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Effects of Bacopa monnieri and Metformin on Learning and Memory in Albino Rats

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Abstract: Background*: Learning is defined as acquiring new or modifying existing knowledge, behaviours, skills, values or preferences which may lead to a potential change in synthesizing information, depth of the knowledge, or behaviour relative to the type and range of experience. Memory is defined as retaining information over time. Metformin updates new spatial memories and decreases Acetylcholinesterase levels. It also decreases TNF-α levels in brain tissue. Bacopa monnieri shows anti-inflammatory, antioxidant, and amyloid beta (Aβ) aggregation inhibitor properties and has been shown to be potential therapeutic agent in the treatment of Alzheimer's Disease (AD).*

Materials and Methods*: This experimental study was conducted over a period of 20 days. Wistar albino rats were taken for this study. Rats were divided into five groups of five animals each. The groups were as follows: Group I- consisted of animals which were given 1% Gum acacia, Group II- Scopolamine (1mg/kg body wt), Group III- Metformin (100mg/kg body wt), Group IV- Bacopa monnieri (100mg/kg body wt), Group V- Rivastigmine (0.5mg/kg body wt). The Morris Water Maze test was carried out from 15th to 20th day. Time taken by the rats to find the hidden platform defined as "Escape Latency" was recorded.*

Results*: This study shows that pre-treatment with Metformin, Bacopa monnieri and Rivastigmine were effective in improving memory deficit induced by scopolamine in rats. Rivastigmine caused more improvement in mean escape latency time when compared to Metformin and Bacopa monnieri but there is no statistically significant difference among them.*

Conclusion:*The test drug, Metformin has been used in this experiment to evaluate its potential in drug repositioning in prevention of Alzheimer's disease. Another test drug, Bacopa monnieri has also been used in this experiment. Very few studies have been published on B.monnieri to establish its efficacy in Alzheimer's disease. The aim for using Bacopa monnieri in this experiment was to further prove its usefulness in the treatment of Alzheimer's disease.*

Key Word*:Dementia; Morris Water Maze; Metformin; Bacopa monnieri; Alzheimer's Disease.*

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I. Introduction

Many different forms of dementia exist among which Alzheimer's disease is the most common form which may contribute to 60–70% of cases. Other major forms include vascular dementia, dementia with Lewy bodies (abnormal aggregates of protein that develop inside nerve cells), and various diseases that contribute to frontotemporal dementia (degeneration of the frontal lobe of the brain). Alzheimer's disease (AD) is characterized by a continuous decline in cognitive function. AD is found to be increased among people aged 65 years or more, with a progressive decline in memory, language, thinking and learning capacity. The pathophysiology of AD is related to the injury and death of neurons, which begins in the hippocampus brain region that is involved with memory and learning, then atrophy affects the entire brain[1,2]. It is projected that the total number of people with dementia will rise to 82 million in 2030 and 152 million in 2050[3].

Many drugs have been approved for the treatment of Alzheimer's disease such as Donepezil, Rivastigmine, etc. and some natural and herbal nootropics, such as Ginkgo biloba, Bacopa monnieri and Panax quinquefolius(American Ginseng) have been studied in boosting the brain function. Bacopa monnieri or Brahmi is derived from the family of Scrophulariaceae, found throughout the Indian subcontinent in a wet, damp, and marshy area[4]. It has purple flowers with numerous branches and small leaves. This plant is known to be used for various nervous system disorders, including insomnia, anxiety, and epilepsy. According to Ayurvedic medical practitioners, Bacopa monnieri is categorised as a medhyarasayana, a compound that stimulates and enhances the memory and intellect. These properties have been studied preclinically and clinically[5].Administration of B. monnieri is known to increase the level of ACh and upregulation of receptor binding for cholinergics in the frontal cortex and hippocampus[6].

Metformin is the most commonly used drug in the treatment of Diabetes Mellitus, so it has been used in this experiment to evaluate its potential in drug repositioning in prevention of Alzheimer's disease.Metformin is a biguanide developed from galegine, a guanidine derivative found in Galega officinalis (French lilac). It is an antihyperglycemic agent, which improves glucose tolerance in patients with type 2 diabetes, lowering both basal and postprandial plasma glucose. Its mechanisms of action are different from other classes of oral antihyperglycemic agents. Metformin decreases hepatic glucose production, decreases intestinal absorption of glucose, and improves insulin sensitivity by increasing peripheral glucose uptake and utilization. Metformin improves the ability to update new spatial memories, a task related to hippocampal neurogenesis[7]. There is evidence that metformin decreases the activity of acetylcholinesterase (AChE), which is responsible for the degradation of acetylcholine (Ach), a neurotransmitter involved in the process of learning and memory[8]. Treatment with metformin is associated with attenuation of neuro-inflammation, as evidenced by decreasing Tumor Necrosis Factor (TNF-α) in brain tissues. Metformin was reported to increase Akt phosphorylation through activation of the AMPK/phosphatidyl inositol3 kinase (PI3K) pathway[9]. Phosphorylated Akt is known to inhibit the enzyme glycogen synthase kinase (GSK-3β), which is increasingly thought to play a pivotal role in production of hyperphosphorylated tau and neurofibrillary tangles[10].

Both the test drugs taken in this study, Metformin and Bacopa monnieri were tested through Morris water maze on albino rats for assessment of learning and memory.Mazes are traditional tools in assessing learning and memory performance in laboratory animals. They are the simple but effective tools to evaluate learning and memory function in rodents. Morris Water Maze is the most widely used test for studying the psychological processes and neural mechanisms of spatial learning and memory. This test was first established by neuroscientist Richard G. Morris in 1981 in order to test hippocampus-dependent learning and acquisition of spatial memory[11].

II. Material And Methods

The experiment was carried out in the Department of Pharmacology, RIMS, Ranchi, a tertiary care hospital. The study was approved by the Institutional Animal Ethics Committee (IAEC).

Study Location: Department of Pharmacology, RIMS, Ranchi
Study Duration:20 days
Sample size: 25 animals

Inclusion criteria: Healthy male albino rats weighing 150-200 grams were taken for the study.
Exclusion criteria:
1. Male albino rats <150 grams &>200 grams were excluded.
2. Unhealthy and Inactive animals were excluded.
3. Female albino rats were excluded due to cyclical hormonal changes.

Procedure methodology

Healthy Male Wistar albino rats weighing 150-200 grams were taken for the present study. These rats were divided into five different groups, five animals in each group. The groups were as follows : Group I- consisted of animals which were given 1% Gum acacia, Group II- Scopolamine (1mg/kg body wt), Group III- Metformin (100mg/kg body wt), Group IV- Bacopa monnieri (100mg/kg body wt), Group V- Rivastigmine (0.5mg/kg body wt). The doses of drugs were determined on the basis of surface area of rats.

The study duration was of 20 days. The Morris Water Maze Test was carried out from 15^{th} to 20^{th} day. During this period, the rats were trained to learn the task, which was to find the hidden platform. The training days were designated as day1, 2, 3, 4 and the Escape Latency time was noted. A gap of 48 hrs was given and the retention memory time was noted. This time gap simulates normal forgetting, which occurs due to lack of rehearsal of pre-learned task. The memory test day was designated as day 6.

Statistical analysis

One way Analysis Of Variance (ANOVA) test was used for statistical analysis of data to compare the effect of drugs on different groups, and differences were considered to be statistically significant if $p<0.05$. Tukey's HSD test was used for post-hoc analysis of significant overall differences. All the computations were done by using IBM SPSS statistics tool.

III. Result

The experimental data in Table 1 shows thecomparison of Mean and SD (standard deviation) of Escape latency time in seconds for all the groups and days in Morris Water Maze test. The data shows that there was a significant impairment of learning in Scopolamine (Group 2) treated group when compared to Control

group(Group 1) as shown in Figure 1 and 2(a). Metformin treated animals (Group 3) showed an improvement in learning of task in Morris Water Maze Test which is evident by decrease in mean escape latency time from day 1 to day 6 as shown in Figure 2(b). Bacopa treated animals (Group 4) also showed an improvement in learning in MWM test shown in Figure 2(c).When compared with B. monnieri and Metformin, Rivastigmine (Group5) showed better improvement in learning of task.This improvement was significant from day 2 to day 6 when compared to Scopolamine treated rats (Group 2) as shown in Figure 2(d).Both test drugs showed an improvement in learning of task, which was significant from day 2 onwards when compared with scopolamine treated group. When compared to Control (Group 1), Rivastigmine treated rats showed no statistically significant difference in results.

Comparison between Rivastigmine + Scopolamine (Group 5) and Metformin + Scopolamine (Group 3), and comparison between Rivastigmine + Scopolamine (Group 5) and Bacopa monnieri + Scopolamine (Group 4), shows that in all the groups an improvement in learning and memory has occurred. But there is no statistically significant difference among them i.e., both the test drugs are nearly identical in reversing amnesia produced by scopolamine, but Rivastigmine pretreated rats had better learning of task than Metformin and Bacopa treated rats which can also be concluded from Table 1 and Figure 1.

Table 1: Comparison of Mean and SD (standard deviation) of Escape latency time in seconds for all the groups and days in Morris Water Maze test

	Group 1 (Control)	**Group 2 (Scopolamine)**	**Group 3 (Metformin & Scopolamine)**	**Group 4 (Bacopa monnieri &Scopolamine)**	**Group 5 (Rivastigmine&Scopolamine)**
	Mean ± SD	**Mean ± SD**	**Mean ± SD**	**Mean ± SD**	**Mean ± SD**
Day1	70.26 ± 15.34	82.61 ± 8.10	79.20 ± 8.53	80.72 ± 6.12	73.10 ± 12.48
Day2	52.16 ± 9.36	78.60 ± 11.01	57.80 ± 10.57	60.00 ± 11.14	54.46 ± 17.84
Day3	29.30 ± 5.34	74.06 ± 9.87	42.12 ± 9.55	44.84 ± 9.13	38.54 ± 9.02
Day4	22.66 ± 4.84	71.94 ± 11.59	29.20 ± 2.58	32.80 ± 5.46	26.66 ± 6.39
Day6	17.02 ± 4.80	70.82 ± 9.34	24.40 ± 2.78	27.12 ± 3.66	22.44 ± 2.90

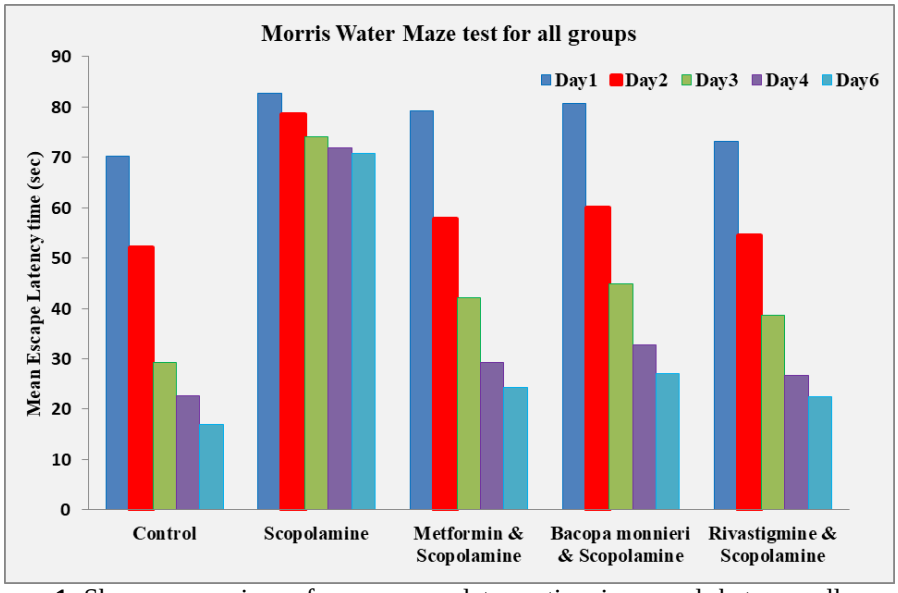

Figure 1: Shows comparison of mean escape latency time in seconds between all groups

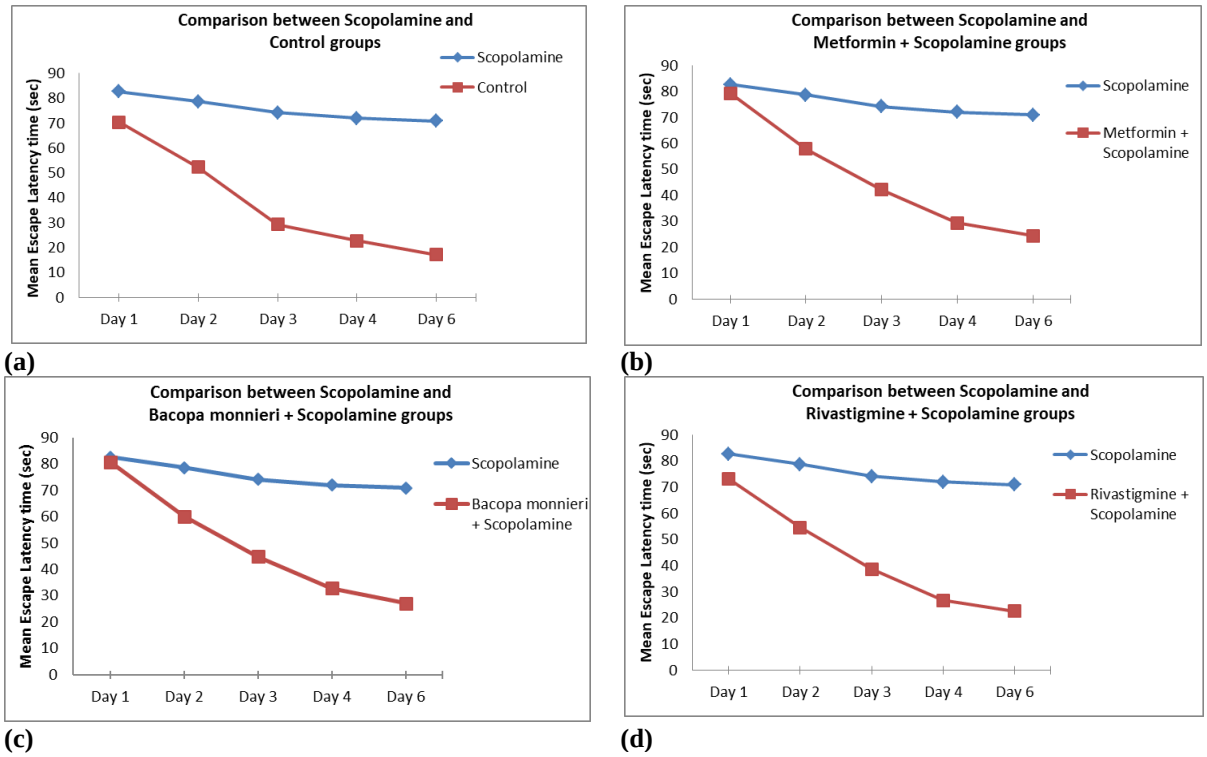

Figure 2: Learning curve between groups (a)Control group (Group 1) and Scopolamine (Group 2), (b) Scopolamine (Group 2) and Metformin + Scopolamine (Group 3), (c) Scopolamine (Group 2) and Bacopa monnieri + Scopolamine (Group 4), and (d) Scopolamine group (Group 2) and Rivastigmine + Scopolamine group (Group 5).

IV. Discussion

Present study showed that exposure to Scopolamine, which was used as an amnesic agent, impaired the learning and memory processing activities in rodents, i.e. there was delayed learning in this group which was measured by an increased escape latency time. The data shows that there was a significant impairment of learning in Scopolamine treated group (Group 2), which was significant from day 2 to day 6. These observations were quite similar to the previous studies done on the differential metformin dose-dependent effects on cognition in rats: role of Akt (Dalia K. Mostafa et al., 2016) which showed memory enhancing effects[12]. In this study, the scopolamine-treated group showed a significant decrease in learning performance versus the control rats. Scopolamine was given at a dose of 1mg/kg and was given intraperitoneally, 30 minutes before any behavioural session.

Metformin treated animals (Group 3) showed an improvement in learning of task in Morris Water Maze Test which is evident by decrease in mean escape latency time from day 1 to day 6.Dalia K. Mostafa et al., 2016 postulated that the modulation of Akt by metformin treatment provides a novel mechanism explaining its protective role in AD-like memory impairment[12]. Another study done by Mohammad Hossein Esmaeili, Mahine Mafe Esmaeili, 2016in which Metformin 100mg was used in Morris water Maze test also reversed the amnesia induced by Scopolamine[13].Bacopa monnieri treated animals (Group 4) also showed an improvement in learning in MWM test which is evident by decrease in mean escape latency time from day 1 to day 6. Previous studies done on neurocognitive effect of nootropic drug Brahmi (Bacopa monnieri) in Alzheimer's Disease (Kaustubh S. Chaudhari et al., 2017) also showed memory enhancing effects[14].

Basso et al., 2002 proved that the role of Akt in AD pathogenesis is complex as it was reported to increase tau accumulation by either interfering with its degradation or enhancing abnormal tau hyper-phosphorylation[15].Wang et al., 2012 showed that Metformin in addition to promoting neurogenesis also improved spatial memory formation in mice. It has been suggested that oxidative stress might be the cause ofneurodegeneration. Oxidative stress even may be the initial cause of neuronal damage.Also, in process of aging, Reactive Oxygen Species(ROS) production increases and ability of body to fight with them decreases. Hence, Metformin, which is usually started in midlife, can arrest the process of neurodegeneration at its start and bring down the prevalence of cognitive impairment. Wang et al., 2012 in the study showed that Metformin, by activating an aPKC-CBP pathway, recruits adult neural precursors and enhances neural function, thereby providing a candidate pharmacological approach for nervous system therapy[16].

V. Conclusion

The test drug, Metformin has been used in this experiment to evaluate its potential in drug repositioning in prevention of Alzheimer's disease. Another test drug, Bacopa monnieri has also been used in this experiment. Very few studies have been published on Bacopa monnieri to establish its efficacy in Alzheimer's disease. The aim for using Bacopa monnieri in this experiment was to further prove its usefulness in the treatment of Alzheimer's disease.The results from this study have shown that Metformin and Bacopa have markedly improved learning and memory in rats as compared to Scopolamine group. Rivastigmine caused more improvement in mean escape latency time when compared to Metformin and Bacopa monnieri but there is no statistically significant difference among them. This is a short-term study and long-term studies are required for better evaluation of these drugs.

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