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Synergistic effect of folic acid and galantamine against experimentally induced oxidative stress in IMR 32 cells

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Galantamine is an active constituent obtained from *Galanthus nivalis* L., a traditional herb known for its pharmacological properties, particularly nootropic effect. Folic acid is a dietary supplement that enhances neuronal activity. Effect of galantamine and folic acid on human neuronal cells is well known. In the present study, we explored the protective effect of galantamine and folic acid, both independently as well as in combination, over antioxidant defence system and nootropic effects on human neuroblastoma cells IMR-32. The treatment galantamine, folic acid and their combination 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 viz. total protein, protein carbonyl, lipid peroxidation and glutathione were analyzed along with super oxide dismutase and catalase. Result of cytotoxicity showed dose dependent increase in percent viability and significant decrease was observed in apoptosis and necrosis. Moreover, exposure to Galantamine, Folic acid and their combination significantly decreased lipid peroxidation and protein carbonyl formation along with the enhancement in antioxidant defence mechanism. Findings of these dose reliant toxicity study of Galantamine , Folic acid and their combination suggest that these has higher potency when given together and shows synergistic effect. They also causes repair of human neuronal cells IMR-32 cells enhancing the cell viability and consumption of Galantamine and Folic acid together will help in prevention of CNS disorders and neurodegeneration.

Keywords: *Galanthus nivalis*, Nootropic, Neuroprotection, Snowdrop

Neurodegenerative diseases are one of the major health challenges especially in elderly population. It includes numerous diseases categorized by progressive loss of neurons. Various genetic and environmental factors have been found to modulate the risk for neurodegeneration[1]. Neurodegeneration brings various challenges in the day to day life of an individual leading to difficulty in the survivals of the individual several nootropic agents have shown their effect on neurodegeneration. Nootropic agents, also known as smart drugs are highly acknowledged supplements that boost the cognitive performance. Nootropics increase the mental ability like learning, memory, attention, etc.[2].

Galantamine hydrobromide is a tertiary alkaloid which belongs to the *Amaryllidaceae* family[2]. It is isolated from many species including *Leucojum* species, *Narcissus* species and *Galanthus* species. It is an important therapeutic option in various diseases[3], including 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[6,7]. With the route of improvement, it has emerged as a massive beneficial preference in different neurological degeneration. Galantamine has been a clinically accepted drug for the remedy of Alzheimer's sickness. The medicine half way is going approximately as an AChE inhibitor that isan expected cholinergic nicotinic receptors[8].

Folic acid is a dietary supplement that possesses fundamental roles in CNS and related functions in all age groups[9]. It is an inexpensive and multifunctional component that has shown its benefits in prevention of CNS disorders[10]. It also enhances the cellular neuronal differentiation by enabling biomechanical and biochemical pathway[11]. Folic acid has also shown promising effects in functional neuronal recovery[12]. It also possesses neuroprotective function as it inhibits the neuronal apoptosis via microRNAs[13]. In the current study, we tried to evaluate the neuroprotective effect of galantamine and folic acid on H_2O_2 and glutathione induced cytotoxicity in IMR-32 cells, which mimic cerebral cortex for better biological correlation.

Materials and Methods

Chemicals

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

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Fig. 2 — Effect on the Levels of (A) Total protein;, (B) Protein carbonyl; (C) Lipid peroxidation; (D) GSH; (E) SOD; and (F) CAT in control (Gr. 1); and Galantamine (Gr. 2, 5, 8); Folic acid (Gr. 3, 6, 9); and Galantamine+Folic acid (Gr. 4, 7, 10) treated groups at low, mild and high dose with @2, 5, 10 mM, respectively [Values are expressed as mean± SEM (P <0.01) and (P <0.001)]

the cellular neuronal differentiation by enabling biomechanical and biochemical pathway[32].

Folic acid has also shown its notable effects in functional neuronal recovery[33]. It also possesses neuroprotective function as it inhibits the neuronal apoptosis via micro RNAs[34].Folic acid is an essential source of the single carbon group used in DNA methylation and plays a pivotal role in the development, function, regeneration, and repair of the central nervous system (CNS)[35]. In this study, the level of glutamate was found to be elevated in cultured human neuroblastoma cell line IMR-32 studied using MTT assay. The cell viability was negligible and the cell mortality was decreased up to 5% in case of galantamine treated group whereas it was 5.6% in folic acid treated group but even more promising result was seen in case of galantamine + folic acid treated group i.e. up to 7% which exclaimed the synergistic neuroprotective and neuroregenerative effect. The ratio of live and dead cells clearly showed that treatment of galantamine, folic acid and galantamine + folic acid treated group increased cell viability decreasing the rate of mortality. Moreover, propidium iodide and Hoechst 33342 double staining results revealed that

galantamine, folic acid and galantamine + folic acid treated group reduces apoptosis as well as necrosis in dose dependent manner. Excitotoxicity exerted by glutamate is also responsible for increase in the cytosolic Ca^{2+} level, which is due to either influx from the extracellular space or release from the intracellular stores[36]. In this condition the survival of a cell depends largely on functioning of the mitochondria[37].

As mitochondria not only satisfy the cellular energy demands but also involved in ROS generation, which in turn are suspected to cause cell death if they get out of control[38]. Increased ROS level causes damage to cell in several ways and oxidation of macromolecules like lipid, proteins and DNA. Increase in lipid peroxidation level due to ROS production results in loss of function and integrity of neuronal cell membranes, which in turn results in increase in non-specific permeability to ions, leading to disruption of membrane structure and cell functions[39]. The malondialdehyde, a marker of lipid peroxidation was decreased significantly in galantamine + folic acid treated group , as well as in galantamine and folic acid treated groups. Oxidation of proteins and amino acids induced by ROS generation also resulted in increase of protein carbonyl level and these could be determined by carbonyl groups (aldehydes and ketones) that are produced on protein side chains when they are oxidized[40]. In this study, a decrease in protein carbonyl level with corresponding increase in total protein confirms the notable rise in neuronal protein content. Antioxidant defense system consist mainly glutathione (GSH), superoxide dismutase (SOD) and catalase (CAT) as they are protagonist in our body[41]. The combination of Galantamine and folic acid protects the alteration of the antioxidant defense system and reduces oxidative stress. GSH protects the cells against free radical peroxides and other toxic compounds[42].

In current study, dose dependent increment of GSH level was found after galantamine and folic acid treatment. Superoxide dismutase defends the cell against free radical injury by converting O_2- radical to hydrogen peroxide (H_2O_2) and prevents the formation of OH^- radicals through O_2- driven Fenton reactions[43]. The H_2O_2 formed by SOD is removed by catalase. Hence, if the activity of CAT is not adequate to degrade H_2O_2 into H_2O and O_2, than more H_2O_2 is converted into toxic hydroxyl radicals and finally responsible for cellular damage[44,45]. Here, galantamine with folic acid was found to increase GSH levels as well as adhere the SOD and CAT activities, ultimately leading to the inhibition of neuronal cell damage.

Conclusion

The above results have demonstrated significance of both galantamine and folic acid treatments in neuroprotection. Galantamine and folic acid combination has shown more promising results in neuroprotection and as well as oxidative stress as indicated by cell viability and decreased apoptotic and necrotic behaviour of the cells. Apparent changes in the levels of total protein, protein carbonyl, lipid peroxidation, GSH, SOD and CAT in the treated groups have further shown synergistic effect of increased neuroprotection and decreased damage to the cells caused by oxidative stress. The neuro-protective effect of galantamine and folic acid on H_2O_2 and glutathione induced cytotoxicity in IMR-32 cells have also been shown.

Conflict of interest

Authors declare no competing interests.

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