ABSTRACT

The present study deals with evaluation of the prolonged effect of galantamine hydrobromide one of the memory enhancing drug on the overall behaviour and ATPases system in mice brain. Male albino mice weighing 30 ± 10 grams of 30 ± 2 days age obtained from Sri Venkateswara Enterprises, Bangalore was the experimental animal and galantamine hydrobromide, the drug. Galantamine hydrobromide ED₅₀ evaluated and it was found to be 5mg/kg body weight and it was also supported by Sweeney et al., (1990)⁵⁸. For prolonged dose studies, the mice were administered with galantamine hydrobromide once in a day orally for 360 days continuously. Observations on the general growth aspects such as weight and also the performance skills⁴⁸ of both control and the experimental mice were recorded on selected days as per the schedule. The levels of various constituents of ATPase system were determined in different regions of mice brain such as Olfactory Lobe (OL), Hippocampus (Hc), Cerebral Cortex (CC), Cerebellum (Cb), Ponsmedulla (Pm) and Spinalcord (Spc) at selected time intervals/days under prolonged dosage with galantamine hydrobromide as per Tirri et al., 1973⁵⁵. The results revealed that the galantamine hydrobromide causes significant perturbations in behaviour of mice and levels of all ATPases were inhibited to different extent in all the above mentioned areas of brain upon galantamine hydrobromide dosage thus exhibiting region specific sensitivity of mice brain. While under prolonged galantamine hydrobromide exposure all different types of ATPases registered maximum inhibition on 180th day and by 360th day almost near normalcy was restored. All the above changes in the ATPase system were finally manifested in the behaviour of mice exhibiting the symptoms of vomiting, weight loss, tremors and convulsions etc. From these observations, it was obvious that galantamine hydrobromide dosage caused severe perturbations in the functions of the nervous system through ATPases. Further an interesting observation was that the levels of all ATPases on 360th day and 30th day was similar indicating that the prolonged effect of galantamine hydrobromide getting reduced.

Key words: Alzheimer’s Disease, ATPases System, Morphometric and Behavioural changes, Galantamine hydrobromide and Mice Brain.

INTRODUCTION

Neurological disorders are quite diverse, chronic, challenging to treat and often disabling. Major symptoms of these neurological disorders are headache, stupor, coma, dementia, seizure, sleep disorders, trauma, infections, neoplasms, neuroophthalmology, movement disorders, demyelinating diseases, spinalcord disorders and peripheral nerves, muscle and neuro muscular junctions. The World Health Organization⁶¹ reported that neurological disorders ranging from migraines to epilepsy and dementia affect up to 1 billion people worldwide and the number is raising year after year due to various reasons such as pollution, increase in the life span of people. These neurological disorders kill around 6.8 million people each year equating to 12 percent of global deaths. Further, these reports also reveal that 2 percent of dementia cases appear before the age of 65 years and during later stages, the prevalence of dementia doubles for every 5 years.

This incurable, neurodegenerative and terminal disease was first described by German Psychiatrist and neuropathologist Alois Alzheimer in 1906 and was named after him⁶⁵. Alzheimer’s disease is the most common cause of dementia in adults worldwide. In 1991 the Canadian study of Health and Aging (CSHA) documented the prevalence rates of dementia (8.0% among people over age 65, 28.5% among those over 85 and 58% among those over 95) and found that Alzheimer’s disease was the cause in 64% of all cases of dementia, where as vascular dementia was present in only 19% of all cases⁷⁰¹⁸. Alzheimer’s disease is characterized by loss of neurons and synapses in the cerebral cortex and certain sub cortical regions. This loss results in gross atrophy of the affected regions, including degeneration in the temporal lobe, parietal lobe, parts of the frontal cortex and cingulate gyrus⁸⁶⁵. Both amyloid plaques and neurofibrillary tangles are clearly visible by microscopy in the brain of those afflicted by Alzheimer’s Disease⁶⁶⁵. Plaques are dense, mostly insoluble deposits of amyloid-beta peptide and cellular material outside and around neurons. Neurofibrillary tangles are aggregates of the microtubule-associated protein tau which has become hyperphosphorylated and accumulate inside the cells themselves. Although many older individuals develop some plaques and tangles as a consequence of aging, the brain of AD patients have a higher content of these plaques and tangles in specific regions such as the temporal lobe. Lewy bodies are not rare in AD patient’s brains⁶³⁵.

Further, it has been noticed that there is a marked loss of basal forebrain cholinergic neurons and also large reduction in cholinergic markers such as ChAT, acetylcholinesterase (AChE) and synaptosomal choline uptake in cholinergic target areas such as the cortex, hippocampus and amygdala of Alzheimer’s Disease patients²¹¹. Noradrenergic deficits were also found in the cortex and in hypothalamus²¹⁸. The serotonergic system is likewise compromised in Alzheimer’s disease. In the cortex there is some loss of both presynaptic (5-HT concentrations and 5-HT uptake sites) and postsynaptic (5-HT1 and 5-HT2 receptors) serotonergic markers²¹¹. These findings reveal that the neurotransmitter system is disturbed in Alzheimer’s Disease patients.

In view of the severity and magnitude of this disease, a number of drugs have been designed or developed and after clinical trials, they were put into market.
for treatment of Alzheimer’s Disease. Currently there are several drugs that have been approved for the treatment of Alzheimer’s disease. Galantamine is one of the latest drugs recommended to improve the cognitive functions and subsequently to treat the Alzheimer’s patients.

Now-a-days a number of memory enhancing drinks, foods and drugs are available in the market. People are very much fascinated to take these memory enhancing products to boost up their memory. Fizzy drinks, high proteinaceous and vitamin foods can improve the memory according to experts today[34]. Brain Speed Shake, Brain Speed Smoothie, Mocha Focus Delight etc., are widely used as memory enhancing drinks. It was observed that these drinks are prepared by mixing some FDA approved memory enhancing drugs like Detox, Phosphatidylserine etc., and have some structural similarities to memory enhancing drugs such as donepezil, galantamine, rivastigmine which are exclusively meant for the treatment of Alzheimer’s Disease[39]. In addition to these, Nootropics, also referred as smart drugs, memory enhancers and cognitive enhancers (Examples : hydergine, vinpocetine, bifenemalene, huperzine A and dimethylaminooethanol) are drug supplements, nutraceuticals and functional foods that are purported to improve cognitive functions such as memory, intelligence, motivation, and concentration[34][37]. These are also having some structural and functional similarities with drugs which are used to treat Alzheimer’s disease[38]. Among these, huperzine A may possess almost similar skeletal structure to galantamine hydrobromide except some side groups (Fig :1). The mode of action of galantamine and donepezil may also have close relation with huperzine A.

Structural similarities between galantamine hydrobromide (meant for treatment of A.D.) and huperzine A (a plant product in health drinks)

Galantamine hydrobromide
(C_{18}H_{32}BrNO_{4})

Huperzine A (C_{18}H_{49}N_{2}O)

The foregone literature clearly reveals that the drugs meant for treatment of a neurological disorders viz. Alzheimer’s Disease are misused by the public in order to improve their cognitive functions, to get relief from jet lag, to set their biological clock. In a recently issued United States patent, it is claimed that galantamine is effective in combating jet lag[12] to reset the body’s biological clock, the recommended procedure is to take galantamine once in a “day” in the time zone to which the person is going to travel. Further, these drugs are also consumed by employers in companies, factories, organizations etc. where shift system in duties is necessary. In view of this, it is proposed to study the prolonged effects of memory enhancing drug viz. Galantamine hydrobromide on the neurotransmitter system of albino mice, during its postnatal stages of development upto adult.

MATERIALS AND METHODS

One month old male albino mice weighing 30 ± 10 grams, obtained from Sri Venkateswara Enterprises, Bangalore were elected as experimental animals and Galantamine hydrobromide from Appollo pharmacy, Hyderabad was selected as the drug. The mice were fed with food pellets (Lipton Indian Ltd., Bangalore) and drinking water adlibitum. The animals were maintained in the laboratory conditions according to the instructions given by Behringer (1973)[21] 15 days prior to experimentation.

Parameters studied
1. Morphometric and Behavioural changes
2.ATPases System:
   a) Total ATPases: Tirri et al., 1973[61]
   b) Ca^{2+} ATPases: Fritz and Hamrick, 1966 as supported by Desaiah and Ho, 1979[13].
   c) Inorganic phosphates: Fiske and Subba Row, 1925[19].

2. Statistical Treatment of Data:
The mean, Standard Deviation (SD), Standard Error Mean (SEM) and test of significance or student’s ‘t’ test were calculated following the method of Pillai and Sinha (1968)[47].

Isolation of tissues
Animals were sacrificed on the 30th day, 60th day, 90th day, 120th day, 150th day, 180th day, 210th day, 240th day, 270th day, 300th day, 330th day and 360th day after oral treatment of Galantamine hydrobromide. After cervical dislocation the brain was isolated quickly and placed in ice. Different areas of the brain such as Olfactory lobe, Hippocampus, Cerebral Cortex, Cerebellum, Ponsmedulla and Spinalcord were isolated by following standard anatomical marks[35]. The isolated brain areas were immediately homogenized in suitable media for biochemical analysis. The results obtained were analysed statistically.

RESULTS
1. Morphometrical Studies ( Fig. 1)
From the results on morphometric studies, it was obvious that in general all the experimental mice gained relatively more body weight from 30th day to 240th day compared to their corresponding controls. On 30th day there was 29% increase in the weight of experimental mice compared to control. From 30th day onwards up to 210th day, the experimental mice recorded further gain of 35% in their body weights. However, on 240th day, the body weights of control and experimental mice were same. After 240th day the experimental mice started losing their body weights gradually up to 360th day.

Behavioural Changes (Fig. 2)
The results on water maze experiment revealed that the experimental mice treated with galantamine hydrobromide were very active compared to their corresponding control groups and at any given time the experimental mice took less time to reach the hidden platform. For example, the control mice on 180th day took 127 seconds whereas the experimental mice only 15 seconds. Thus there was a nine fold increase in the learning and memory activity and in over all performance skills of the experimental mice. However, after 300th day onwards the performance skills of the experimental mice started dwindling down with respect to their corresponding controls throughout the rest of period of experimentation thus revealing the ill effects of galantamine hydrobromide on the mice brain. In support of this observation, the experimental mice developed a number of side-effects like vomiting, anxiety, dizziness, indigestion, weight loss, sleeplessness, urinary tract infection, tremors and convulsions etc., on continuous exposure to galantamine hydrobromide.

2. ATPases System ( Figs. 3 to 5)
Na^+, K^+ - ATPase activities were assayed in different brain areas of control and galantamine hydrobromide administered mice. The enzyme activity was expressed as µ moles of inorganic phosphate / hour. Invivo studies were conducted in prolonged doses of galantamine.

Na^+, K^+ - ATPase activity (Figs 3)

Even though, the Na^+, K^+ - ATPase activity in general in all areas of mice brain
was inhibited under prolonged exposure to galantamine hydrobromide. During subsequent periods, maximum decrease in Na⁺, K⁺ - ATPase activity on 180th day in all selected regions of mice brain. However, from 180th day onwards up to 360th day the depleting trend in of Na⁺, K⁺ - ATPase activity showed gradual improvement in all the regions of mice brain and it was maximum in Ponsmedulla and minimum in Hippocampus.

Mg²⁺ – ATPase activity (Figs 4)
Similar to Na⁺, K⁺ - ATPase, Mg²⁺ – ATPase activity also recorded significant inhibition in different brain areas of albino mice treated with galantamine hydrobromide. From 30th day up to 180th day inhibition in Mg²⁺ – ATPase activity was recorded and maximum depletion on Mg²⁺ – ATPase activity was observed in different regions of mice brain on 180th day except in Ponsmedulla and Cerebellum. From 210th day onwards up to 360th day, Mg²⁺ – ATPase activity exhibited a reversal trend.

Ca²⁺ – ATPase activity (Figs. 5)
Along with Na⁺, K⁺ and Mg²⁺ – ATPase, Ca²⁺ – ATPase activity was also inhibited in different regions of mice following prolonged treatment with galantamine hydrobromide. Eventhough, from 30th day up to 180th day, different regions of mice brain recorded significant inhibition, it was highly fluctuating among these regions. However, on 180th day, maximum inhibition was noticed in Spinal cord and minimum in Hippocampus. Between 210th day to 360th day, Ca²⁺ – ATPase activity showed gradual increase in all regions of mice brain and on 360th day, the level of Ca²⁺ – ATPase was absolutely equivalent to the level noticed on 30th day.

An interesting observation was that the levels of all Na⁺/K⁺, Mg²⁺ and Ca²⁺ ATPases on 360th day and 30th day was similar indicating that the prolonged effect of galantamine hydrobromide getting reduced.
Fig: 3: Graphical representation of Percent change from control in Na+,K+ ATPase activity in different regions of mice brain exposed to chronic dosage of galantamine hydrobromide
Cerebellum

Exposure Period

Ponsmedulla

Exposure Period

Spinal cord

Exposure Period
Fig: 4: Graphical representation of Percent change from control in Mg+2 ATPase activity in different regions of mice brain exposed to chronic dosage of galantamine hydrobromide
Fig: 5: Graphical representation of Percent change from control in Ca+2 ATPase activity in different regions of mice brain exposed to chronic dosage of galantamine hydrobromide
In the present study we well supported by Gorini and Colleagues, 2002[24]. By the treatment of memory enhancing or Alzheimer’s disease treated drugs decreased Mg\(^{2+}\). ATPase activity in the frontal cortex of old aged rats was reported by Gorini and Colleagues (2002)[23]. In the present study also the decreased levels of Mg\(^{2+}\) - ATPase activity was observed upto 360 days in without Alzheimer’s disease mice also. During Alzheimer’s disease the calcium release was enhanced and transported through ER calcium channels and the drugs which are used to treat AD were act as inhibitors of Endoplasmic Reticulum calcium channels and protects cell from apoptosis agents including AB[189].

The results of the present study clearly state that galantamine hydrobromide causes significant changes in the body weight and behavioural aspects of mice. Compared to control the experimental mice gained body weight upto 240 days but after 240 days they started losing their body weight gradually. The significant observation on behavioural aspect was the experimental animals were more active on 180th day.

The pharmacologists observed in the time of evaluating the efficacy of donepezil, rivastigmine and galantamine in control trials they showed significant improvement in cognitive functions up to six months after that they were become normal[30]. There was also report which supported to the present study the drugs used to treat AD are having the ability to improve all neurotransmitters systems and causes activeness and learning and memory improvements in disease or in without disease condition for only some period and after that the remaining normal[42]. There was also evidence that the long-term efficacy of galantamine is delaying the cognitive function, activities of daily living and behavioural disturbances has recently began to be reported[43].

It was also reported that the galantamine hydrobromide has a unique character to inhibits the Na\(^+\)/K\(^+\) Pump due to non specific reduction of Na\(^+\) influx which stimulates the Na\(^+\)/Ca\(^{2+}\) exchanger and inhibits K\(^+\) conductance[25]. The drugs used to treat Alzheimer’s disease like galantamine hydrobromide, Memantine act as non-competitive antagonist of NMDA calcium channel receptors predominantly localized at dendritic spines belong to the group of very few drugs that have been proven to have some benefit to Alzheimer’s patients[59][60]. These earlier reports further substantiate the results in the present study where inhibition in Ca\(^{2+}\) - ATPase activity levels under disease free conditions was observed on treatment with galantamine hydrobromide.

Eaten and Salt (1989)[17] have stated that in neurons from ventrobasal thalamus 5-HT enhances both NMDA and non-NMDA – mediated effects; such action of 5-HT, however is indirectly mediated by an inhibition of Na\(^+\)/K\(^+\) current[17]. The cytoplasmic free Ca\(^{2+}\) is elevated in aged neurons and neurodegeneration encountered during Alzheimer’s Disease[59][64]. So, the drugs used to treat Alzheimer’s disease were shown to have inhibitory activity on Ca\(^{2+}\) - ATPase activity.

There was also evidence that the drugs act as Monoamine oxidase inhibitors also exhibit inhibitory effects on ATPases[11][16][14]. It is the best support to the present study, where galantamine hydrobromide one of the MAO inhibitor also lowered ATPases activities in all regions of mice brain. So the drugs used to treat memory impairments have the character to decrease Ca\(^{2+}\) - ATPase levels and MAO levels also[27][67].

The results presented in this study indicating that the administration of galantamine hydrobromide decreases the activities of the ATPase enzymes related to energy metabolism and membrane transport functions in different regions and to different extent. The reason for different regions of the brain exhibiting different levels of sensitivity to galantamine hydrobromide treatment perhaps may be because of the heterogeneity of the brain and also the
functions with which they are associated. For example olfactory lobe is implicated in the manifestation of sensory functions, hippocampus in memory and cognitive function, cerebral cortex in reflex actions and locomotor activities, cerebellum in locomotory functions and co-ordination of movements, pons medulla in respiratory functions and spinal cord in voluntary movements and co-ordination of muscles.

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