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ORIGINAL RESEARCH ARTICLE

A Study on the Effects of Scyllo-Inositol on Learning and Memory in Senile Mice

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ABSTRACT

The aim of the present study was to evaluate the effects of scyllo-inositol, an inositol stereoisomer on learning and memory in senile mice model. Its role in ameliorating the disease pathology in Alzheimer's disease is well established. The study was done in senile swiss albino mice in the department of Pharmacology after the approval of the Institutional Animal Ethics Committee approval. The animals were allocated to the young control, senile control and treatment groups based on their age in months. The animals in the treatment group were administered scyllo-inositol at a dose of 30 mg/kg in the drinking water for a period of 7 days. Learning and memory was assessed in each of the groups using passive avoidance test, novel object recognition test and open field test. In the passive avoidance test, non-entry into the dark compartment during retention trial for the treatment group was 25% higher compared to that of senile control. A statistically significant increase in the preference index ($p< 0.01$) was obtained in the novel object recognition test for the treatment group and the open field test also demonstrated an increase in the rearing activity ($p=0.007$) and the number of squares crossed ($p=0.01$) in the animals treated with scyllo-inositol compared to their age matched senile controls. Scyllo-inositol increases learning and memory in senile mice and could be a potential therapeutic agent in senile dementia.

Key words: Scyllo-inositol, Senile mice, Dementia, Alzheimer's.

INTRODUCTION

According to the World Health Organization, there is a dramatic increase in the number of people with dementia due to increased longevity. The risk of dementia rises sharply with age with an estimated 25-30% of people aged 85 years or older having some degree of cognitive decline [1]. People with dementia in low and middle income countries generally do not have access to the affordable long-term care that their condition may warrant.

Scyllo-inositol is an inositol stereoisomer that has shown promise in the phase 2 studies of Alzheimer's dementia as a potential therapeutic agent in mild to moderate disease [2]. It ameliorates the cognitive and memory decline by inhibiting the beta amyloid aggregation and fibrillogenesis which is central to the pathogenesis of Alzheimer's disease [3]. There are studies which demonstrate the efficacy of scyllo-inositol in the transgenic mice models like TgCRND8 mice and other animal models of Alzheimer's disease which express the amyloid pathology [4,5]. But no studies have been done to assess the effects of scyllo-inositol in senile mice model which is devoid of specifically induced changes in the brain amyloid pathway. Therefore the present study aims to evaluate the effects of scyllo-inositol on learning and memory in the senile mice model.

MATERIALS AND METHODS

The study was done after Institutional Animal Ethics Committee approval. Adult, male Swiss albino mice of age 4-5 months (young control), 15-16 months (senile control and treatment group), and weighing 25- 40 g were randomly allocated to 3 groups. Each group included 8 animals.

- Group I - young control
- Group II - senile control
- Group III - treatment group

The animals were group housed, 4 per cage at a constant ambient temperature, on a 12 - h light, 12

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- h dark cycle. Pellet diet and tap water were provided *ad libitum.* The treatment group animals were administered scyllo-inositol (TCI Chemicals Ltd) at a dose of 30 mg/kg per orally for a duration of 7 days. The drug was administered to the animals in the drinking water and the concentration was adjusted to achieve the total daily dose based on average daily drinking water consumption.[6] At the end of 7 days treatment, all the animals in each of the three groups were subjected to the passive avoidance test, the novel object recognition test and the open field test.

Step through passive avoidance test:

This was done to test the non-spatial memory and was done using light dark shuttle box which consisted of two separate chambers, one illuminated and other dark, joined by a guillotine door. The test involved two sessions separated by 24 hours. Animals were left within the illuminated chamber initially. After 30 sec, the connecting door was opened. On crossing into dark compartment, the door was closed. Mild electric foot shock (0.4 mA for 2 sec) was given before returning the animal to home cage. After 24 hours interval, during the retention trial, animals were subjected to the same test. Memory was operationally defined as failure to enter or non-entry into the dark compartment within 3 minutes.

Novel object recognition test:

This test was done to assess the episodic memory using visual clues. Mice were individually habituated to an open field box for 5 minutes. During acquisition phase, two objects (A and B) of identical material were placed in symmetric position for 5 minutes duration. After 240 minutes, one of these A or B was randomly substituted by a novel object (C). Exploratory behavior was evaluated for 5 minutes. Successful recognition was revealed by preferential exploration of the novel object. Preference index was determined as: (time near the new object) / (time near the new + time near the old object) and expressed as percentage.

Open field test:

This was done to test the locomotor activity, activities like grooming and rearing of the animals. The arena of open field was divided into 16 squares - 4 inner squares and 12 squares in the periphery along the walls. After acclimatization to the lab and the open field apparatus, mice were placed individually in the centre of the open field. The parameters which were observed for 5 minutes included the number of squares crossed, number of rearing and the number of times the animal groomed.

Statistical analysis:

All data was analyzed using SPSS software version 19. In the passive avoidance test, the non entry into the dark compartment was expressed in percentage and the frequency distributions of the percentages between the groups were compared. All values in the novel object recognition test and the open field test were expressed as mean ± standard deviation (**Table 1**). The exploration time for the novel object and the preference index between the groups in the novel object recognition test was analyzed using ANOVA test. Paired t test was done to assess if there was any statistically significant difference in the exploration time for the familiar and the novel object in each group. The number of squares crossed, the number of rearing and the number of times the animal groomed in the open field was compared using ANOVA test.

Table 1: Novel object recognition test and open field test expressed as mean± standard deviation

Groups	Familiar object Exploration time (s)	Novel object Exploration time (s)	Number of grooming activity	Number of rearing activity	Number of squares crossed
I	14.5±3.78	19.5±4.27	5.3±4.08	46.3±8.33	151.5±51.34
II	14.1±2.56	14.8±1.94	7.6±4.71	16.6±11.87	76.6±27.09
III	14±2.96	17.8±3.18	5.6±2.58	41.6±20.81	107.3±27.82

RESULTS

In the passive avoidance test (**Figure 1**) non entry into the dark compartment during the retention trial for the treatment group (group III) was 75% compared to that of the control groups, senile control (group II) 50% and young control (group I) 87.5%. A 25% increase in the non-entry response was noted in the treatment group compared to the senile control group.

ANOVA test done showed that there was a statistically significant difference in the preference index (**Figure 2**) between the control and the treatment groups with $p < 0.01$ whereas there was no statistically significant difference in the exploration time of the novel object between the three groups with p=0.073(**Figure 3**).Paired t test (**Table 2**) done to assess if there was any statistically significant difference in the exploration time of the novel and the familiar object in the three groups showed that there was a significant difference only in the treatment group (p=0.002) and the young control group (p<0.001). The senile control group showed no statistically

significant difference in the exploration time of the novel and familiar objects (p=0.175).

The open field test (**Figure 4**) showed that there was a statistically significant difference in the number of rearing (41.6±20.81) and the number of grooming activities (5.6±2.58) of the treatment group compared to the control groups with p = 0.007 for rearing and p = 0.01 for the squares crossed using ANOVA test of significance whereas there was no statistically significant difference seen in the number of grooming activity between the three groups, p = 0.547.

Table 2: Paired t test result for novel object and familiar object exploration times

Groups	Exploration time of F=familiar & N= novel object	Mean(s)	Standard deviation	t value	p value
I	F	14.5	3.78		
	N	19.5	4.27	11.180	.000
II	F	14.1	2.56		
	N	14.8	1.94	1.581	.175
III	F	14.0	2.96		
	N	17.8	3.18	5.861	.002

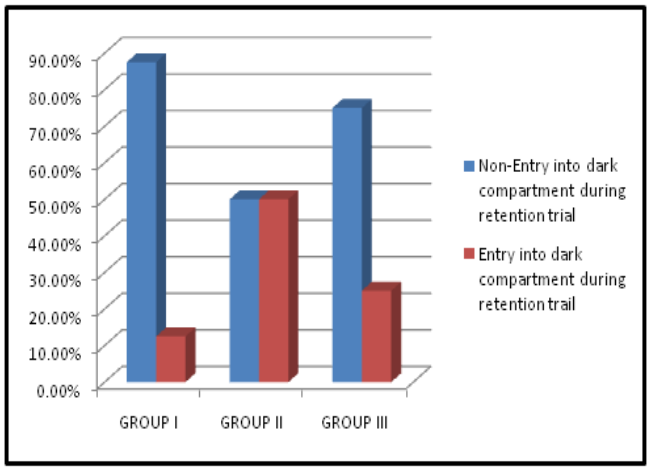

Figure 1: Passive avoidance test- Percentage of non-entry into dark compartment during retention trial

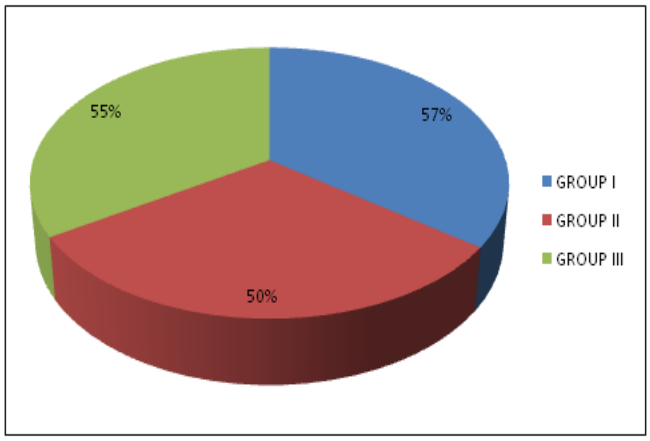

Figure 2: Preference index in percentage

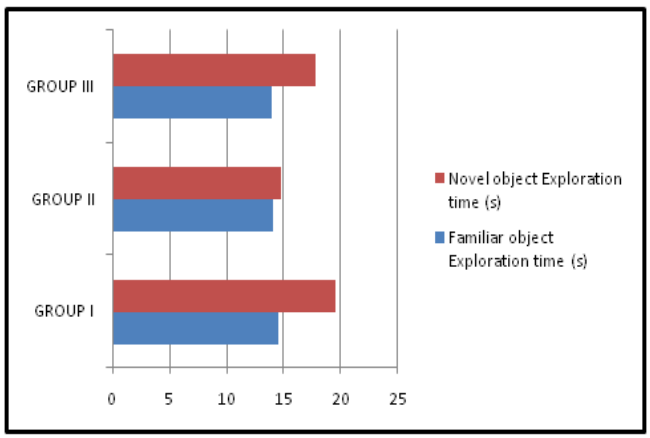

Figure 3: Novel object recognition test - Exploration time of familiar and novel object in seconds

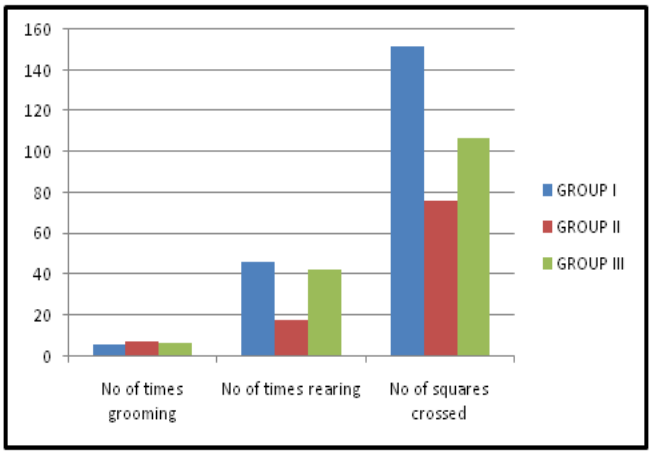

Figure 4: Open field test - Number of times the animal groomed, reared and the number of squares crossed expressed as mean

DISCUSSION

Inositol has 8 stereoisomers, four of which are physiologically active that includes scyllo-inositol. Scyllo-inositol is structurally *scyllo*cyclohexanehexol. This inositol stereoisomer has been demonstrated to inhibit amyloid beta aggregation and fibrillogenesis. The small size, neutral pH, stability, stereo-selectivity, and favorable toxicity profile of the molecule has made it a highly attractive candidate to test for therapeutic benefit in Alzheimer's disease and is currently under phase 2 clinical trial [2,7]. It is well known that Alzheimer's disease is characterized by deposition of amyloid beta protein forming plaques and neurofibrillary tangles contributing to neuronal loss and cognitive decline. The effect of scyllo-inositol in ameliorating the disease pathogenesis in various animal models of Alzheimer's disease has been well studied. Our study has brought out the effectiveness of scyllo-inositol in improving learning and memory in senile mice model which has no specifically induced changes in the amyloid pathway as in Alzheimer's model.

The exact mechanism contributing to this effect in the senile mice model is not known. The probable

mechanism could be a reduction in the amyloid beta burden in the brain which increases with normal ageing. Autopsy studies in the past decade have demonstrated that significant Aβ amyloid can be found post-mortem in 25% - 45% of normal older individuals, and that the extent and distribution of pathology may be indistinguishable from that found in Alzheimer's disease and imaging studies also have shown that amyloid deposition is an early event on the path to dementia, beginning insidiously in cognitively normal elderly individuals [8]. This could lead to complex functional alterations in cognitively relevant neural networks and the largely overlapping episodic memory networks specifically related to Aβ-mediated reversible disruption of synaptic plasticity rather than a direct consequence to neurodegenerative pathological processes [9]. Thus reduction in Aβ deposition that is distributed differentially across the cortex and progressing at varying rates with age [10,11] could be the likely mechanism for scyllo-inositol's favorable effect on learning and memory in senile mice. This is further supported by the fact that there is an increase in the levels of natural metabolite scyllo-inositol in normal ageing human brain detected using magnetic resonance spectroscopy [12, 13] which could possibly be a compensatory mechanism by the body to ameliorate the amyloid burden in aged individuals.

The study has brought out that scyllo-inositol improves learning and memory in senile mice model through the passive avoidance test, novel object recognition test and the open field test. This indicates that scyllo-inositol is a potential therapeutic candidate for senile dementia which has to be further confirmed in senile transgenic models and clinical trials.

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