig. 1.c. Standard

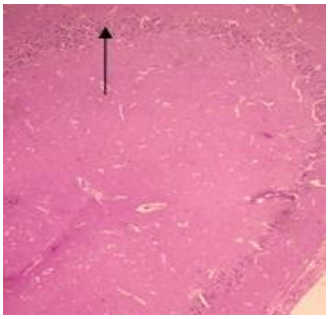

Fig. 1.d. Lower dose(250mg/kg) of 70%EESCF

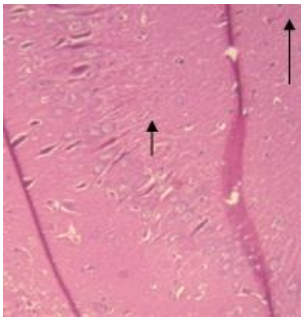

Fig. 1.e. Higher dose (500mg/kg) of 70%EESCF

DISCUSSION

During oxidative stress, the free radicals initiate a disastrous chain of oxidative reactions of thousands of events long causing denaturation of proteins, lipids, carbohydrates, DNA, RNA, etc., leading to cell death. Thus, a consistent and persistent oxidative stress leads to numerous diseases[12].

Plants are conceived as source of antioxidants due to presence of poly phenols and flavonoids, which possess wide spectra of biological properties. Recent studies showed that many flavonoids and related polyphenols contribute significantly to the total antioxidant activity. Antioxidants further helps as organ protectants. This study a widely grown plant *Spathodea campanulata* reported to possess polyphenolic compounds, the preliminary phytochemical screening of plant showed that, they possess polyphenols, flavonids, tannins and saponins, which are reported to possess antioxidant property.

In present the administration of MSG (2 g/kg, i.p. for 7 days) produced significant decrease in body weight, motor and muscle grip related behaviours and antioxidant status in the brain. These findings are well co-relates to histopathological observations which indicates the presence of degenerated nerve cell and

loosening of nerve fibers in striatal region of the brain. The reduction of body weight in MSG induced rats could be due to metabolic impairment caused by neurotoxic agents i.e. altered in energy metabolism, mobilization of energy stores and lipid peroxidation which constitute peripheral effects. There was no weight reduction in the pretreated 70%EESCF treated rats. The attenuation of body weight change produced due to MSG toxicity by 70%EESCF indicates its mitochondrial protection ability from toxins. Treatment with MSG was associated with both hypoactivity and hyperactivity depending on frequency and time of dosing. Animals treated with MSG showed significant hypoactivity along with marked neuronal loss in the dorsolateral striatum depicting that rigidity and movement disorders are related to basal ganglia lesions. In present study, 70%EESCF treatment improved the hypoactivity, as evident by behavioural investigations such as balance beam and locomotor activity. Striatal lesion indicated by treatment with MSG caused impairment in the locomotion observed in actophotometer. MSG have been reported to cause lesion in hippocampal and pyramidal neurons. Animals showed poor locomotion in the treated groups. This observation indicates that the treatment with MSG causes motor dysfunction[13]. In the present study 70%EESCF treatment significantly improved locomotor performance and showed improved motor activity. The MSG increased the tissue lipid peroxidation and decreased tissue GSH, SOD, CAT and TP levels. Co-administration of test extract reversed all the above mentioned parameters to the near normal levels. Since the MSG induced neurotoxicity was reported to be via free radicals, the neuroprotective activity of test extract in this model is also attributed to the antioxidant activity of the plant. The neuroprotective property of the extract is further confirmed by significant improvement of the brain architecture by reversing the disintegrated neuropil fibres over MSG group.

CONCLUSIONS

The 70%EESCF has neuroprotective potential against MSG induced neurotoxicity in rats. EESCF attenuates the behavioural impairment and oxidative stress induced by MSG. It also prevented the neurodegeneration in striatal and hippocampal regions due to MSG toxicities. The observed protective effects may be due to antioxidant properties of EESCF.

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REFERENCES

[1] National Institute of Environmental Health Science (https://:www.niehs.nih.gov/research/supported/health/neurodegenarative/index.cfm).
[2] Vijay K Ramanan, Andrew J Saykin. Pathways to neurodegeneration: mechanistic insights from GWAS in Alzheimer's disease, Parkinson's disease, and related disorders. Am J Neurodegener Dis 2013;2(3):145-175.
[3] Patil T R, Patil S, Patil A, Patil S T. Garlic and Neurodegenerative Disorders: A Review. International Journal of Pharmacognosy and Phytochemical Research 2016; 8(10); 1634-1644.
[4] Markesbery, W. Damage to Lipids, Proteins, DNA, and RNA in Mild Cognitive Impairment. Arch Neurol. 64(7):954-956; July, 2007.
[5] Bertram, L. "The Genetic Epidemiology of Neurodegenerative Disease." *Journal of Clinical Investigation* 115.6 (2005): 1449-457. Web.
[6] Chin-Chan, Miguel, Juliana Navarro-Yepes, and Betzabet Quintanilla-Vega. "Environmental Pollutants as Risk Factors for Neurodegenerative Disorders: Alzheimer and Parkinson Diseases." *Frontiers in Cellular Neuroscience* 9 (2015): n. pag. Web.
[7] Manish Agrawal, Nandini D, Vikas Sharma, Chauhan N. S. Herbal Remedies For Treatment of Hypertension. Int J Pharm Sci Res. 2010; 1(5): 01-21.
[8] Oluwole, Amusan O.O.G, Ezekiel K, Antimalarial active principles of *Spathodea campanulata* stem flower, Phytotherapy research 10(8), 1996, 692-693.
[9] Coldin GA, Branch LG, Lipnic RJ, Willet WC and Rosner B.et.al. Increased green and yellow vegetables intake and lowered cancer death in elderly population AmJ.Clin.Nutr 1985; 41:32.
[10] Elija Khatiwora, Vaishali B. Adsul, Manik M. Kulkarni, N.R. Deshpande and R.V Kashalkar Spectroscopic determination of total phenol and flavonoid contents of *Ipomoea carnea***.** International Journal of ChemTech,July-Sept 2010.Vol.2,No.3,pp 1698-1701.

[11] Rekha Rajendran, Saleem Basha N, Ruby S. Evaluation of *in-vitro* antioxidantactivityof stem-bark and stem wood of *premna serratifolia*. Res J pharmacognosy and phytochemistry. July 2009; 1(1): 11- 14.
[12] Niki E. Antioxidant defenses in eukaryotic cells. In: Poli G, Albano E, Dianzani MU, editors. Free radicals: From basic science to medicine. Basel, Switzerland: Birkhauser Verlag; 1993; 365-73.
[13] Neils Tygstrup, Soren Astrup Jensen, Bjorg Krog and Kim Dalhoff. Expression of liver-specific functions in rat hepatocytes following sublethal acetaminophen poisoning. J Hepatology. 25: 1996, 183-190.