**International Journal of Global Science Research** **ISSN: 2348-8344 (Online)**
**Vol.3, Issue 5, April 2016, pp. 295-301**
***Available Online* at www.ijgsr.com**


Research Paper

# Mitochondrially targeted antioxidants prevents oxidative damage in brain

Deepali Jat
Department of Zoology, Dr. Hari Singh Gour University, Sagar, MP, India
Corresponding author Email: deepalipunia@gmail.com

Received: 07/11/2015
Revised: 13/12/2015
Accepted: 30/03/2016

**Abstract:** Glutamate induced mitochondrial impairments are associated with brain injury and neuronal cell degeneration. Therefore, targeting mitochondria with mitochondrially targeted antioxidants can be beneficial in preventing mitochondrial impairment in brain. In the present study we synthesized mitochondrially targeted antioxidant and observed the efficacy of antioxidant against glutamic acid induced neurotoxicity. The intra-peritoneal injections of glutamic acid for 14 days produced brain mitochondrial impairments reflected by increased in lipid peroxidation, protein carbonylation and decline in glutathione. Supplementations of mitochondrially targeted antioxidant prevent mitochondrial impairments. Mitochondrially targeted gallic acid was found more efficacious in preventing mitochondria from oxidative damage. In conclusion our studies showed that mitochondrially targeted antioxidant gallic acid can be used for preventing mitochondrial impairment in brain mitochondria against glutamic acid neurotoxicity.
**Keywords**: Neuroprotection, excitotoxicity, oxidative stress, mitochondrially targeted antioxidant.

## INTRODUCTION

Excitotoxicity created by glutamate is known to be the major causative factor for the oxidative damage and antioxidant depletion in the brain mitochondria. Impairment in the functions of mitochondria is due to excitotoxic action of glutamate as measured by elevation in the lipid peroxides and protein carbonylation and lowering the glutathione level in the mice brain mitochondria. Mitochondria is known to be the major site of free radical induced damage and due to its greater membrane potential it can accumulate antioxidants in several fold greater than that of the plasma membrane when targeted with lipophilic triphenylphosphonium ions. Liphophilic cation can take antioxidant to mitochondria and reduces the damage caused by oxidative stress as measured by reduction in lipid peroxidation and protein carbonylation and elevating glutathione pool in the brain mitochondria. This study describes the therapeutic benefits of mitochondrially targeted antioxidants in ameliorating the damage caused by oxidative stress in the brain mitochondria. There are clinical evidences that neurodegeneration can be

ameliorated upon dietary intake or supplementary intake of natural antioxidants. Dietary intake contains variety of antioxidants vitamin supplements those play a vital role in neuroprotection in variety of neurological disorders (Peter et al.2004). These natural antioxidants prevent oxidation of proteins, lipid peroxidations and prevent generation of reactive oxygen species (ROS), thus act as therapeutic barrier to oxidative stress. Our aim is to describe the role of oxidative stress in mitochondrial dysfunctioning and to understand the antioxidant derived defence mechanism in understanding mitochondrial bioenergetics. Targeting antioxidants attached with lipophilic cation can be beneficial in attenuating the damage caused by glutamate as noted in decreased lipid peroxidation and protein carbonyls.

## MATERIALS AND METHODS

### Animals

Normal healthy adult (3 months old) swiss albino mice (*Mus musculus albinus*) of both the sexes, were procured from institute of Animal Health and Veterinary Biologicals, Mhow. These mice were reared and maintained under an automated light/dark (12h:12h) cycle with the provision of standard chow and water available ad libitum. Four animals were housed in each cage. The size of the cage was 30×20×12 cms. The bedding used for mice was sterile paddy husk, which was changed every week. Animals were used in compliance with all applicable laws and regulations.

### Modulating antioxidants to target it to mitochondria

Reactions were carried out under a nitrogen atmosphere. To synthesize targeted derivative, a solution of bromo-antioxidants (Gallic acid; 2gm, 1.06 mmol ) was refluxed and evaporated the volatiles under vaccum below $100^0$C. The compound obtained was hygroscopic, dissolved in methanol and precipitated after adding n-hexane. Filtered precipitate was again dissolved in methanol containing triphenylphosphine [1.5gm, 0.098 mmol (for gallic acid)]. After refluxing the solution, evaporated the volatiles under vaccum to obtain targeted derivative of antioxidants. The obtained derivatives are mitochondria-targeted derivative of mitochondria-targeted derivative of gallic acid (mtGA).

### In vivo experiment on mice brain mitochondria

Swiss albino mice (Mus musculus albinus) weighing 28±2 gm were acclimatized in laboratory conditions for at least one week before experimentation. Stock solutions of mitochondrially targeted antioxidants were prepared in PBS ($Na_2HPO_4$ and $NaH_2PO_4$, pH 7.4) containing dimethylsulfoxide (DMSO). The effects of mitochondrially targeted antioxidants (20-30 mg/kg/day) were observed against the treatment of glutamic acid [0.3 mg/gm, bw (i.p.)] for 14 days. All animals were weighed on last day of the experiment after an overnight fasting and then sacrificed by cervical dislocation. Brains were removed quickly cleaned and washed twice in ice-cold phosphate buffered saline (pH 7.4), cut into small pieces and homogenized in 10% (w/v) ice-cold buffer (pH 7.4) containing sucrose (250 µM), EDTA (1 µM) and Tris-Cl (10 µM) with the help of a motor drives teflon homogenizer. The samples were stored at $-20^0$C for mitochondrial isolation (Parihar et al., 2014).

### Biochemical estimations

Lipid peroxidation (LPO) was determined following the method of (Ohkawa et. al. ,1979). Protein carbonyl content was determined following the method of (Levine et. al. 1990). Reduced glutathione was measured in the samples following the method of (Jollow et. al. ,1974). The protein

**International Journal of Global Science Research** **ISSN: 2348-8344 (Online)**
**Vol.3, Issue 5, April 2016, pp. 295-301**
***Available Online* at www.ijgsr.com**


content from mitochondrial fraction and homogenates of brain subregions was determined following the method of (Lowry et al.,1951) using bovine serum albumin (BSA) as a standard.

**Statistical analysis:** All data expressed as mean ± SE. Statistical comparisons were made relative to the appropriate control groups by student's 't' test and analysis of variance. The 0.05 level was selected as the point of minimal statistical significance in every comparison.

## RESULTS

Effect of glutamate (0.3 mg/gm i.p., b.w.) and protective effect of mitochondrially targeted antioxidant (mtGA, 20 mg/kg) on lipid peroxidation in brain mitochondria *in vivo*. Results are summarised in table1, 2, and 3.

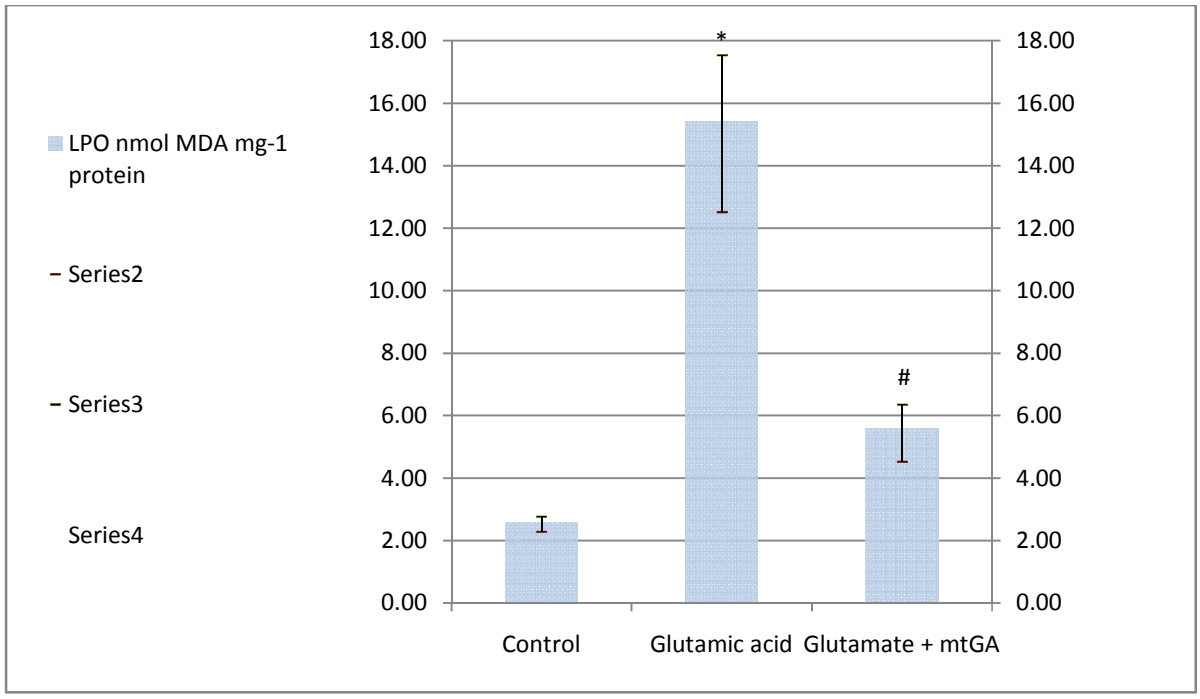


**Figure 1: Effect of glutamate (0.3 mg/gm i.p., b.w.) and protective effect of mitochondrially targeted antioxidant ( mtGA, 20 mg/kg) on lipid peroxidation in brain mitochondria in vivo.**

International Journal of Global Science Research
Vol.3, Issue 5, April 2016, pp. 295-301
*Available Online* at www.ijgsr.com

ISSN: 2348-8344 (Online)

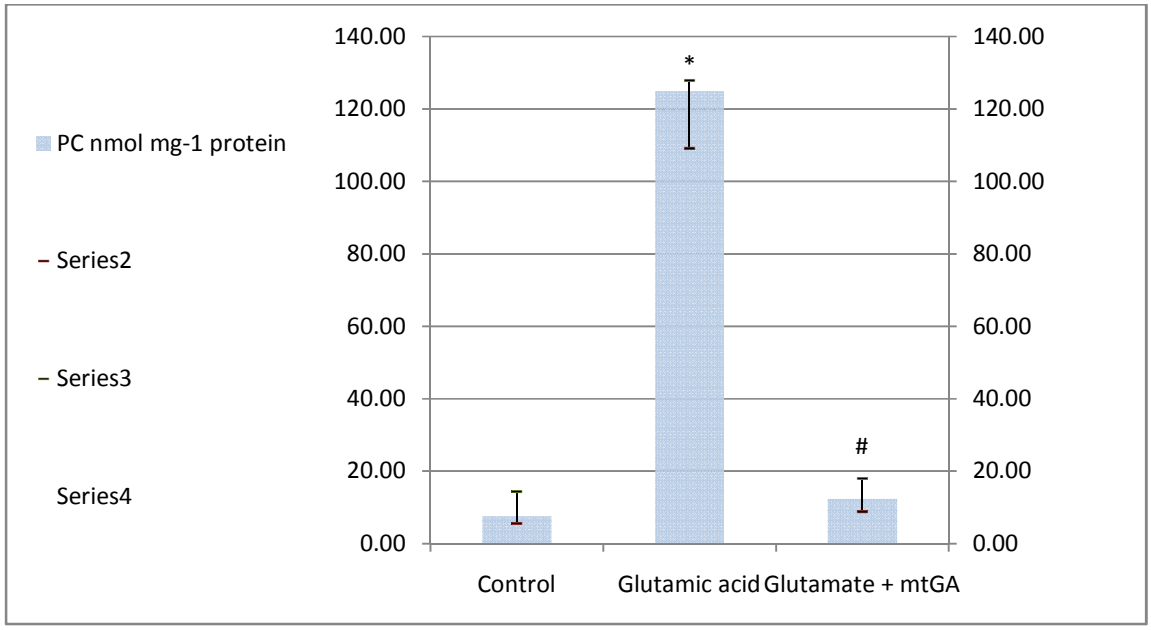


**Figure 2: Effect of glutamate (0.3 mg/gm i.p., b.w.) and protective effect of mitochondria targeted antioxidant (mtGA, 20 mg/kg) on protein carbonyls in brain mitochondria in vivo.**

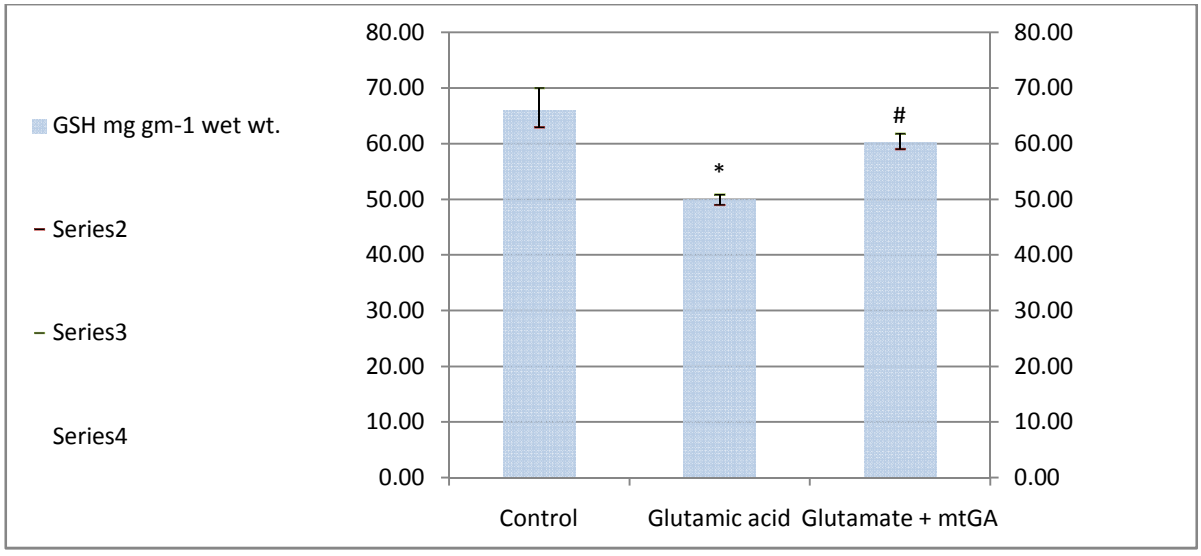


**Figure 3: Effect of glutamate (0.3 mg/gm i.p., b.w.) protective effect of mitochondria targeted antioxidants (mtGA, 20 mg/kg) on reduced glutathione in brain mitochondria in vivo.**

The administration of glutamic acid (0.3 mg/gm b.w.) for 14 days by intraperitoneal route increased the lipid peroxidation significantly ($p<0.05$) in mice brain mitochondria in vivo as compared to its respective vehicle control during 14 days experimentation period. The increase was noted 15.42±2.12 nm /MDA/mg protein in comparison to its control value 2.56±0.21 nm/MDA/mg protein. Mitochondria-targeted derivative of gallic acid treatment reversed the effects of glutamic acid administration, resulting in a significant ($p<0.05$) decrease in LPO levels. The decrease was noted 3.59±0.77 nm/MDA/mg protein in mtGA treated group as compared to their respective toxicant values. Values of protein carbonyl content increased significantly ($p<0.05$) in group receiving 0.3 mg/gm of glutamic acid. The value of PC content was noted 120.69±8.20 nm/mg protein in comparison to its respective control value 9.20±3.72. The administration of targeted derivative of antioxidant gallic acid significantly ($p<0.05$) decreased protein carbonyl content. The value of PC content was noted 12.42±3.98 nm/mg protein in mtGA treated group as compared to glutamic acid treated groups. Administration of glutamic acid significantly decreased glutathione level in mice brain mitochondria in vivo ($p<0.05$, as compared to the control value). The decrease was noted 50.0±1.73 mg/g protein in comparison to its control 62.93±2.11 mg/g protein value. However, a significant ($p<0.05$) increase in GSH content was noted after administration of 20 mg/kg mitochondrially targeted antioxidant during 14 days experiment. The increase was noted 60.01±2.67 mtGA treated group.

## DISCUSSION

During this investigation it is shown that mitochondrially targeted antioxidants containing lipophilic triphenylphosphonium cation accumulated within the brain mitochondria when administered oral. Experiments with the mitochondrially targeted antioxidants suggested that these compounds were predominantly incorporated into brain mitochondria. Oral administration of triphenylphosphonium cations taken up into brain mitochondria by non mediated movement through the lipid bilayer of the plasma membrane, assisted by the plasma membrane potential. Lipophilic cation accumulated within mitochondria driven by large membrane potential that is greater than the cytosol. After 14 days of drug administration, the excitotoxicity induced by glutamate in mitochondria comes near control values as noted by decrease in lipid peroxidation and protein bound carbonyls values. Membrane lipid peroxidation and protein carbonyls play a major role in mitochondrial chain dysfunctioning. Delivering mitochondrially targeted antioxidants to brain mitochondria reduces TBARS generated as a result of lipid peroxidation and protein carbonyls in experimental animal. We have shown that uptake of antioxidants targeted to mitochondria within brain can reduce the risk of oxidative stress by enhancing cellular antioxidant level. This approach enables a range of small molecules to be targeted to mitochondria and extends earlier approaches designed to target DNA and related molecules to mitochondria (Seibel, 1995; Chrzanowska-Lightowlers, 1995; Vestweber and Schatz 1989). Mitochondrially targeted antioxidant in its therapeutically effective concentration can be delivered to brain mitochondria without toxicity. Triphenylphosphonium ions can be toxic at high concentrations because their high accumulation in mitochondria disrupts oxidative phosphorylation. Instead of that 20 mg/kg/day oral doses of mitochondrially targeted antioxidants were well tolerated.

The maximum tolerated acute doses by i.v. injection were 6, 10, and 20 mg/kg for TPMP, MitoVit E, and MitoQ, respectively were noted (Robin et al., 2002). Therapeutically effective concentration (20 mg/kg/day) were used during this study is able to elevate cellular antioxidant level in brain mitochondria against glutamate induced toxicity. As these compounds will be accumulated further into cells, similar protective effects should be found by incubating cells with lower concentrations, and consistent with this 500 nM to 1 μM concentrations of mitochondria-targeted antioxidants are protective in cultured cells (Kelso, et al.2002; Hwang et al., 2001). In present study it is shown that mitochondrially targeted antioxidant can be delivered to the brain mitochondria. Glutamate induced excitotoxicity and deregulated calcium handling are one of the major causes of mitochondria dysfunctioning. Therefore, targeting drug can be important tool to overcome deleterious consequences occur due to glutamate toxicity and provide novel drugs to protect neurons from oxidative stress.

**Acknowledgements:** This work was supported by grant from Madhya Pradesh Council of Science and Technology, Bhopal. The author is grateful to Prof. M. S. Parihar, Professor & Head, Department of Zoology & Biotechnology, School of Studies in Zoology, Vikram University, Ujjain M.P. for his guidance and valuable suggestions for this entire work and Er. Birendra S. Punia for his help in statistical analysis.

## REFERENCES

Peter P. Z., James C., Anthon A. S., Khachaturian S., Stone V., Deborah G., JoAnn T., Tschanz M. C., Norton K. A., Welsh-Bohmer John C., Breitner S. (2004) Reduced risk of Alzheimer disease in users of antioxidant vitamin supplements-The Cache County Study. Arch. Neurol., 61, 82–88.

Parihar P., Jat D., Ghafourifar P., Parihar M. S. (2014) Efficiency of mitochondrially targeted gallic acid in reducing brain mitochondrial oxidative damage. Cell Mol. Biol. 60(2), 35-41.

Ohkawa H., Ohishi N., Yogi K. (1979) Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction, Analyt. Biochem. 95, 351-358.

Levine R. L., Garland D., Oliver C. N., Amici A., Climent J., Lenz A.G., Ahn B.W., Shaltiel S., Stadtman E.R., (1990) Determination of carbonyl content in oxidatively modified mice Methods in Enzymology 186 , 464-478.

Jollow D. J., Mitchell J. R., Zampaglione N., Gillete J.R. (1974) Bromobenzene induced liver necrosis, protective role of glutathione and evidence for 3, 4-bromobenzene oxide as the toxic metabolite. Pharmacology 11 , 151-169.

Lowry O. H., Rosenbrough N. J., Farr A. L., and Randall R. J., (1951) Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193, 265-275.

Seibel P. E. A., Trappe J., Villani G., Klopstock T., Papa S., Reichmann H. (1995) Mitochondrial Diseases: Models and Methods Nucleic Acids Res 23:10–17.

Chrzanowska-Lightowlers Z. M. A., Lightowlers R. N., Turnbull D. M. (1995) Gene therapy for mitochondrial DNA defects: is it possible? Gene Ther**.** 2, 311–316.

Vestweber D., Schatz G. (1989) DNA-protein conjugates can enter mitochondria via the protein import pathway Nature 338:170–172.

Robin A. J., Smith Carolyn M., Porteous Alison M., Gane Michael P. Murphy . (2002) Delivery of bioactive molecules to mitochondria *in vivo* 19, 5707-5412.

Kelso G. F., Porteous C. M., Hughes G., Ledgerwood E.C., Gane A. M., Smith R.A. J., Murphy M. P. (2002) Prevention of mitochondrial oxidative damage using targeted antioxidants. Ann. N. Y. Acad. Sci. 959, 263–274.

Hwang P. M., Bunz F., Yu J., Rago C., Chan T. A., Murphy M. P., Kelso G. F., Smith R. A .J, Kinzler K .W., Vogelstein B. (2001) Ferredoxin reductase affects p53-dependent, 5-fluorouracil-induced apoptosis in colorectal cancer cells.Natl. Med. 7(10), 1111–1117