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EFFECTS OF ETHANOL EXTRACT OF *DENNETIA TRIPETALA* ON LEARNING AND MEMORY IN SCOPOLAMINE TREATED RATS

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ABSTRACT

Dennettia tripetala (DT) fruits have been proven to have biochemical, anti- inflammatory and nutritional values. *Dennettia tripetala*, when eaten, causes mild stimulation in the brain which may affect neurobehavioral parameters. Whether it affects learning and memory is not yet certain. This study therefore investigated the effects of ethanol extract of *Dennettia tripetala* on learning and memory in scopolamine treated albino Wistar rats using the Novel Object Recognition test and Morris Water Maze, test. Twenty female wistar rats weighing between 100-150g were divided into four groups of five rats each. Group 1 served as the control group. Group 2(Scopolamine) received scopolamine (1mg/kg) intra-muscularly. Group 3(Scopolamine + *Dennettia tripetala*) received scopolamine (1mg/kg) for 1 week + *Dennettia tripetala* (200mg/kg) Extract orally for 2weeks. Group 4 (extract alone group) received 200mg/kg *Dennettia tripetala* orally for 2weeks. All the groups received rat chow and water daily. The animals were acclimatized for 2 weeks. There was no significant difference ($p<0.05$) in swim latency amongst the groups on acquisition days one and three. On day two, swim latency in scopolamine group was significantly increased ($p<0.05$) compared to control group and significantly decreased ($p<0.05$) in extract group compared to the scopolamine group. On day five and six of the reversal training days, there was a significant decrease ($p<0.05$) in swim latency in the extract group compared to control and scopolamine + extract group. Habituation index was decreased ($p<0.05$) in the extract group compared to control in short term memory, in long term memory, there was a significant decrease in extract group compared to control, scopolamine and scopolamine + extract group. Discrimination index in long term memory in the extract group was increased compared to control and scopolamine groups, this shows a preference of the rats for the novel object, hence better retention, and better memory in the extract group. This indicates that oral administration of *Dennettia tripetala* improved learning and memory in albino Wistar rats.

KEYWORDS: *Dennettia tripetala*, Scopolamine, Learning, Memory, Morris.

1.0 INTRODUCTION

The Nervous system primarily affects feelings, movement, memory, learning and other cognitive and non-cognitive functions, as a result, several disorders associated with these functions are also common. Learning is defined as the acquisition of information and skills, subsequent retention of the information is called memory. They are closely related concepts which can be differentiated by the speed with which they occur, skills acquired slowly and laboriously is learning whereas that acquired instantly is memory.[1] Learning is of different types which include associative learning and non-associative learning.[2] Types of memory include sensory memory[3], short and long term memory.[4] There are problems related to memory and learning such as memory loss (amnesia).[5] This depending on the cause, memory loss may only last a brief period of time. Activities of daily living may be hampered by severe memory impairments.[6] Memory loss can occasionally accompany mental health issues like bipolar disorder following a big traumatic incident or stressful situation, despair anxiety, or schizophrenia.[7]

Scopolamine, also known as hyoscine or devil's breath is a high affinity muscarinic acetylcholine receptor antagonist used as a research tool to cause cognitive

impairment and to alleviate post-operative nausea, vomiting and motion sickness.[8] Blood-brain barrier is easily crossed by scopolamine and it's believed that inhibiting muscarinic receptors in the brain causes a cholinergic deficit that weakens memory.[9] Scopolamine therefore, has been widely employed in pre-clinical and clinical trials of drugs to treat cognitive impairments.[10]

It is well known that plants are used to maintain good health.[11] According to reports, plant-based products serve as the foundation for many contemporary medications used to treat a wide range of illnesses.[12] A wide range of orthodox drugs have been employed as therapeutic intervention in the treatment of health conditions, but some have proven to be ineffective, exorbitant or have debilitating side effects. Hence, an alternative approach which involves the use of plants is being explored for more effective and accessible drugs with less or no side effects. World Health Organization estimates that plant-based medicine is used to treat about 80% of the world's population. One of such plants is *Dennettia tripetala.*[13]

Dennettia tripetala (pepper fruit) is a native fruit belonging to the Annonacea family.[14] It is commonly cultivated in Nigeria's rain forests and some parts of West Africa. It is locally called Ako in Edo, Mmimi in Igbo, Ata Igbere in Yoruba and Nkarika by the Efiks of Calabar.[15] It is widely consumed in Nigeria because of its distinct peppery flavor. Additionally, it has been used for many years as a treatment for nausea, diabetes, toothaches, fever and cough.[16] The fruits have a very distinct smell, but because they are spicy, both the fruits and the seed can be eaten. This food is abundant in vitamins A, C and E as well as fatty acids, carbohydrates, proteins, calcium, potassium, magnesium and phosphorus. Fruit is extremely nutrient-dense. Phytochemicals found in the have been demonstrated to have antibacterial, insecticidal, analgesic and anti-inflammatory activities. Additionally, the plants chemotherapeutic, antihyperglycemic and antioxidant capabilities have been demonstrated.[17] Ikpi and Nku[18] investigated the *Dennettia tripetala* ethanol extract's toxicity and its effects on haematological indicators in healthy, normal rats. The neuropharmacological properties of *Dennettia tripetala's* essential oil derived from its fruits, leaves and seeds have recently been linked to a component that researchers have identified. This compound 1-nitro-2-phenyl ethane, exhibits hypnotic, anxiolytic and anticonvulsant effects in mice.[19] Literature abound on the medicinal use of *Dennettia tripetala* seeds which have been proven to have biochemical, anti-inflammatory and nutritional values on Wistar rats.[20] Further studies have shown that *Dennettia tripetala* possess many nutritive properties that can improve motor cognitive functions and anxiety.[17] *Dennettia tripetala* fruit when eaten causes a mild stimulatory effect which improves alertness and may have an effect on learning and memory.[15] However, there is paucity of data regarding this, the outcome of this study will enlighten the general public on the possible effect of *Dennettia tripetala* on learning and memory especially when combined with scopolamine.

This study was therefore conducted to investigate the effect of ethanol extract of *Dennettia tripetala* on Wistar rats.

2.0 MATERIALS

The following materials were used for this research work: wooden cages, 80% ethanol, *Dennettia tripetala* extract, Scopolamine Hydrobromide Injection (Omega Laboratories Ltd), cotton wool, ti ssue, methylated spirit, water (350liters), large container, tempers paint, platform, electronic weighing balance, hand gloves, syringes (1 and 2ml) sawdust, needles, specimen bottles, disinfectant, feeding plates, water bottles, vital growers feeds and marker.

Apparatus: Morris water maze, novel object recognition box, light-dark transition box and beam walking balance.

Preparation of Ethanol Extract of *Dennettia tripetala*

The pepper fruits of (*Dennettia tripetala*) were purchased from Akpabuyo Local Government Area of Cross River State. They were then identified by a botanist in the Department of Botany University of Calabar, Calabar.

Before extraction, the plant was properly washed, dried and separated from foreign materials such as topsoil, pebbles, rocks, weeds.

The maceration extraction method[21] was applied in all the processes of the extraction. *Dennettia tripetala* fruits were cut into smaller samples to increase contact between the plant materials and the liquid solvent. The coarse smaller plant materials were added into a stripped container and 80% ethanol was added as a solvent. The mixture was allowed undisturbed at room temperature for a period of 3 days with frequent agitation (this process was intended to soften and break down the plant cell walls to release the soluble phytochemicals). After 3 days, the mixture was then filtered and the extract collected into a clean heater. A rotary evaporator was used to remove the ethanol from the plant extract. The extract was placed on a water bath at 40°C temperature. The dry crude plant extract was then collected into a dry container and tightly sealed.

Experimental Animals

Following approval of the study (Approval #232PHY2523) Twenty (20) female Wistar rats weighing 100-150g used for this study were purchased from the Department of Physiology University of Calabar and housed in wooden cages with iron mesh (5 rats per cage) and kept under hygienic and favorable conditions (i.e ventilated cages at room temperature of 25°C and exposed to a normal 12/12 hours light/dark cycle) in the animal house of the Faculty of Basic Medical Science University of Calabar, Calabar for six

weeks. The first two weeks were for acclimatization and the next 4 weeks were for administration of *D. tripetala* extract and scopolamine. The rats were allowed unrestricted access to pelletized feed and clean water. Their beddings (sawdust) were changed daily. The rats were handled based on the 1985 guidelines of the National Institute of Health publication for laboratory animals.

Experimental Design

The rats were randomly assigned into 4 groups (n = 5)

Group 1: (control group) were given clean water and feed only.

Group 2: (Scopolamine group) was administered scopolamine (1mg/kg) intramuscularly.

Group 3: (Scopolamine + *D. tripetala* extract group) was administered scopolamine (1mg/1kg) intramuscularly and extract of *Dennettia tripetala* (200mg/kg) orally.

Group 4: (Extract group) was administered extract of *Dennettia tripetala (*200mg/kg) orally. All groups freely had access to rat feed and tap water.

At the end of the four week administration, the rats were taken to the Physiology Neurobehaviour Laboratory for experimentation.

Behavioural Assays and scores

Morris water maze and novel object recognition test for learning and memory.

Statistical Analysis

Statistical Package for Social Sciences (SPSS) (Version 20). Data obtained was analyzed using analysis of variance (ANOVA) followed by Turkey's post hoc test $p<0.05$.

3.0 RESULTS

Morris Water Maze Results

Swim Latencies During Acquisition Training in the Morris Water Maze Test

In fugure 1, Swim latencies on day 1 of the acquisition training in the control, scopolamine, scopolamine + 200mg/kg *D. tripetala* and 200mg/kg *D. tripetala* treated groups were 34.35 ± 3.50, 42.17 ± 3.45, 40.41 ± 0.47, 39.90 ± 4.47s respectively.

There was no significant difference in the swim latencies of the animals in the different experimental groups on day 1 of the training (Figure 1).

Swim latencies on day 2 of the acquisition training in the control, scopolamine, scopolamine + 200mg/kg *D. tripetala* and 200mg/kg *D. tripetala* treated groups were 20.47 ± 4.20, 39.82 ± 3.19, 30.38 ± 2.72, 24.91± 4.05s respectively.

There was a significant ($p<0.05$) increase in the swim latencies of the animals in the scopolamine treated group compared to the control. Also, there was a significant ($p<0.05$) decrease in the 200mg/kg *D. tripetala* treated group compared to the scopolamine treated group (Figure 1).

Swim latencies on day 3 of the acquisition training in the control, scopolamine, scopolamine + 200mg/kg *D. tripetala* and 200mg/kg *D. tripetala* treated groups were 24.89 ± 5.76, 20.42 ± 1.68, 17.86 ± 2.11, 18.71 ± 3.34s respectively.

There was no significant difference in the swim latencies of the animals in the different experimental groups on day 1 of the training (Figure 1).

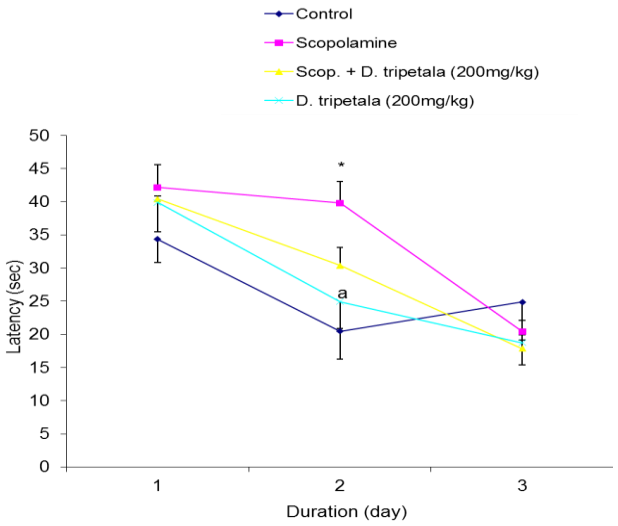

Figure 1: Swim latency during the acquisition training period of the Morris water maze test.

Values are expressed as mean +SEM, n = 5.
* = $p<0.05$ vs control
a = $p<0.05$ vs scopolamine

Swim Latencies during Reversal Training in the Morris Water Maze

In figure 2, Swim latencies on day 1 of the reversal training in the control, scopolamine, scopolamine + 200mg/kg *D. tripetala* and 200mg/kg *D. tripetala* treated groups were 33.95 ± 7.38, 24.45± 3.21, 33.46 ± 1.63, 26.17 ± 2.56 seconds respectively.

There was no significant difference in the swim latencies of the animals in the different experimental groups on day 1 of the reversal training (Figure 2).

Swim latencies on day 2 of the reversal training in the control, scopolamine, scopolamine + 200mg/kg *D. tripetala* and 200mg/kg *D. tripetala* treated groups were 21.67 ± 7.91, 12.83 ± 1.78, 22.82 ± 1.32, 8.75 ± 1.58 seconds respectively.

There was a significant ($p<0.05$) decrease in the swim latencies of the animals in the 200mg/kg *D. tripetala* treated group compared to the control. There was also a significant ($p<0.05$) decrease in the 200mg/kg *D. tripetala* treated group compared to the scopolamine + 200mg/kg *D. tripetala* group (Figure 2).

Swim latencies on day 3 of the reversal training in the control, scopolamine, scopolamine + 200mg/kg *D. tripetala* and 200mg/kg *D. tripetala* treated groups were 11.87 ± 2.32, 10.60 ± 0.84, 12.42 ± 0.77, 7.65 ± 0.97 seconds respectively.

There was a significant ($p<0.05$) decrease in the swim latencies of the animals in 200mg/kg *D. tripetala* group compared to control and scopolamine + 200mg/kg *D. tripetala* groups on day 3 of the reversal training. (Figure 2).

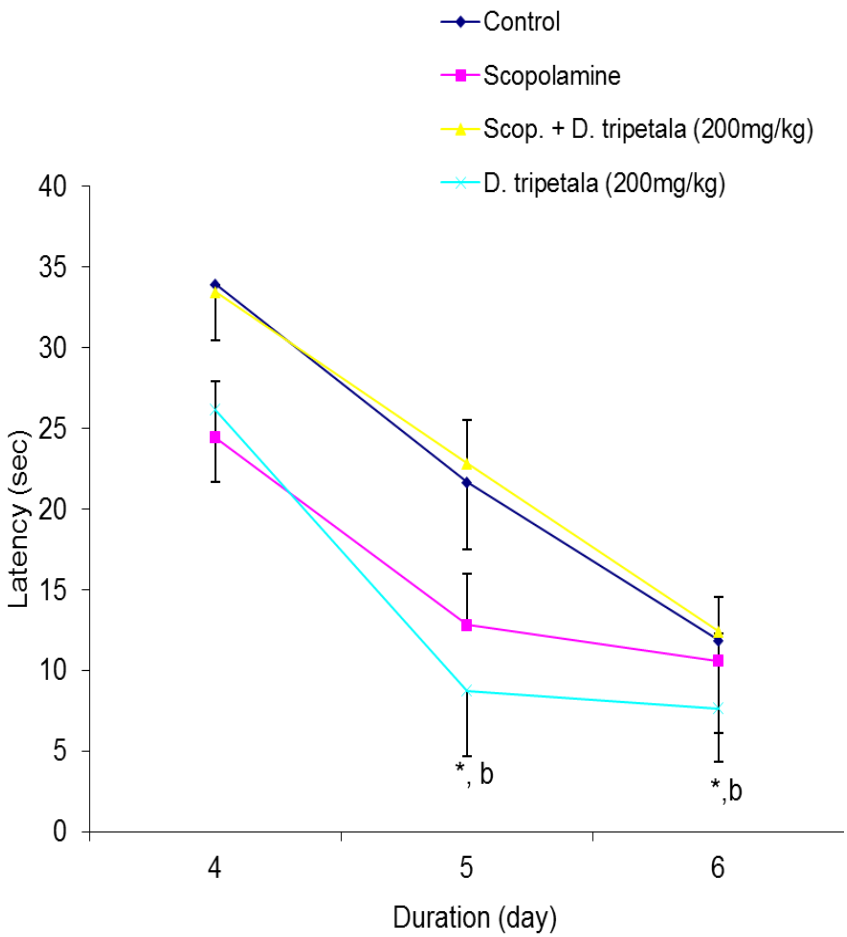

Figure 2: Swim latency during the reversal training period of the Morris water maze test.

Values are expressed as mean +SEM, n = 5.
* = $p<0.05$ vs control
b = $p<0.05$ vs scop. + Extract (LD)

Retention Quadrant Duration during the Probe Trial in the Morris Water Maze Test

In figure 3, the Mean and SEM values for the North East quadrant in the retention probe trial for control scopolamine, scopolamine + 200mg/kg *D. tripetala* and 200mg/kg *D. tripetala* treated groups were 10.83± 1.61, 8.80 ± 1.66, 7.07 ± 2.76, 12.16 ± 2.11 seconds respectively.

There was no significant difference in the swim latencies of the animals in the different experimental groups during the probe trial in the Northeast quadrant (Figure 3).

The mean and SEM values for the south west quadrant in the retention probe trial for control scopolamine, scopolamine + 200mg/kg *D. tripetala* and 200mg/kg *D. tripetala* treated groups were 26.51 ± 2.02, 32.46 ± 4.01, 27.11 ± 3.67, 21.60 ± 2.56 seconds respectively.

The results show a significant decrease in duration the south west quadrant in the 200mg/kg *D. tripetala* compared to the scopolamine treated group (Figure 3).

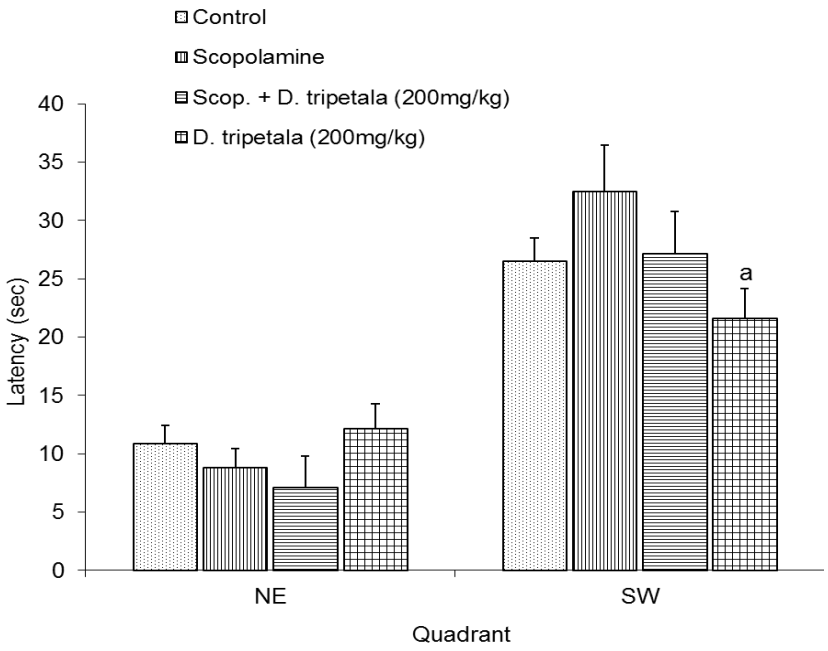

Figure 3: Swim latency during the probe trial period of the Morris water maze test.

Values are expressed as mean +SEM, n = 5.
a = p<0.05 vs scopolamine

Frequency of Annulus Acquisition and Annulus reversal Crossings in the Probe Trial of the Morris Water Maze Test

In figure 4, Frequency of annulus crossing during acquisition training for control scopolamine, scopolamine + 200mg/kg *D. tripetala* and 200mg/kg *D. tripetala* treated groups were 2.80 ± 0.37, 2.40 ± 0.51, 3.40 ± 0.24, 3.20 ± 0.66 seconds respectively**.**

The results showed no significant difference in the annulus crossing during the acquisition training in the experimental groups compared to control (Figure 4)

Frequency of annulus crossing during reversal training for control scopolamine, scopolamine + 200mg/kg *D. tripetala* and 200mg/kg *D. tripetala* treated groups were 5.00 ± 0.32, 4.00 ± 0.55, 5.00± 0.32, 3.80 ± 0.66 seconds respectively**.**

The results showed no significant difference in the annulus crossing during the reversal training in the experimental groups compared to control (Figure 4)

Swim latency during the visible platform test of the Morris water maze test

In figure 5, Swim latencies during the visible platform in the control, scopolamine, scopolamine + 200mg/kg *D. tripetala* and 200mg/kg *D. tripetala* treated groups were 13.44 ± 2.24, 8.06 ± 1.15, 6.28 ± 0.79, 6.76 ± 1.15 seconds respectively.

The results show a significant decrease in the swim latencies of scopolamine, scopolamine + 200mg/kg *D. tripetala* and 200mg/kg *D. tripetala* treated groups compared to control. (Figure 5)

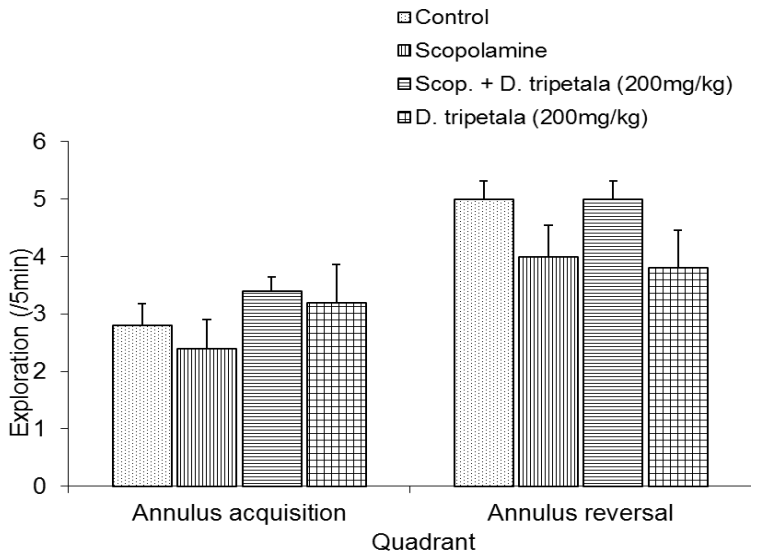

FIG. 4: Annulus crossing during the acquisition and reversal training period of the Morris water maze test.

Values are expressed as mean +SEM, n = 5.

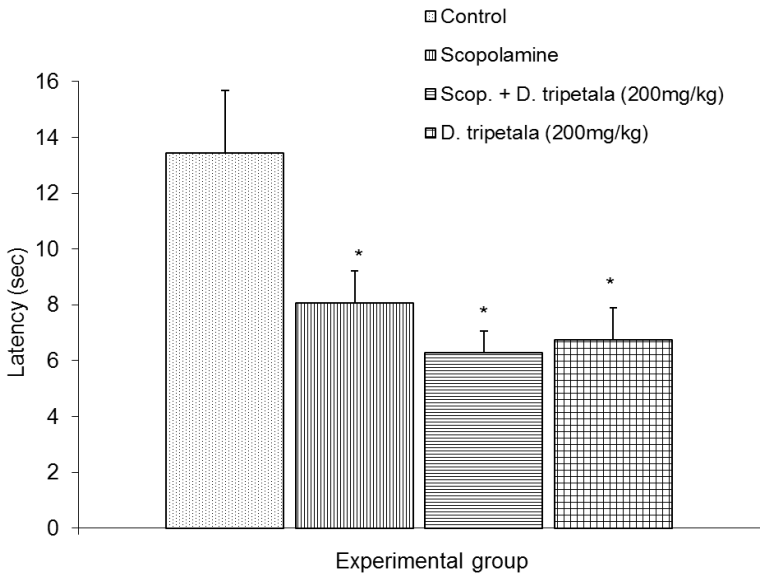

Figure 5: Swim latency during the visible platform test of the Morris water maze test.

Values are expressed as mean +SEM, n = 5.
* = p<0.05 vs control

Novel Object Recognition Task results

Habituation index for short term memory during the novel object recognition task in control and test groups

In figure 6, Mean and SEM values for habituation index for short term memory in control, scopolamine, scopolamine + 200mg/kg *D. tripetala* and 200mg/kg *D. tripetala* treated groups were 6.37 ± 1.82, 1.82 ± 1.11, 2.51 ± 3.71, -1.80 ± 1.88 respectively.

The results show a significant decrease in the habituation index in the 200mg/kg *D. tripetala* treated group compared to control.

Habituation index for long term memory during the novel object recognition task in control and test groups

In figure 7, Mean and SEM values for habituation index for long term memory in control, scopolamine, scopolamine + 200mg/kg *D. tripetala* and 200mg/kg *D. tripetala* treated groups were -2.66 ± 1.38, -2.45 ± 0.65, 6.28 ± 2.32, -0.72 ± 1.58 respectively.

The results show a significant increase in the habituation index in the scopolamine + 200mg/kg *D. tripetala* treated group compared to control and scopolamine treated groups. The results also show a significant decrease in the habituation index for long term memory in 200mg/kg *D. tripetala* treated group compared to the scopolamine + 200mg/kg *D. tripetala* treated group. (Figure 7)

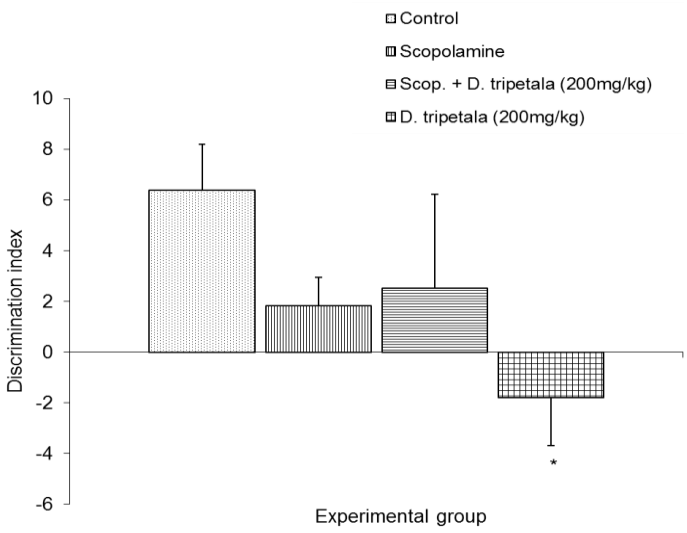

Figure 6: Habituation index for short term memory during the novel object recognition task in control and test groups.

Values are expressed as mean +SEM, n = 5.
* = p<0.05 vs control

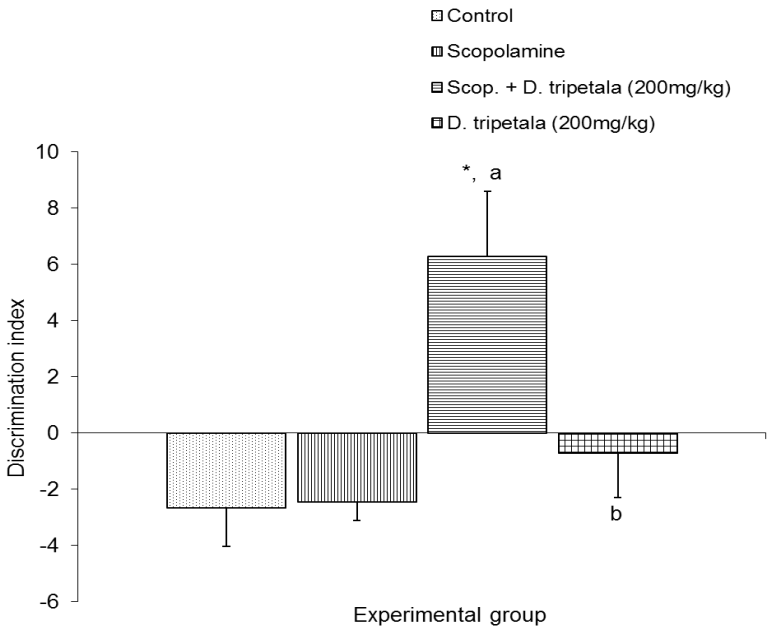

Figure 7: Habituation index for long term memory during the novel object recognition task in control and test groups.

Values are expressed as mean +SEM, n = 5.
* = p<0.05 vs control
a = p<0.05 vs scopolamine
b = p<0.05 vs scop. + Extract (LD)

Discrimination index for short term memory during the novel object recognition task in control and test groups

In figure 8, Mean and SEM values for habituation index for short term memory in control, scopolamine, scopolamine + 200mg/kg *D. tripetala* and 200mg/kg *D. tripetala* treated groups were 0.47 ± 0.17, 0.40 ± 0.24, 0.03 ± 0.09, 0.04 ± 0.26 respectively.

The results showed no significant difference between tests groups and control (Figure 8)

Discrimination index for long term memory during the novel object recognition task in control and test groups

In figure 9, Mean and SEM values for habituation index for long term memory in control, scopolamine, scopolamine + 200mg/kg *D. tripetala* and 200mg/kg *D. tripetala* treated groups were -0.45 ± 0.23, -0.65 ± 0.22, 0.80 ± 0.20, 0.08 ± 0.24 respectively.

The results showed a significant ($p<0.05$) increase in the discrimination index in scopolamine + 200mg/kg compared to control. Also, there is an increase in the scopolamine + 200mg/kg *D. tripetala* and 200mg/kg *D. tripetala* treated groups compared to the scopolamine treated group (Figure 9).

There is a significant ($p<0.05$) increase in the discrimination index in the 200mg/kg *D. tripetala* compared to the scopolamine + 200mg/kg *D. tripetala* treated group. (Figure 9)

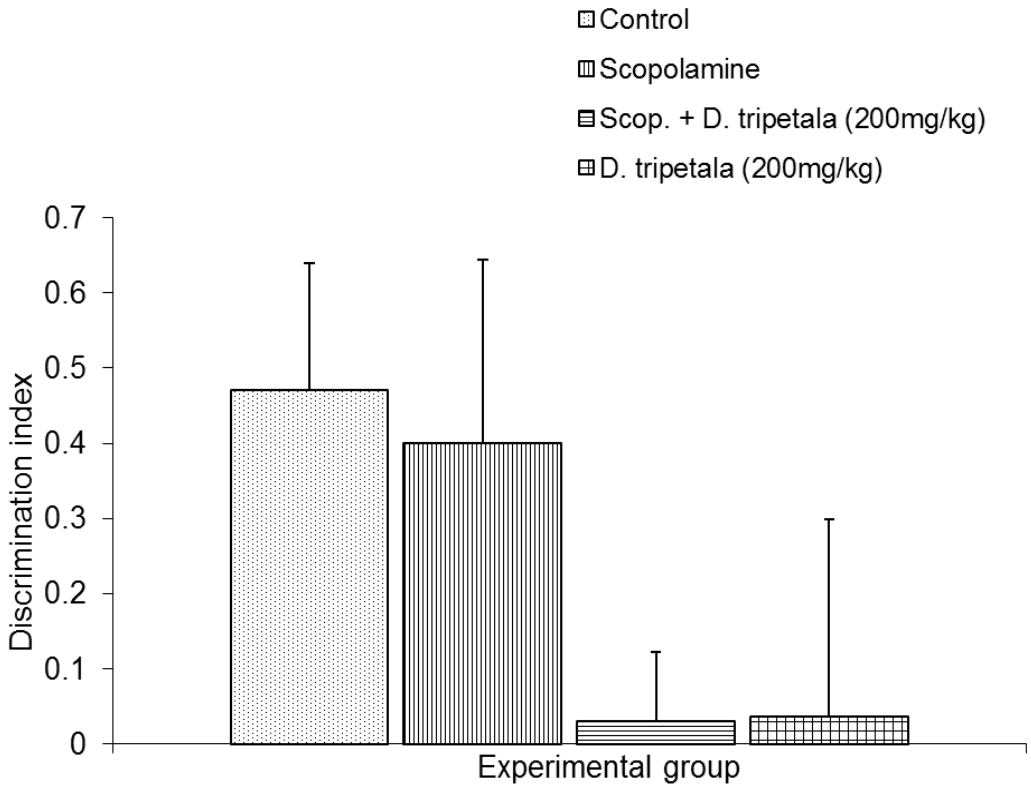

FIG. 8: Discrimination index for short term memory during the novel object recognition task in control and test groups.

Values are expressed as mean +SEM, n = 5.

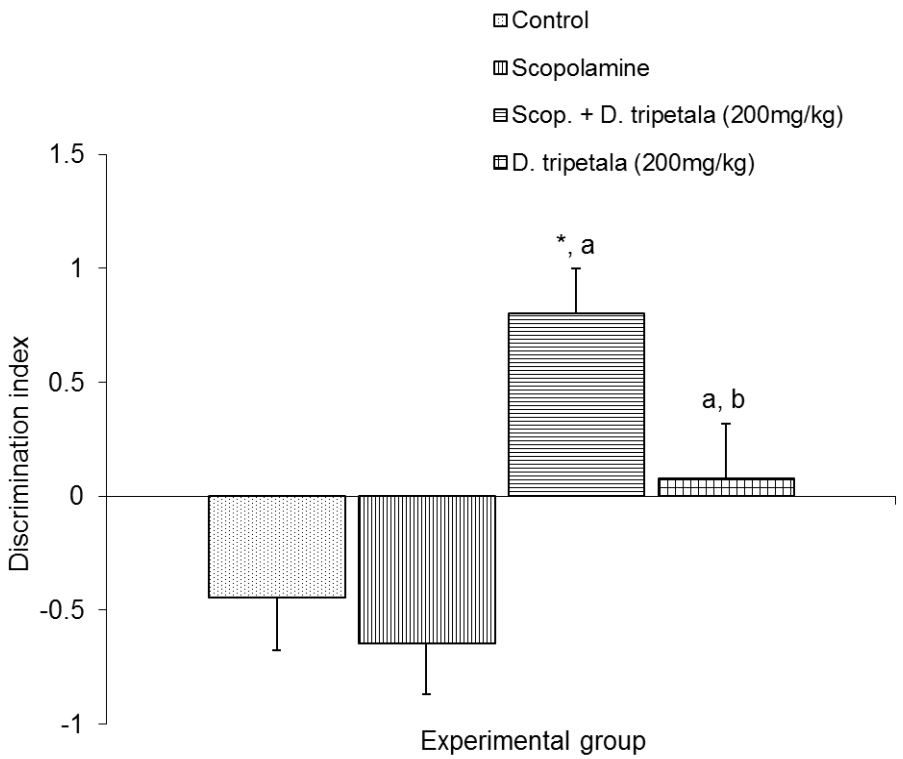

Figure 9: Discrimination index for long term memory during the novel object recognition task in control and test groups.

Values are expressed as mean +SEM, n = 5.
* = p<0.05 vs control
a = p<0.05 vs scopolamine
b = p<0.05 vs scop. + Extract (LD)

4.0 DISCUSSION

Results from the Morris Water Maze Test showed no significant difference in swim latency amongst the groups on the first day (acquisition training day one). Possible explanation could be that the rats were not acquainted or familiar with the positioning of the escape platform.

On day two (2), there was no significant difference in swim latency in scopolamine+ 200mg/kg *D.tripetala* compared to control, however, swim latency was significantly raised in scopolamine group compared to control and significantly reduced in the 200mg/kg *D.tripetala* group. The increase in scopolamine group could be due to its muscarinic acetylcholine receptor (mAChR) antagonistic properties which altered learning and memory in animals and humans as reported by Bubser, Byun, Wood and Jones,[22] The decrease in extract group is suggestive that the 200mg/kg *D.tripetala* improved learning. This improvement might have been exerted by the alkaloid present in the extract.

On day 3, there was no significant difference in swim latency amongst the groups.

On the fourth day (day 1 of reversal training), there was no significant difference, the escape platform was also moved from the NE (north-east) quadrant to SW (south-west).

On day 5 and 6, swim latency was significantly reduced in the extract group which may be attributed to the 'said' memory enhancing properties of *D.tripetala*.[23]

On the probe trial day (day 7) there was no significant difference in the NE (north-east) and SW (south-west) in all the groups. The extract group continued to reduce, promoting its memory enhancing action on the albino wistar rats.

No significant difference amongst the groups was recorded for the annulus acquisition and reversal crossings.

Day 8 (the visible platform test) there was significant increase in the control group when compared with scopolamine, scopolamine + extract and extract groups.

Results for the novel object recognition test showed that habituation index for the short and long term memory in the extract only group (200mg/kg *D.tripetala*) was significantly reduced compared to control. Habituation is a decrease in response to a stimulus after repeated exposure, it is a learned adaptation to the repeated presentation of a stimulus, not a reduction in sensory or motor activity.[24] In this experimental protocol, a lower habituation index shows that the animals spent less time investigating a familiar object which is expected for animals which have good cognitive memory of the familiar object.[25] This simply means that treatment with the 200mg/kg *D.tripetala* improved cognitive memory.

Discrimination index for the long term memory in the scopolamine +200mg/kg *D. tripetala* and 200mg/kg *D.tripetala* was significantly increased compared to control and scopolamine groups. A high discrimination index shows a higher preference for the novel object because, rodents have an innate preference for novelty over the familiar object. Hence, better retention and better memory.[26] The index of discrimination was higher for the extract group (*200mg/kg D. tripetala*) indicating an improved retention.

After intracerebral injections of scopolamine, behavioural processes such as short term memory and attention are found to be affected.[8] This could be a possible explanation for the decrease in scopolamine group in habituation and discrimination index for both long and short term memory.

5.0 CONCLUSION

The significant decrease in swim latencies of the 200mg/kg D.tripetala group in both acquisition and reversal training, probe trial and visible platform test in the Morris water maze task and decreased habituation index in both short term and long term memory as well as increase in the discrimination index of the novel object recognition task showed that visuo-spatial learning, memory retention and preference for the novel object occurred.

D. tripetala treatment at a dose of 200mg/kg body weight alone or in combination with scopolamine was able to enhance memory and learning in scopolamine treated rats.

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