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NEUROPROTECTIVE EFFECT OF GALANTAMINE AGAINST EXPERIMENTALLY INDUCED OXIDATIVE STRESS IN IMR 32 CELLS

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ABSTRACT

Galantamine isa traditional herb has also been explored for a number of pharmacological effects. Today, galantamine has been observed for its nootropic effect. There is affluence of data regarding supporting the effect of Galantamineon human neuronal cells. The present study was designed to investigate the alteration in antioxidant defense system and nootropic effects after Galantamine exposure on human neuroblastoma cells IMR-32. The treatment of Galantamine was given for 24 h and cytotoxicity study was carried out by trypan blue dye exclusion assay. Apoptosis and necrosis were observed using Propidium iodide (PI) and Hoechst double staining method. Biochemical assays like total protein, protein carbonyl, lipid peroxidation and glutathione level were analyzed along with enzymatic activity of super oxide dismutase and catalase. Result of cytotoxicity showed dose dependent increase in percent viability and significant decrease was observed in percent of apoptosis and necrosis. Moreover, exposure of Galantamine significantly decreased lipid peroxidation and protein carbonyl formation along with the enhancement in antioxidant defense mechanism. Findings of these dose reliant toxicity

study of Galantamine suggest that Galantamine has higher potency as a nootropic and causes repair of human neuronal cells IMR-32 cells enhancing the cell viability and consumption ofgalantamine will help in prevention of CNS disorders and neurodegeneration.

Keywords: Nootropic, IMR-32, Galantamine, Neuroprotection

INTRODUCTION

Neurodegenerative diseases are one of the major health challenges especially in elderly population. It includes hundreds of diseases categorized by progressive loss of neurons. Various genetic and environmental factors have been found to modulate the risk for neurodegeneration **[1]**. Galantaminehydrobromide is a tertiary alkaloid which belongs to the Amaryllidaceaefamily **[2]**. It has been isolated from many species including Leucojum species, Narcissus species and Galanthus species. It has become an important therapeutic option in various diseases **[3]**. The pharmacological history of galantamine shows that the bioactive compound was discovered accidentally in the early 1950s, and the plant extracts were initially used to treat nerve pain and poliomyelitis **[4]**. It is a clinically approved drug for the treatment of Alzheimer disease which acts as a CNS AChE inhibitor and allosteric potentiating ligand of the neuronal cholinergic nicotinic receptors **[5-7]**. Owing to the neuroprotective effect shown by galantaminethe current study was designed with intent to evaluate neuroprotective effect of galantamine over H_2O_2 and glutathione induced cytotoxicity on IMR-32 cells, which mimic cerebral cortex for better biological correlation. Also the study aims to study the alteration in antioxidant system galantamine exposure on human neuroblastoma cells IMR-32.

MATERIALS AND METHODS

Chemicals

All chemicals were procured from HiMedia, Mumbai, India and Sigma-Aldrich, USA.

Cell Culture and Treatment

IMR-32 cell line was obtained from National Center for Cell Science (NCCS), Pune, India. Cell line were maintained in Minimal Essential Media (MEM) supplemented with 2 mM L-glutamine, 0.1 mM non-essential amino acids and 10% Fetal bovine serum (HiMedia, Mumbai, India) in CO_2 incubator at 37 0C with 5% CO_2.

Cytotoxicity Testing by Trypan Blue Dye Exclusion Assay

In our previous study, results of MTT assay showed that LC50 of MSG after 24 h of treatment to IMR-32 cells was 7 mM **[8]**.

Based on this data, three different doses (1.7 mM, 3.5 mM and 7 mM) of MSG were selected to evaluate the cytotoxic effects on human neuroblastoma cell line IMR-32 by trypan blue dye exclusion assay **[9]**.

Apoptosis and Necrosis Observation by Propidium Iodide (PI) and Hoechst 33342 Double Staining

Cultures of IMR-32 cells were set up on coverslip for different experimental groups **(Table 1)** and after 24 h of MSG treatment (1.7 mM, 3.5 mM and 7.0 mM doses) apoptosis and necrosis were observed using PI- 1 µL (10 mg/mL) and Hoechst 33342- 1 µL (10 mg/mL). Cells were examined under fluorescence microscope **[10]** and the percentage of apoptotic and necrotic cells were calculated **[11, 12]**.

Percentage of Apoptotic Cells =LA+DA/ LN+LA+DN+DA × 100

Percentage of Necrotic Cells =DN/LN+LA+DN+DA ×100where,

LN= live cells with normal nuclei (PI/ Hoechst 33342: blue chromatin with organized structure)

LA= live cells with apoptotic nuclei (PI/ Hoechst 33342: bright blue chromatin that is highly condensed or fragmented)

DN= dead cells with normal nuclei (PI/ Hoechst 33342: pink chromatin with organized structure)

DA= dead cells with apoptotic nuclei (PI/ Hoechst 33342: bright pink chromatin that is highly condensed or fragmented).

Table 1: Experimental Groups

Groups	Doses
I. Control	-
II. Galantamine low dose	2.5 nM
III. Galantamine mid dose	5 nM
IV. Galantamine high dose	10 nM

Oxidative Stress Indices

IMR-32 cells were seeded in 12 well plates (10^5 cells/well) and cultured for 24 h. After 24 h of MSG treatment, these cells were used to make cell lysate **[13]**. The cells were trypsinized and treated with lysis buffer (pH 7.5) containing 1% Triton X-100, 130 mMNaCl, 10 mMTris-HCl and 10 mM NaH_2PO_4 **[14]**. The mixture was incubated for 30 minutes at 4 °C. The supernatant was used for biochemical assays like total protein (TP) **[15]**, protein carbonyl (PC) **[16]**, lipid peroxidation (LPO) **[17]** and total glutathione (GSH) **[18]** along with the activity of superoxide dismutase (SOD) **[19]** and catalase (CAT) **[20]**.

RESULTS

Cell Viability Study

The effect of Galantamine on cell viability was Neuroprotective when treated with trypan blue dye exclusion. The exposure of IMR-32 cells at lower doses Galantamine was somewhat similar to the control group i.e. 89.9 % cell viability. At increasing dose of 5 mM it was exactly similar to the control group i.e, 90.6%, live cells. When compared to the higher dose of 10 mMgalantamine the cell mortality was decreased upto 5 %. Thusa promising neuroprotection over IMR32 cell line was displayed by Galantamine (**Figure 1**).

Apoptosis and Necrosis Observation

Galantamine treatment with lower dose i.e. 2.5 nM showed non-variant decrease in apoptosis, whereas at higher doses i.e. 5 mM and 10mM respectively showed promising decrease ($p<0.001$) in apoptosis in comparison to the control group. Whereas the effect of Galantamine with all the three doses showed effective decrease ($P<0.001$) in necrosis in comparison to the control group **(Table 2)**.

Oxidative Stress Analysis

Treatment of Galantamine showed that value of total protein increased significantly at mid and higher doses ($p<0.05$ and $p<0.01$ respectively) whereas non-significant alteration was observed at low dose treatment as compared to control **(Figure 2)**. Protein carbonyl can be formed inside the cell due to the breakage of protein backbone by generation of ROS or direct oxidation of amino acids and it showed no significant change at low dose of Galantamine. At higher dose of Galantamine the results where somewhere close to normal ($p<0.001$) as there was no breakage of protein **(Figure 3)**. In order to determine the level of malondialdehyde the level of lipid peroxidation (LPO) is measured and it showed no change at low doses (2.5mM) of galantamine treatment. On the other hand the mid and high doses of Galantamine (Group III and IV respectively) showed promising decrease ($p<0.001$) in LPO as compared to control **(Figure 4)**. Glutathione is a major defense molecule against ROS. The level of GSH was observed to be increasing significantly ($p<0.05$, $p<0.01$ and $p<0.001$ respectively for 2.5mM, 5 mM and 10mM doses of Galantamine) in dose dependent manner as compared to control. These parameters showed an excellent linear dose response relationship in cultured IMR-32 cells after 24 h of Galantamine exposure **(Figure 5)**. Superoxide dismutase is an enzyme that catalysis the disputation of O2- into oxygen and H_2O_2 whereas, catalase converts H_2O_2 to non-toxic water molecule. The SOD activity was non prominent in any

groups ($p<0.001$) in comparison to control **(Figure 6)**. The CAT activity was noticed to moderately increase ($p<0.01$) for the group of cells treated with 10 mMGalantamine (Group VI) **(Figure 7)**.

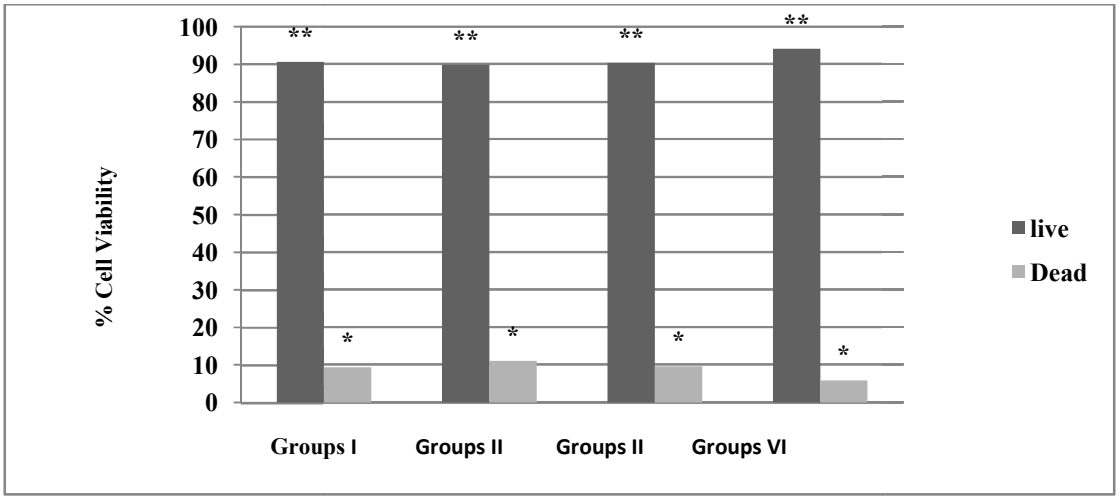

Figure 1: Percentage of live and dead cells after trypan blue dye exclusion assay in control and all Galantamine treated groups

Table 2: Percentage of apoptosis and necrosis by Propidium iodide and Hoechst double staining method

Parameter	Group I	Group II	Group III	Group IV
Apoptosis	4.9 ±0.45	4.6±0.30	4.1±0.21	3.6 ±0.17
Necrosis	11.6 ± 0.54	10.9 ±0.26	10.6±0.28	9.1±0.14

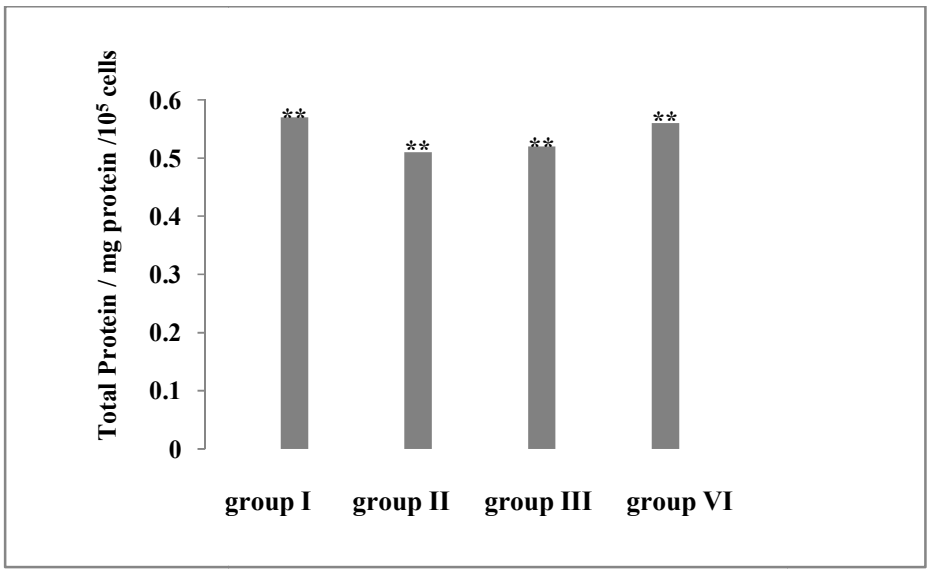

Figure 2: Result of total protein in control and all Galantamine treated groups

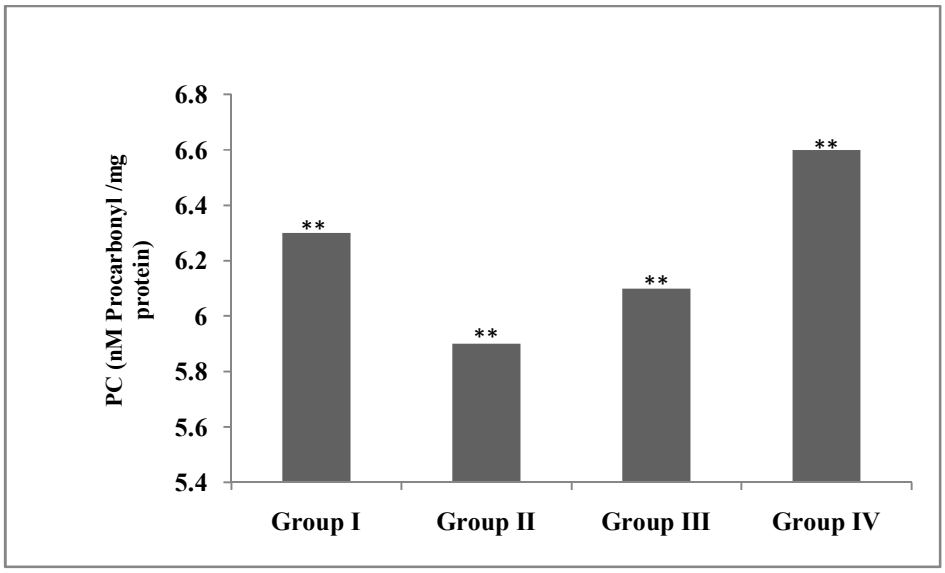

Figure 3: Result of Protein carbonyl control and all Galantamine treated groups

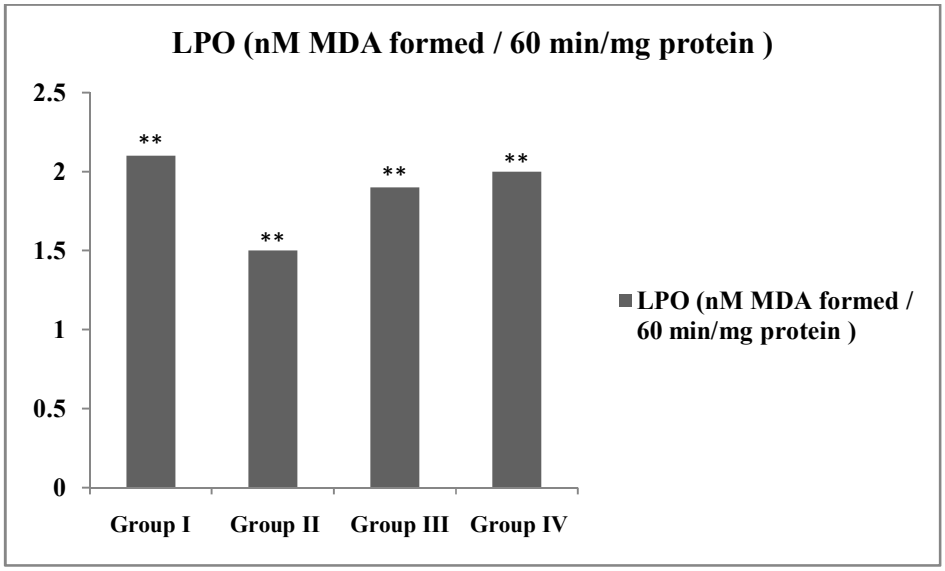

Figure 4: Result of lipid peroxidationcontrol and all Galantamine treated groups

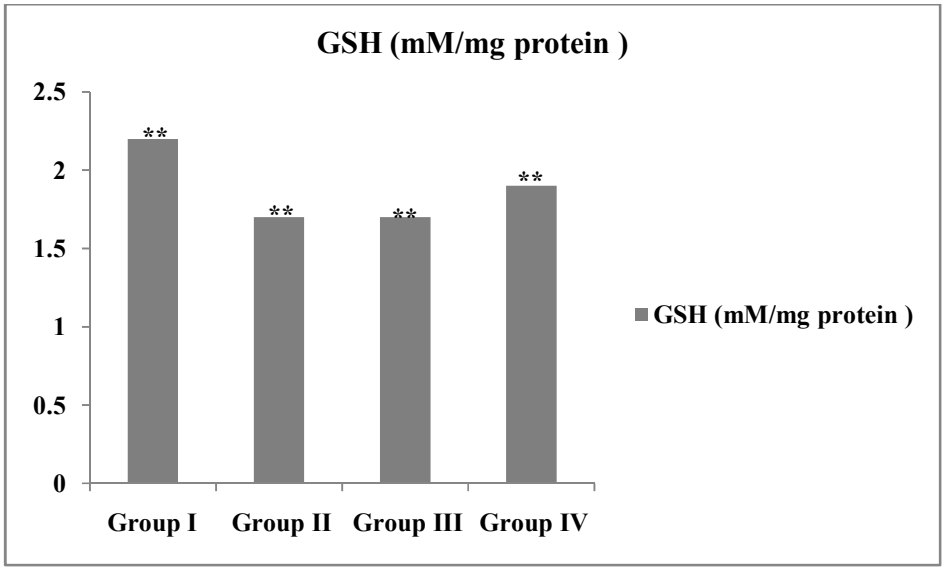

Figure 5: Result of GSH control and all Galantamine treated groups

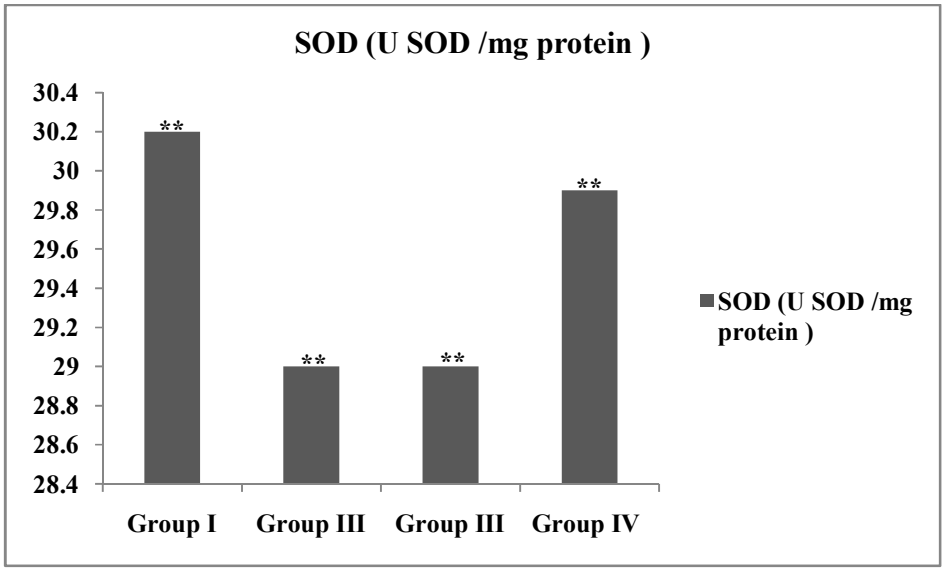

Figure 6: Result of SOD control and all Galantamine treated groups

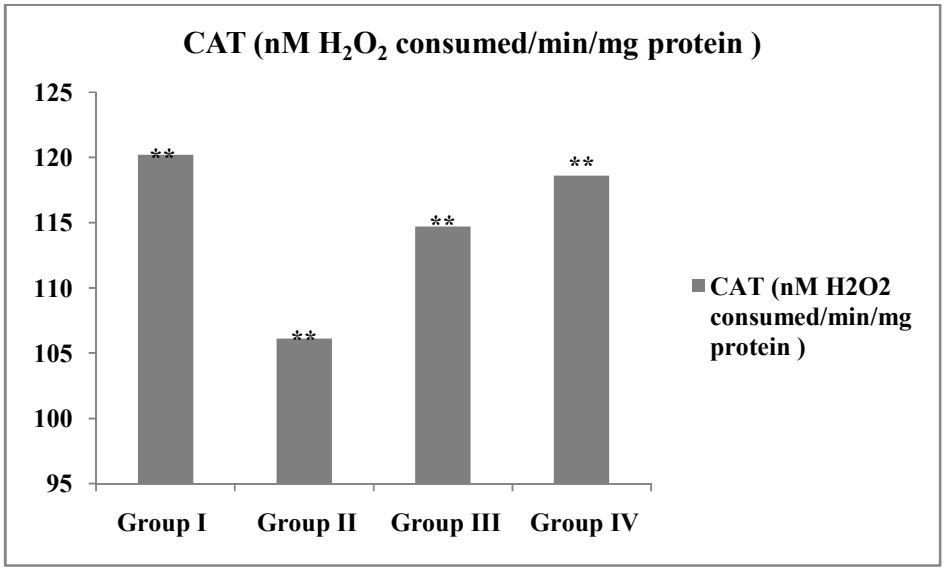

Figure 7: Result of CAT control and all Galantamine treated groups

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