ORIGINAL ARTICLE

INTERNATIONAL JOURNAL OF RESEARCH IN PHARMACEUTICAL SCIENCES

Published by JK Welfare & Pharmascope Foundation Journal Home Page: https://ijrps.com

Neuroprotective activity of *Garcinia morella desr* against monosodium glutamate-induced neurotoxicity in rats

Iyappan R[*1], Neeraj K Sharma[2], Senthilkumar S K[3]

[1]Research Scholar, Pacific University, Udaipur, Rajasthan-313003, India
[2]Faculty of Pharmacy, Bhagwant University, Ajmer, Rajastan- 305004, India
[3]Arunai College of Pharmacy, Tiruvannamalai-606611, Tamilnadu, India

Article History:

Received on: 15 Aug 2020
Revised on: 25 Sep 2020
Accepted on: 24 Oct 2020

Keywords:

Antioxidants, Dextromethorphan, Monosodium glutamate, Neurotransmitters

ABSTRACT

The aim of the present study is to evaluate the neuroprotective effect of methanol extract of *Garcinia morella Desr* leaves in monosodium glutamate (MSG) induced neurotoxicity in rats. The MSG 2 g/kg, i.p. was used to induce the neurotoxicity. The rats were administrated with methanol extract of *Garcinia morella Desr* leaves (MEGM) 200 and 400 mg/kg, p.o. and Dextromethorphan 30 mg/kg, p.o. after 1 h injection of MSG. The animals were evaluated for its behavioural factors such as muscle grip and locomotor activity. At the end of the study, the antioxidant enzymes levels, neurotransmitter levels, TNFα, ß-amyloid and mineral levels were estimated in brain homogenate. The MEGM treated group shows significant improvement of behavioural and locomotor activity and muscle strength against MSG induced neurotoxicity. The glutathione, SOD, catalase and total protein levels were significantly increased in MEGM treated group. However, significant reduction within the level of varied neurotransmitters like AChE, Dopamine, TNFα, ß-amyloid were observed on treatment with MEGM extract. Further, MEGM also significantly decrease the MSG induced toxicity through declined levels of Ca^{+2} and Na^{+} with increased levels of K^{+}. In conclusion, the present study suggests that the MEGM has significant neuroprotective activity against MSG induced neurotoxic rats.

[*]Corresponding Author

Name: Iyappan R
Phone:
Email: iyps2001@gmail.com

ISSN: 0975-7538

DOI: https://doi.org/10.26452/ijrps.v11iSPL4.4448

Production and Hosted by

IJRPS | https://ijrps.com

INTRODUCTION

Neuroprotection is a treatment which means to shield the rest of the nerve cells from further harm. It is a broadly uncovered treatment for many central nervous system (CNS) illnesses such as stroke, neurodegenerative diseases, spinal cord injury, brain injury, paralysis and neurotoxin consumption. Any substance from natural molecule that has a protective influence in the nervous system against neurodegenerative disease or brain injury will possess neuroprotective activity (Djaldetti *et al.*, 2003; Ha *et al.*, 2011). Common neurotoxicity mechanisms are elevated levels of oxidative stress, mitochondrial dysfunction, excitotoxicity, inflammatory changes, iron accumulation, and protein aggregation (Seidl and Potashkin, 2011; Andersen, 2004; Zádori *et al.*, 2012).

The world health organization assesses that, 80% of the world's population (more than six billion people) fundamentally rely upon animal and plant based medicine (Alves and Rosa, 2005). Medicinal

floras are a best foundation to discover new solutions for neurological diseases. *Garcinia morella Desr* (GM) is a medicinal plant under the family of Guttiferae. GM is distributed in Asian, African and Polynesian countries and itis locally called as kujithekera. GM is commonly used for the management of stomach disorders, bowel problems and inflammations. Different extracts of Garcinia species has been revealed for its anticancer (Choudhury *et al.*, 2016), anti-oxidative (Yamaguchi *et al.*, 2000), antifungal (Gopalakrishnan *et al.*, 1997), antibacterial (Rao and Verma, 1951; Sani and Narasimha-Rao, 1969) activities. And also the Garcinia species contains secondary metabolites such as xanthones, flavonoids, lactones, benzophenones and phenolic acids. The Morello flavone, Gambogic acid and guttiferic acid has reported in GM (Karanjgaokar *et al.*, 1967; Chantarasriwong *et al.*, 2010). The fruits of GM are rich in hydroxyl citric acid, garcinol, guttiferones, camboginol and xanthochymol (Hemshekhar *et al.*, 2011). The flavonoids, xanthones, and prenylated benzophenones have been reported for its Neuropharmacological property. But there is no scientific validation of GM for neuroprotective activity. Therefore, this research work was aimed to evaluate the neuroprotective activity of GM.

MATERIALS AND METHODS

Plant Collection and Identification

Raw leaves of GM were acquired in Tirunelveli region of Tamil Nadu, India. The GM was verified by the taxonomist Dr.P.Jayaraman, Institute of Herbal Science, Plant Anatomy Research Center, Chennai. The voucher specimen numbers is PARC/2017/4338.

Preparation of Extracts

Unprocessed leaf of GM was completely cleaned, shadow dried and pulverized. Then it was extracted using continuous extraction (Soxhlet extraction) method. The methanol was utilized for extraction for 48 h. The concentrate product was concentrated by using vacuum evaporation at 45°C. Then the end product was collected in a well closed container for pharmacological screening (Gopalasatheeskumar, 2018).

Laboratory animals

Wistar albino rat (either sex; 125-150 g body weight) were kept in a well-organized room temperature for 12 h. The animals were free admittance to clean food and water. The investigations were led in sound proof research centre. This investigation was done with the approval of the animal ethical committee no. KPCP/2017-2018/CPCSEA/0006/1c.

Experimental protocol

Animal were randomly divided into five groups 6 animals in each as follows,

Group I

Served as normal control, rats received 1% w/v carboxy methyl cellulose (CMC) (5 ml/kg, p.o.).

Group II: Served as negative control, rats were treated with Mono sodium glutamate (MSG) (2 g/kg, i.p.).

Group III: Served as negative control, rats were administered with dextromethorphan (30 mg/kg. p.o.) after 1 h injection with MSG (2 g/kg, i.p.).

Group IV

Rats were administered with methanol extract GM (MEGM) (200 mg/kg, p.o.) after 1 h of injection with MSG (2 g/kg, i.p.).

Group V

Rats were administered with MEGM (400 mg/kg, p.o.) after 1 h of injection with MSG (2 g/kg, i.p.).

The entire group received the treatment for a period of 7 days. After MSG administration the general behavioural changes, were observed for 30 min daily, on 8th day, the rats were evaluated for locomotor and muscle relaxant activity. The locomotors activity was studied using actophotometer. In this, animals of all the groups were placed in actophotometer for 10 min and the score were recorded. Muscle relaxant activity was investigated using rota rod apparatus. Rats were placed on the rotating rods and the time of fall from the rotating rods were recorded (Mahalaxmi *et al.*, 2018).

Biochemical parameters

On 9th day, the experimental rats were sacrificed by cervical dislocation. The brain was isolated carefully and perfused in 0.85% NaCl to eliminate blood. The brain tissue 10% w/v was homogenised in hand-held homogeniser using Tris–HCl buffer at pH 7.4 and it is centrifuged at 5000 rpm for 10 min. Supernatant obtained was used for estimation of antioxidant enzymes, minerals and neurotransmitter levels (Hemshekhar *et al.*, 2011).

Estimation of antioxidant enzyme levels

The antioxidant parameters including Superoxide dismutase (SOD), Reduced glutathione (GSH), Catalase (CAT) and Lipid peroxidation (LPO) levels were estimated as per the standard protocols followed by (Hemshekhar *et al.*, 2011; Yuvaraja *et al.*, 2020). The 0.05 ml of brain homogenate was used for the estimation of these parameters.

Estimation of neurotransmitters and minerals

The Acetylcholinesterase (AChE), Glutamate, Serotonin, Dopamine, calcium and sodium levels were estimated from 250 μL of brain homogenate and followed by standard assay methods (Usama *et al.*, 2017; Boopathi and Thangavel, 2020).

Estimation of β-amyloid and tumor necrosis factor-α (TNF-α)

The level of β-amyloid peptide and TNF-α in brain homogenate were estimated by the rat ELISA Kit with a microplate reader as per the ventors protocol (Usama *et al.*, 2017; Silva *et al.*, 2015).

Statistical analysis

Results are communicated as mean$\pm$SEM. Results were analysed by one-way analysis of variance (ANOVA) trailed by Dunnet's multiple comparison test utilizing Graph Pad Prism software. <0.05 is reflected as significant.

RESULTS AND DISCUSSION

Effect of MEGM on Locomotor and muscle relaxant activity

Treatment with MSG treated group shows significant decrease in the time of fall from the rotating rod compared with control group. While on Treatment with MEGM at the both doses and standard drug dextromethorphan it is reversed (Table 1). The locomotor activity is decreased significantly in MSG treated groups as compared with control. On Treatment with MEGM at the dose of 200 and 400 mg/kg as well as standard drug, dextromethorphan locomotor activity score is increased as compared with negative control (Table 2).

Effect of MEGM anti-oxidant parameters

Significant accumulation of lipid peroxides in brain tissue of rat was observed and effect of antioxidant levels including SOD, Catalase and GSH was decreased significantly in MSG injected group when it was compared with control, whereas MEGM and dextromethorphan treated rat produce a significant reduction in LPO activity and significant elevation in SOD, CAT and RGSH in brain tissues as compared with MSG treated rat it is shown in Table 3. (Gopalasatheeskumar *et al.*, 2020)

Effect of MEGM on minerals

The MSG treated groups shows the significant elevated levels of calcium and sodium when compared to normal control group (, P<0.01). However, potassium levels were significantly decreased. These altered levels were significantly normalized in MEG and Dextromethorphan treated groups (Table 4).

Effect of MEGM on neurotransmitters, β-amyloid and TNFα

The MSG treated groups shows the significant elevated levels of glutamate, AChE, TNFα, dopamine and β-amyloid when compared to normal control group (P<0.01). However, GABA and Serotonin levels were significantly decreased. These altered levels were significantly normalized in MEG and Dextromethorphan treated groups (Table 5).

MSG a flavouring agent causes neuronal symptoms like numbness, weakness, dizziness and headaches and also it's reported as MSG changes antioxidant levels and lipid peroxidation of mitochondria in brain (Meldrum, 2000). Treatment of MSG in rat may cause severe behavioural abnormalities like increased irritability, hypoactivity, and deficits in spontaneous alternation behaviour (Shivasharan *et al.*, 2013). MSG produces excitotoxicity by activating NMDA receptors, which successively disturb the calcium homeostasis, which can cause glutamate-induced neuronal damage. Earlier report says that there's association between increased glutamate neurotransmission and cognitive dysfunction (Swamy *et al.*, 2013) . Glutamate elicits synaptic responses via metabotropic mGLU and ionotropic NMDA receptors resulting in increased cellular excitation and induce the discharge of Ca^{+2} from intracellular stores. On the opposite hand activation of ionotropic receptors increases cellular permeability to Ca+2 (Weil *et al.*, 2008).

Acetyl choline is a most vital neurotransmitter of CNS and it plays a major role in behavioural also as learning and memory and neurodegenerative diseases. Our results showed that on treatment with MSG shows that there's an increased AChE level within the brain may lead to a reduction of cholinergic neurotransmission efficiency due to a decrease in acetyl choline level within the synaptic cleft, thus contributing to progressive cognitive impairment (Jiang and Zhang, 2008). Additionally, MSG causes oxidative stress by increasing lipid peroxides and reactive oxygen species (ROS), which could enhance AChE level. Significant alterations in serotonin, dopamine, and β amyloid levels within the brain of MSG-treated rats were recorded in our study. This decrease in serotonin level in brain may cause neurotoxicity. While on treatment with MEGM at the both doses as well as standard drug dextromethorphan significantly increased the serotonin level which gives neuroprotection property and therefore the report was supported by a previous report on exposed of MSG (Sylvie *et al.*, 2015). Moreover, the MSG induced rats were raised in Aβ accumulation which cause development of

Table 1: Effect of MEGM in MSG induced neurotoxocity in Rota rod test in albino rats

S. No	Treatment	Time (sec) of animals remained without falling from revolving rod		
		30	60	90
1	Control	258.10±6.32	271.60±6.37	220.50±4.77
2	Negative control	186.80±8.47	14.80±9.86	82.10±2.77
2	Standard	251.80±8.75	255.80±7.12	186.60±8.00
3	MEGM 200mg/kg	263.30±4.38	245.10±8.90	137.50±10.47
4	MEGM 400mg/kg	263.80±5.66	268.30±4.59	183.30±6.66

No. of Animals 6, Data were analysed as Mean±SEM, One way ANOVA followed by Dunnett's test, All groups were matched with negative control, *p<0.05, **p<0.01,***p< 0.001

Table 2: Effect of MEGM in MSG induced neurotoxocity in actophotometer in albino rats

S. No	Treatment	Before treatment	After treatment	% reduction activity
2	Negative control	280±9.31	165.80±4.16	41.07%
2	Standard	280.80±8.07	310±3.65	10.39%
3	MEGM 200mg/kg	278.30±7.49	295±7.63	6.00%
4	MEGM 400mg/kg	270±7.30	293.30±10.54	8.62%

No. of Animals 6, Data were analysed as Mean±SEM, One way ANOVA followed by Dunnett's test, All groups were matched with negative control, *p<0.05, **p<0.01,***p< 0.001

Table 3: Effect of MEGM on the antioxidant parameters in MSG induced neurotoxocity in albino rats

Group	GSH (U/L)	LPO (U/L)	SOD (U/L)	CAT(mg/dl)
Control	3.72±0.437***	3.76±0.1607***	18.33±0.4084***	63.90±1.69***
Negative control	1.13±0.10	11.19±0.10	10.45±0.23	22.84±0.51
Standard	3.12±0.30***	4.81±0.21***	18.67±0.21***	54.63±0.98***
MEGM 200mg/kg	2.35±0.10***	7.25±0.14***	15.11±0.15***	40.09±0.44***
MEGM 400mg/kg	3.11±0.09***	5.42±0.20***	17.08±0.11***	65.50±1.30***

No. of Animals 6, Data were analysed as Mean±SEM, One way ANOVA followed by Dunnett's test, All groups were matched with negative control,*p<0.05, **p<0.01,***p< 0.001

Table 4: Effect of MEGM on the mineral, β-amyloid and TNFα levels in MSG induced neurotoxocity in albino rats

Group	Calcium	Sodium	Potassium	β-amyloid	TNFα(ng/L)
Normal control	80.90±2.28***	9.03±0.17***	72.24±0.75***	15.23±0.14***	23.74±0.40***
Negative control	136.75±3.27	14.22±0.11	51.98±0.32	53.44±0.18	56.18±1.59
Standard	86.10±1.03***	12.05±0.13***	57.18±0.30***	18.65±0.30***	25.26±0.98***
MEGM 200mg/kg	106.13±1.43***	11.08±0.09***	67.10±0.37***	24.84±0.32***	35.73±0.75***
MEGM 400mg/kg	75.18±6.60***	10.01±0.11***	67.98±0.62***	20.79±0.50***	28.38±1.12***

No. of Animals 6, Data were analysed as Mean±SEM, One way ANOVA followed by Dunnett's test, All groups were matched with negative control,*p<0.05, **p<0.01,***p< 0.001

Table 5: Effect of methanolic extract of *Garcinia morella* on the neuro transmitters parameters in MSG induced neurotoxocity in albino rats

Group	Dopamine (ng/mL)	(AchE) activity	Serotonin	GABA (pg/mL)	Glutamate (nmol/μL)
Control	0.51±0.01***	15.22±0.53***	153.08±2.80***	8.08±0.21***	7.51±0.21***
Negative control	1.35±0.02	20.83±0.29	24.60±5.03	2.47±0.18	13.08±1.27
Standard	0.67±0.01***	15.79±0.19***	167.2±1.07***	7.02±0.24***	7.45±0.17***
MEGM 200mg/kg	0.9±.01***	16.75±0.20***	195.95±3.08***	5.12±0.09***	9.77±0.44***
MEGM 400mg/kg	0.66±0.02***	15.59±0.40***	167.39±2.89***	6.48±0.17***	7.89±0.16***

No. of Animals 6, Data were analysed as Mean±SEM, One way ANOVA followed by Dunnett's test, All groups were matched with negative control,*p<0.05, **p<0.01,***p< 0.001

amyloid plaques within the rat hippocampus and induced neurobehavioral abnormalities, this effect was reversed by MEGM and standard drug dextromethorphan. Furthermore, MSG treated group shows increased levels of TNF-α, it confirms the neuro inflammation caused by MSG. While on treatment with MEGM at the both doses as well as standard drug dextromethorphan significantly reduced the amount of these inflammatory mediators and mainly it suppress the expression of TNF-α (Jain *et al.*, 2009).

CONCLUSIONS

The results getting from this research clearly revealed the neuroprotective effect of MEGM against MSG induced neurotoxic rats. Since MSG is extremely dangerous its use must be limited. This effect may be due to the presence of antioxidants like Xanthones, flavonoids and prenylated benzophenones present within the extract. Further the isolation of active principles for find out the exact mechanism of neuroprotective activity.

Conflict of Interest

The authors declare that they have no conflict of interest for this study.

Funding Support

The authors declare that they have no funding support for this study.

REFERENCES

Alves, R. R., Rosa, I. L. 2005. Why study the use of animal products in traditional medicines? *Journal of ethnobiology and ethnomedicine*, 1(1).

Andersen, J. K. 2004. Oxidative stress in neurodegeneration: cause or consequence? *Nature Medicine*, 10(S7):S18–S25.

Boopathi, E., Thangavel, C. 2020. CDK4/6 inhibition protects normal cells against cancer therapy-induced damage. *Translational Cancer Research*, 9:405–408.

Chantarasriwong, O., Batova, A., Chavasiri, W., Theodorakis, E. 2010. Chemistry and Biology of the CagedGarciniaXanthones. *Chemistry - A European Journal*, 16(33):9944–9962.

Choudhury, B., Kandimalla, R., Bharali, R., Monisha, J., Kunnumakara, A. B., Kalita, K., Kotoky, J. 2016. Anticancer Activity of Garcinia morella on T-Cell Murine Lymphoma Via Apoptotic Induction. *Frontiers in Pharmacology*, 7.

Djaldetti, R., Lev, N., Melamed, E. 2003. Neuroprotection in progressive brain disorders. *IMAJ-The Israel Medical Association Journal*, 5(8):576–580.

Gopalakrishnan, G., Banumathi, B., Suresh, G. 1997. Evaluation of the Antifungal Activity of Natural Xanthones fromGarcinia mangostanaand Their Synthetic Derivatives. *Journal of Natural Products*, 60(5):519–524.

Gopalasatheeskumar, K. 2018. Significant role of Soxhlet extraction process in phytochemical research. *MJPMS*, 7(1):43–47.

Gopalasatheeskumar, K., Ariharasivakumar, G., Kalaichelvan, V. K., Sengottuvel, T., Devan, V. S., Srividhya, V. 2020. Antihyperglycemic and antihyperlipidemic activities of wild musk melon (Cucumis melo var. agrestis) in streptozotocin-nicotinamide induced diabetic rats.

Ha, K. Y., Carragee, E., Cheng, I., Kwon, S. E., Kim, Y. H. 2011. Pregabalin as a Neuroprotector after Spinal Cord Injury in Rats: Biochemical Analysis and Effect on Glial Cells. *Journal of Korean Medical Science*, 26(3).

Hemshekhar, M., Sunitha, K., Santhosh, M. S., Devaraja, S., Kemparaju, K., Vishwanath, B. S., Niranjana, S. R., Girish, K. S. 2011. An overview on genus garcinia: phytochemical and therapeutical aspects. *Phytochemistry Reviews*, 10(3):325–351.

Jain, S. K., Rains, J., Croad, J., Larson, B., Jones, K. 2009. Curcumin Supplementation Lowers TNF-α, IL-6, IL-8, and MCP-1 Secretion in High Glucose-Treated Cultured Monocytes and Blood Levels of TNF-α, IL-6, MCP-1, Glucose, and Glycosylated Hemoglobin in Diabetic Rats. *Antioxidants & Redox Signaling*, 11(2):241–249.

Jiang, H., Zhang, X. J. 2008. Acetylcholinesterase and apoptosis. *FEBS Journal*, 275(4):612–617.

Karanjgaokar, C. G., Radhakrishnan, P. V., Venkataraman, K. 1967. Morelloflavone, a 3-(8-) flavonylflavanone, from the heartwood of. *Tetrahedron Letters*, 8(33):3195–3198.

Mahalaxmi, M., Gangurde, S. K., Kadam, V. 2018. Protective effect of Solanum torvum on monosodium glutamate-induced neurotoxicity in mice. *Indian Journal of Natural Products and Resources*, 8(4):351–359.

Meldrum, B. S. 2000. Glutamate as a Neurotransmitter in the Brain: Review of Physiology and Pathology. *The Journal of Nutrition*, 130(4):1007S–1015S.

Rao, P. N., Verma, S. C. L. 1951. The antibiotic principles of Garcinia morella. I. Preparation and antibacterial activity of morellin. morellin-T. morellin-M. morellin-L and iso-morellin. *J. Sci. Ind. Res. B*, 10:184–185.

Sani, B. P., Narasimha-Rao, P. L. 1969. Antibiotic principles of Garcinia morella. XIV. neomorellin & X-guttiferin. *Indian journal of chemistry*.

Seidl, S. E., Potashkin, J. A. 2011. The Promise of Neuroprotective Agents in Parkinson?s Disease. *Frontiers in Neurology*, 2:68–68.

Shivasharan, B. D., Nagakannan, P., Thippeswamy, B. S., Veerapur, V. P. 2013. Protective Effect of Calendula officinalis L. Flowers Against Monosodium Glutamate Induced Oxidative Stress and Excitotoxic Brain Damage in Rats. *Indian Journal of Clinical Biochemistry*, 28(3):292–298.

Silva, B., Sousa, L., Miranda, A., Vasconcelos, A., Reis, H., Barcelos, L., Arantes, R., Teixeira, A., Rachid, M. A. 2015. Memory deficit associated with increased brain proinflammatory cytokine levels and neurodegeneration in acute ischemic stroke. *Arquivos de Neuro-Psiquiatria*, 73(8):655–659.

Swamy, A. V., Patel, N. L., Gadad, P. C., Koti, B. C., Patel, U. M., Thippeswamy, A. H. M., Manjula, D. V. 2013. Neuroprotective activity of pongamia pinnata in monosodium glutamate-induced neurotoxicity in rats. *Indian journal of pharmaceutical sciences*, 75(6).

Sylvie, C., Bockaert, J., Giannoni, P. 2015. Serotonin: a new hope in Alzheimer's disease.

Usama, H. K., Hassan, N., Elhalwagy, M., Zaki, A., Abubakr, H., Venkata, K. N., Jang, K., Bishayee, A. 2017. Ginger and Propolis Exert Neuroprotective Effects against Monosodium Glutamate-Induced Neurotoxicity in Rats. *Molecules*, 22(11).

Weil, Z. M., Norman, G. J., DeVries, A. C., Nelson, R. J. 2008. The injured nervous system: A Darwinian perspective. *Progress in Neurobiology*, 86(1):48–59.

Yamaguchi, F., Ariga, T., Yoshimura, Y., Nakazawa, H. 2000. Antioxidative and Anti-Glycation Activity of Garcinol fromGarcinia indicaFruit Rind. *Journal of Agricultural and Food Chemistry*, 48(2):180–185.

Yuvaraja, K. R., Santhiagu, A., Jasemine, S., Kumar, K. G. 2020. Hepatoprotective activity of Chloroform and Ethyl acetate extract of Dipteracanthus patulus against Paracetamol induced Hepatotoxicity in rats through Antioxidant mechanism. *Research Journal of Pharmacy and Technology*, 13(1):203–203.

Zádori, D., Klivényi, P., Szalárdy, L., Fülöp, F., Toldi, J., Vécsei, L. 2012. Mitochondrial disturbances, excitotoxicity, neuroinflammation and kynurenines: Novel therapeutic strategies for neurodegenerative disorders. *Journal of the Neurological Sciences*, 322(1-2):187–191.