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Prolonged Effects of an Anti-Alzheimer's Drug, Galantamine Hydrobromide on Morphometric and Behavioural Aspects of Albino Mice.

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Research Article

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ABSTRACT

The present study, emphasises the prolonged effects of an anti-Alzheimer's drug, Galantamine Hydrobromide, on Morphometric and Behavioural aspects of albino mice in the absence of the disease, AD. One month old male albino mice, *Mus musculus* (20±2g) were selected as experimental model and Galantamine Hydrobromide as the test drug. The ED_{50} for GHB was evaluated and was found to be 5mg/kg body weight. This effective dose was given to Experimental groups of mice once in a day up to 180 days continuously. Observations on the general growth aspects such as weight and size and also changes in the behavior pattern of both control and experimental mice were recorded with help of Morris water maze technique. The results on Morphometric aspects revealed that the experimental mice recorded a steady and continuous gain in the body weight with maximum weight (22.15%) gained on 150th day. However, after 150th day, the experimental mice started losing their body weights gradually up to 180th day. As a corollary to these morphometric changes, maximum elevation in performance was noticed on 150th day (56.69%). From then onwards, there was a gradual decline in the performance of the mice. In view of these observations, it was concluded that the memory enhancing drugs meant for treatment of Alzheimer's disease or any formulated health drinks containing chemicals mimicking the memory enhancing drugs should not be consumed in any form by normal people in general and by young children in particular for a long time for improvement of their cognitive skills

INTRODUCTION

Alzheimer's Disease (AD), also called Senile Dementia of Alzheimer Type (SDAT), is the most common form of dementia. This disease was first described by German Psychiatrist and Neuropathologist, Alois Alzheimer in 1906 and was named after him [1]. This disorder usually appears in people older than age 65, but less common forms of the disease appear much early in adulthood [2,3]. AD is becoming a more common cause of death in the populations of the United States and other countries since the life span of human beings is increasing. Although other major causes of death continue to experience significant declines, those from AD have continued to rise. Between 2000 and 2008, deaths attributed to AD increased by 66%, whereas those attributed to the number one cause of death, heart disease, decreased by 13% [4]. An estimated 26.6 million people worldwide had Alzheimer's disease in the year 2006, and this number may quadruple by 2050. The incidence of AD rised from 2.8 per 1000 persons in the age group of 65-69 to 56.1 per 1000 persons in the older people of 90 years [5]. Alzheimer's disease is characterized by a marked loss of cholinergic neurons involved in regulation of learning and memory. Apart from this, neurons and synapses in the Cerebral Cortex, subcortical regions, temporal lobe, parietal lobe, parts of the frontal cortex and singulate gyrus have been atrophied which eventually resulted in manifestation of AD [6]. AD is further characterized by the presence of senile plaques and nerofibrillary tangles (NFTs) which are extra cellular deposits of filamentous ß-amyloid, a product of amyloid precursor protein.

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It has been reported that the fizzy drinks and the scourge of healthy diet campaigners, can improve the memory [7]. Brain Speed Shake, Brain Speed Smoothie, Mocha Focus Delight etc., which are also now-a-days used as memory enhancing drinks are prepared by the mixing some FDA approved memory enhancing drugs like Detox, Phosphatidylserine. Neuroscientists from Glasgow Caledonian University found that, consuming two cans of these soft drinks with high amount of glucose and other sugar substances can boost memory retention and combat dementia in older people. Further, biochemical studies have also revealed that the chemical substances in these soft drinks are having some structural similarities with some memory enhancing drugs such as Donepezil, Galantamine, Huperzine A, Rivastigmine which are exclusively recommended for treatment of Alzheimer's disease [8]. Apart from these, Nootropics, also referred as smart drugs, memory enhancers, and cognitive enhancers, are drugs, supplements, nutraceuticals, and functional foods which improve mental functions such as cognition, memory, intelligence, motivation, attention and concentration [9, 10]. Nootropics are thought to work by altering the availability of the brain's supply of neurochemicals such as neurotransmitters, enzymes, and hormones, by improving the brain's oxygen supply or by stimulating nerve growth. So, these nootropics are now-a-days preferred to be consumed along with memory drinks and food items or sometimes directly. They are also misused by shift workers in companies, industries etc. to reset the body's biological clock in order to lessen the risk of on-the-job injuries caused by impaired alertness.

Currently, among several drugs available for treatment of AD, Galantamine hydrobromide (GHB)is one of the latest drug recommended to improve the cognitive functions, and subsequently to treat Alzheimer's patients [11]. As already mentioned above, people without AD are also using these nootropics and anti-Alzheimer drugs to improve their memory, alertness and intelligence either directly or indirectly along with other foods or drinks. In view of this, in the present investigation, it is proposed to assess the long-term effects of memory enhancing drug, GHB on the Morphometric and Behaviour aspects of male albino mice in the absence of AD.

MATERIALS AND METHODS

Chemicals

All chemicals used in the present study were of Analar grade (AR), and were obtained from Sigma (st. Louis, MO, USA), Fisher (Pittsburg, PA, USA), Merck (Mumbai, India), Ranbaxy (New Delhi, India), Qualigens (Mumbai, India) scientific companies. Standard equipments were used for biochemical analyses.

Animal maintenance

One month old male albino mice, *Mus musculus*, weighing 20±2 grams, obtained from Sri Venkateswara enterprises, Bangalore, was selected as the experimental model The mice were maintained in the laboratory conditions according to the instructions of Behringer, [12] 1973 and as per the approval of the Institutional Animal Ethical Committee (No.:05/(i)/a/ CPCSCA/ IAEC/ SVU/ KY/BNK/Dt. 22.09.2007).

Selection of Drug

GHB (tablets), an anti-Alzheimer's drug which is a memory enhancing drug was selected for the present study.

Selection of the solvent

Saline (900mg of NaCl + 100ml of distilled water) was selected as a solvent since GHB dissolves in it without any turbidity.

Determination of ED_{50}

The Reed-Muench method [13] (1938) is a simple method for determining the ED_{50} value in experimental biology. The ED50 was checked by maintaining ten groups, six mice per group up to one month. In that, five groups were control and another five groups were experimental treated with different doses (1mg, 3mg, 5mg, 10mg and 15mg) of GHB for one month orally with the help of gavage. After the administration of different doses, ED_{50} was found to be 5 mg / kg body weight. In support of this Sweeny et al., [14] (1990) also reported the Effective dose of GHB in mice as 5mg/kg body weight.

Experimental protocol

After the mice were acclimated to the laboratory conditions, they were randomly divided in to two main groups, control and experimental. Each main group was again divided in to six groups of six each, and were housed

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in separate polypropylene cages. The effective dose of GHB (5mg/kg body weight) was administered orally to the experimental group of mice once in a day up to 180 days continuously.

Parameters Studied

Morphometric aspects

The basic morphometric aspects such as size and total body weight of control and experimental mice treated with GHB have been recorded once in five days from 5th day up to 180th days. The data thus obtained was analyzed and used to correlate the morphometric aspects with the behavioral aspects.

Behavioral aspects

Morris Water Maze test

Morris Water Maze task was performed to evaluate learning and memory efficiencies in rodents [15]. A great deal of knowledge has been obtained on the neurochemical, neuroanatomical and neurophysiological basis for the behavior associated with this paradigm. The apparatus consisted of a circular tank, 100 cm in diameter and 50 cm in depth. The tank was filled with water (21-26°C) up to a height of 30cm and the transparent escape platform made of plexiglass (10cm in diameter and 29 cm in height) was hidden at 1.5 cm below the surface of water in a fixed location. The water was made opaque with powdered non-fat milk or non-toxic white colored dye. The platform was not visible from just above the water level and transfer trials have indicated that escape on to the platform was not achieved by visual or other proximal cues [16]. The time spent by the animal to reach the hidden platform was used as the index of memory. Before starting the experiment the mice were acclimatize to the maze environment. The water maze test was conducted for all groups of mice on selected days viz., 30th day, 60th day, 90th day, 120th day, 150th day and 180th day for all six animals in a group separately. For each trial, the time required (in seconds) for individual mouse to find the hidden platform was recorded and the mean data from the tests were used for statistical analysis.

Statistical analyses

Data was expressed as mean ± standard error of mean (SEM). Results were statistically analyzed by student's t-test [17]. The level of significance was at $p<0.05$.

RESULTS AND DISCUSSION

Morphometric studies (Fig: 1, Table: 1)

In the present study, changes in the general growth parameters such as size and weight of control and Experimental (GHB treated) mice were recorded using digital balance at selected time intervals as given in the materials and methods section. The results revealed that the control mice showed a gradual increase in their body weights on all selected days throughout the tenure of the experimental for 180 days. When compared to the control ones, GHB treated mice recorded a phenomenal increase in their weights at all time periods from 30th day (22.8), 60th day (29.6), 90th day (34.9), 120th day (39.5), 150th day (46) and 180th day (43.5). The maximum weight (22.15%) was gained on 150th day. After 150th day, the experimental mice started losing their body weights gradually up to 180th day. The reason may be that GHB, through stimulation of cholinergic functions might have activated the metabolic pathways leading to substantial increase in the overall growth aspects of mice.

Figure 1: Graphical representation of differences in the body weight of control and experimental mice at selected time intervals during the treatment with Galantamine hydrobromide for 180 days.

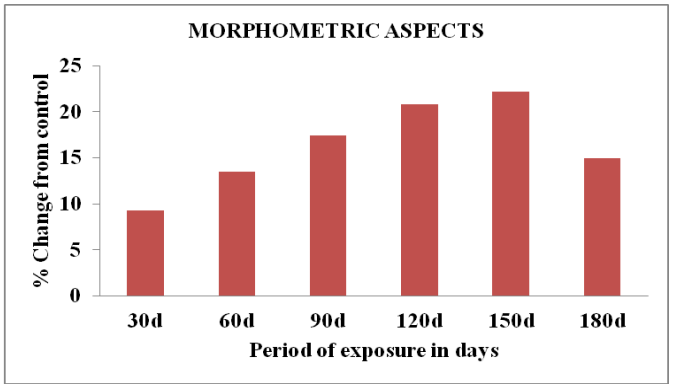

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Table 1: Differences in the body weight of control and experimental mice at selected time intervals during the treatment with Galantamine hydrobromide for 180 days.

	30 DAYS		60 DAYS		90 DAYS	
	C	E	C	E	C	E
Mean	20.717	22.837*	25.667	29.667*	28.85	34.933***
SEM	±0.323	±0.596	±1.192	±0.424	±0.324	±0.697
% Change		9.28		13.48		17.41
	120 DAYS		150 DAYS		180 DAYS	
	C	E	C	E	C	E
Mean	31.332	39.550***	35.85	46.051***	37.064	43.584***
SEM	±0.318	±0.801	±0.374	±1.003	±0.619	±0.732
% Change		20.78		22.15		14.96

Values are mean ± SE of six observations each from tissues pooled from 6 animals.
*** Values are significant at P < 0.0001 ; ** indicate significance at P < 0.001
* indicate significance at P < 0.05; C = Control E = Experimental

Behavioural aspects (Fig: 2, Table: 2)

Our results on Morris Water maze experiment revealed that, on all selected days, GHB exposed mice took significantly less time than the control animals to find hidden platform in water maze experiment, which indicates better performance talents of experimental mice. Maximum elevation was noticed on 150th day (56.69%) wherein, the experimental mice exhibited a 10 fold better performance than their control counter parts. However, from then onwards, there was not only a gradual decline in the performance skills of the experimental mice but several side effects like weight loss, vomiting, tiredness, dizziness etc. were noticed. Behavior is an observable or otherwise measurable muscular and secretory response reflecting the changes in an animal's internal or external environment [18]. It may include components which do not lend themselves to simple quantification. Animal behavior is neurally regulated phenomenon mediated by the brain and neurotransmitters [19]. Memory is defined by the properties of brain circuits sub-serving the neural changes induced by learning experiences [20, 21]. A few research findings demonstrated that Galantamine, on prolonged exposure caused a significant delay in cognitive function, activities of daily living and behavioural disturbances [22, 23] following damage to the cholinergic system. The changes in performance observed in Aged/Gal rabbits further indicated that Galantamine ameliorated the cognitive deficits typically associated with cholinergic functions [24] and improved learning in young as well as in older rabbits [25]. AChE inhibitors, namely, physostigmine and tacrine, donepezil, rivastigmine, and galantamine showed modest improvement in the cognitive function of Alzheimer's patients [26, 27].

The present findings report that uptake of GHB in the absence of disease condition improves learning and memory which is ofcourse short-lived (up to 150 days only), and thence it results in side effects like weight loss, vomiting, tiredness, dizziness etc. these observations derive support from the earlier reports wherein it has been substantiated that the memory enhancing drugs like opiates and other drugs, after prolonged intake cause anxiety like effects [28, 29].

Figure 2: Graphical representation of the results of the Water maze experiments on control and experimental mice treated with Galantamine hydrobromide for 180 days at selected time intervals.

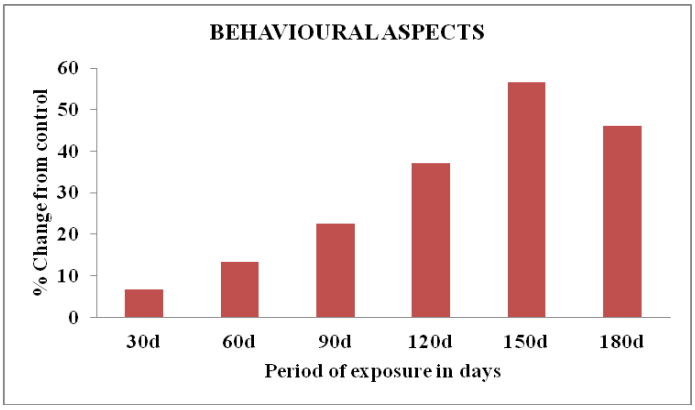

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Table 2: The results of the Water maze experiments on control and experimental mice treated with Galantamine hydrobromide for 180 days at selected time intervals.

	30 DAYS		60 DAYS		90 DAYS	
	C	E	C	E	C	E
Mean	31.797	29.660*	31.64	27.390*	28.45	22.047**
SEM	±0.443	±0.808	±1.197	±1.198	±0.457	±0.996
% Change		6.72		13.43		22.51
	120 DAYS		150 DAYS		180 DAYS	
	C	E	C	E	C	E
Mean	23.654	14.853**	17.18	7.440***	20.488	11.020***
SEM	±1.876	±0.930	±1.107	±0.924	±1.077	±0.349
% Change		37.21		56.69		46.21

Values are mean ± SE of six observations each from tissues pooled from 6 animals.
*** Values are significant at P < 0.0001 ; ** indicate significance at P < 0.001
* indicate significance at P < 0.05; C = Control E = Experimental

In the present study, it has noticed that GHB- induced effects were similar to those of cigarette smoking, which temporarily normalizes sensitomotor gating deficits in schrizophrenics but prolonged usage causes so many health problems [30]. Besides this, it has been observed that GBH also mimics the actions of another cognitive enhancer, Viz. guanfacine which strengthened the working memory, reduced distractibility, improved response inhibition, increased regional cerebral blood flow, in several animal models, as well as [31]. In another report, it was demonstrated that pioglitazone exhibited cognitive and functional improvement [32].

The most significant observations in the present study were that the morphometric and behavioural changes in mice treated with GHB showed positive result up to around 150 days and then onwards the experimental animals lost their body weight and activity levels because they develop side effects. So it may be suggested that the effect of the drug not only depends on its concentration but on the duration of exposure also. The other factors on which the effect depends include the extent and rate of absorption from the site of administration into the blood stream, distribution to various parts of the body from the blood, binding in tissues and mechanism of inactivation. From the above results, it was obvious that GHB significantly increased the weight and performance skills of mice up to certain time only and subsequently they were declined.

If we focus our attention on the undesirable ill effects caused by galantamine during later stages of prolonged exposure of mice in the absence of AD, it must be remembered that if these so called memory enhancing drugs are consumed by normal persons directly or indirectly, severe irreversible changes in the entire neurotransmitter system occur leading to a number of undesirable personality disorders in human beings. Hence, it was finally concluded that the memory enhancing drugs meant for treatment of Alzheimer's disease or any formulated health drinks containing chemicals mimicking the memory enhancing drugs should not be consumed in any form by normal people in general and by young children in particular for improvement of their cognitive skills.

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