

The Effect of Chronic Administration of Clove Extract on Anxiety- & Depression-like Behavioral patterns and Memory Deficit in Rat

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Abstract - Clove is a dried lower bud belonging to the myrtaceas family that is indigenous to the Maluku islands in Indonesia but has recently been farmed in different places worldwide. A study on the effect of chronic administration of clove on anxiety-like, depression-like and memory deficit was carried out using Wister albino rats weighing 180-200g obtained from animal house carried out in the University of Port Harcourt. A total of twenty-five albino rats were weighed and randomly divided into five groups of five rats per group. Group one which is the control group was given feed and water group, group two was given feed, water and 1ml of clove, group three was given feed, water and 1.5ml of clove, group four was given feed, water and 2ml of clove while group five which is the drug group was given 0.5ml/10g of hyoscine. The animals were then opened to different maze tasks such as navigation, passive avoidance, rotarod, handgrip, inverted screen and Barnes test for up to 21 days in order to determine the effect of clove on their memory, anxiety-like behaviour and depression-like behaviour. The result gotten from navigational task showed an expression of depression behaviour coupled with deficit in motor performance. The result gotten from barnes maze task suggested that clove action on memory and motor activities may not be potent enough even with time and dose. In using inverted screen it was observed in our study that there were motor deficits during these trials. Memory and motor deficits were investigated using passive avoidance task and it was observed that cognition and motor activities were enhanced except in the control group as expected. There was also a decline in these activities in the drug group too as expected. The degree of awareness and memory recall was positively up-regulated by the clove administration but worsened by the drug. These observations were consistent in all the trials irrespective of the dose administered. The results from the study suggested that with appropriate doses of the clove extract, appreciable level of enhancement in memory, motor and cognito-motor activities could be achieved and sustained.

Keywords; Clove, Hyoscine, anxiety-like behavior, depression-like behavior, memory.

Introduction

Syzygium (S.) aromaticum, also known as clove, is a dried flower bud belonging to the Myrtaceae family that is indigenous to the Maluku islands in Indonesia but has recently been farmed in different places worldwide (1).

The clove tree is composed of leaves and buds (the commercial part of the tree) and the flowering bud production begins four years after plantation. Afterward, they are collected either by hand or using a natural phytohormone in the pre-flowering stage (1). Interestingly, they are commercially used for many medicinal purposes and in the perfume industry, and clove is considered one of the spices that can be potentially used as preservatives in many foods, especially in meat processing, to replace chemical preservatives due to their antioxidant and antimicrobial properties (1,2). Several reports have documented the antibacterial, antiviral, anticarcinogenic, and antifungal activities of some aromatic herbs including cinnamon, oregano, clove, thyme, and mint. However, clove has gained much attention among other spices due to its potent antimicrobial and antioxidant activities (3). The effective role of clove in the inhibition of different degenerative diseases is attributed to the presence of various chemical constituents in high concentrations with antioxidant activity [4,5].

In a book published in 1878 (*Physiologie des passions*), Charles Letourneau, who was contemporary with the French neuroanatomist Paul Broca, defined emotions as “passions of a short duration” and described a number of physiological signs and behavioral responses associated with strong emotions.

Emotions are “intimately linked with organic life,”⁽⁶⁾ he said, and either result in an “abnormal excitation of the nervous network,” which induces changes in heart rate and secretions, or interrupt “the normal relationship between the peripheral nervous system and the brain.” Cerebral activity is focused on the source of the emotion; voluntary muscles may become paralyzed and sensory perceptions may be altered, including the feeling of physical pain leading to depression in most cases in living organisms.

Materials & Methods

Experimental Animal

A total of twenty-five male wistar albino rats weighing 180-200g were obtained from animal house. The rats were kept in clean disinfected wooden cages with saw dust as beddings in the animal house, with 12hours light/dark cycle and 50-60% humidity at a temperature of about 30°C and were allowed to acclimatize to the new environment for two weeks, with free access to clean water and animal feed. The rats were weighed using an analytical weighing balance at commencement of the experiment.

3.2. Experimental Design

A total of twenty-five albino wistar rats were weighed and randomly divided into four groups of five rats per group.

Table 1 Experimental Design and Grouping of the Rats

Groups	Number of animals	Treatment:
Group I (Normal control)	5	Feed + Water ad libitum
Group II	5	Feed + Water ad libitum + (1 ml clove extract)
Group III	5	Feed + Water ad libitum + (1.5ml clove extract)
Group IV	5	Feed + Water ad libitum + (2ml clove extract)
Group V	5	Drug hyoscine-treated (0.5ml/10mg)

The animals here were subjected clove extract for the period of three weeks with different doses being administered to each group, which are 1ml/100g b.w., 1.5ml/100g b.w., and 2. 0ml/100g b.w. The animals were then opened to different maze tasks for up to 21 days in order to ascertain the impact of clove (*Syzygium aromaticum*) on their memory and an anxiety like behaviour and depression like behaviour.

Chemicals: hyoscine was purchased from Sigma Chemicals (St Louis, MO, USA).

Barnes maze - it is a visual- spatial learning and memory task designed for rats. It consists of an elevated circular surface with holes around the edge.

Principles – it is a dry-land based rat's behavioral model for assessing spatial learning and memory. The rats use extra-maze visual cues to locate an escape hole that allows them to escape from open space and bright light into a dark box beneath the maze. The time it takes to locate the escape hole into the dark box beneath the maze should be recorded.

Passive –avoidance test – it is a useful task for evaluating the effects of novel chemical entities on learning and memory as well as studying the mechanism involved in psychomotor.

Principle – The testing apparatus is a trough-shaped alloy divided into two distinct compartments with an opening door. The white, brightly lit compartment is free of aversive stimulation whereas the black, dark compartment is equipped with shock capability.

Navigational task – it is widely used in behavioral neuroscience to study spatial learning and memory. It is used to measure the effect of neurocognitive disorder on spatial learning and possible neural treatments, to test the effects of lesions to the brain in area concerned with memory.

- **Principle** – it is basically used to test mnemonic function in rats. These tasks are designed in such a way that the rats have to use either spatial or cue information to solve them. The animals find their way through the environment without getting lost, which require memory for locations and routes.

Inverted Screen Test

- Inverted screen test is similar to handgrip test but, in this case, the animals were placed with four limb grip. A wire gauze was hung and the animals hung with their four limbs under the wire gauze. Stopwatch was used to determine how long it took the animals to hang with their four limbs before they fell on the soft pad. The test were repeated for about five times.

DATA ANALYSIS

The quantitative data were represented in the charts and graphs, while qualitative data from the behavioral study was represented in tables. The variation and the statistical significance of the differences between the groups were determined by Analysis of Variance (ANOVA) and Turkey post Hoc test. The Analysis was performed using Statistical package for Social sciences (SPSS) software version 20.

RESULTS

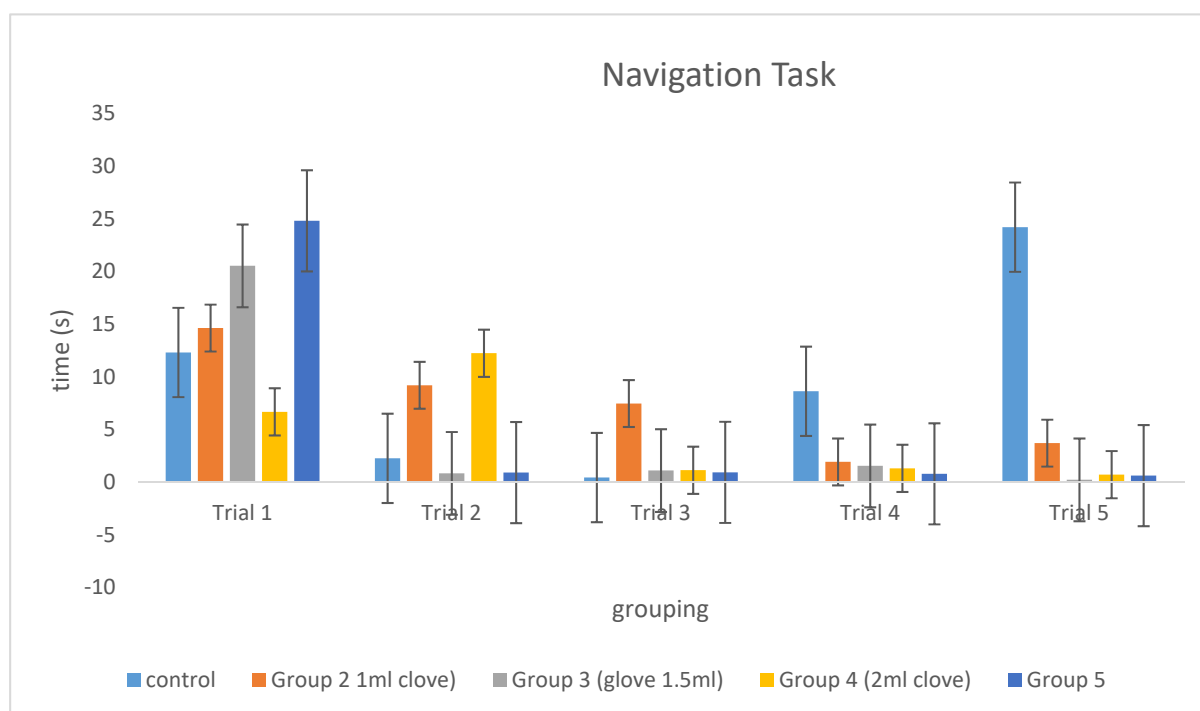


Fig 1. Comparative study of Navigation test across the groups.

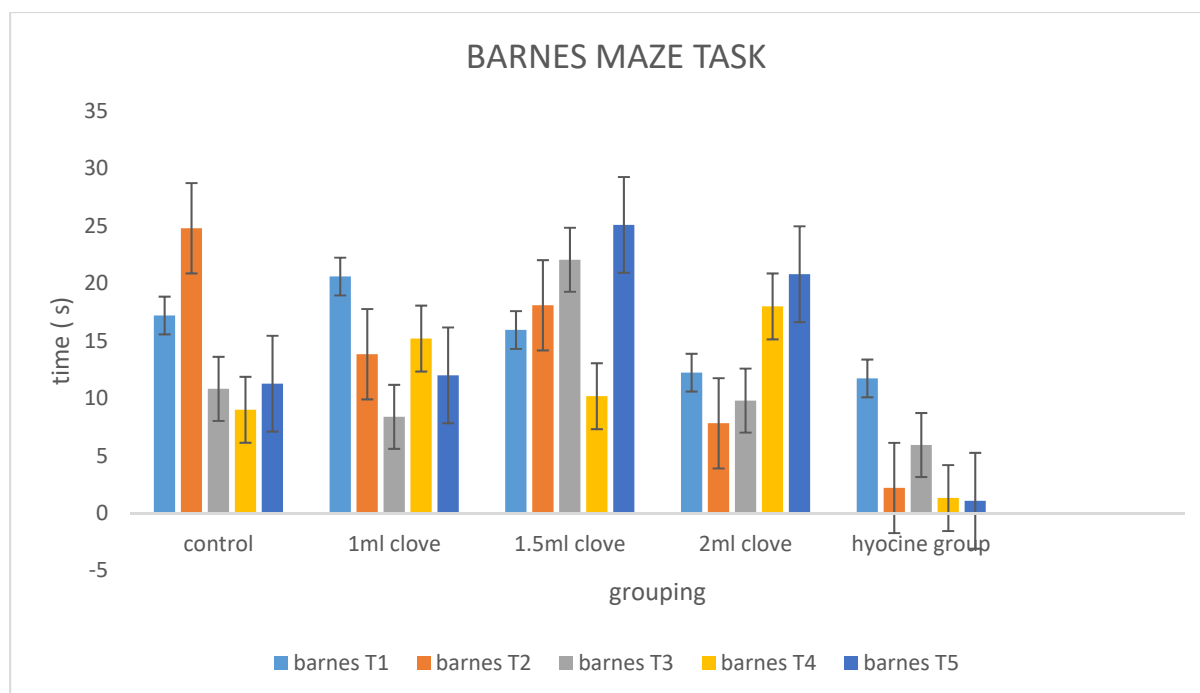


Fig 2. Comparative study of Barnes test across the groups

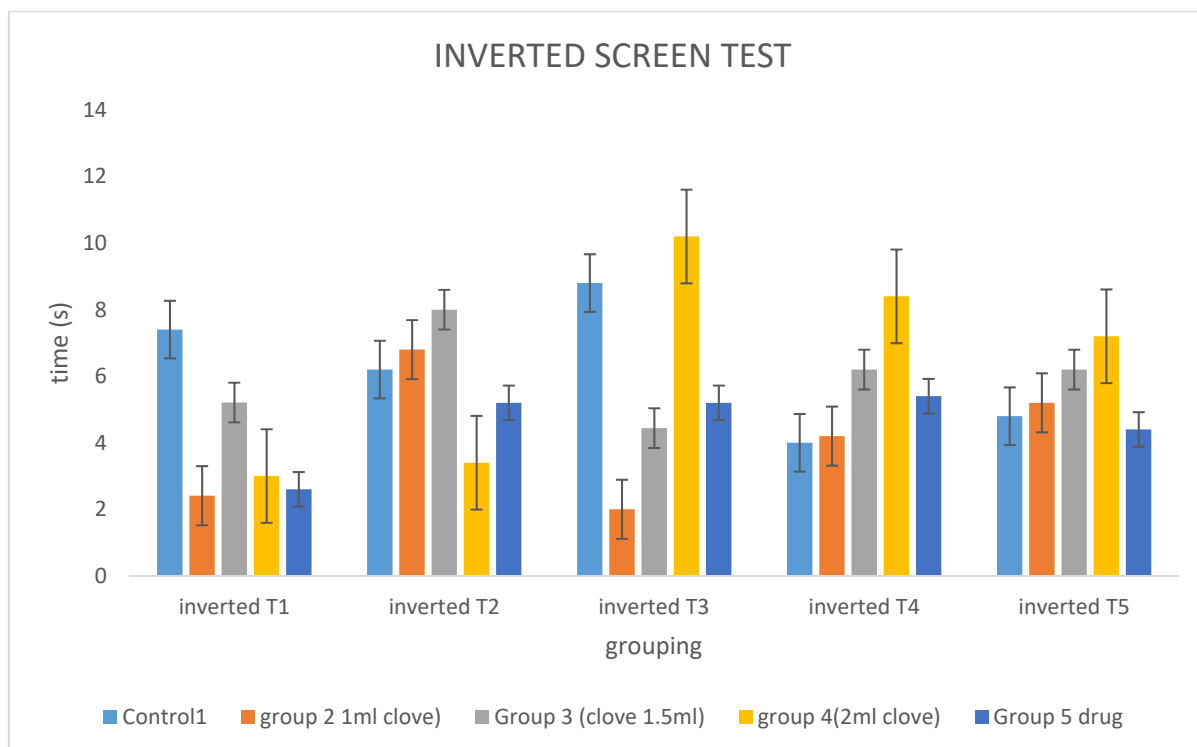


Fig 3. Comparative study of inverted screen test across the groups after week 3

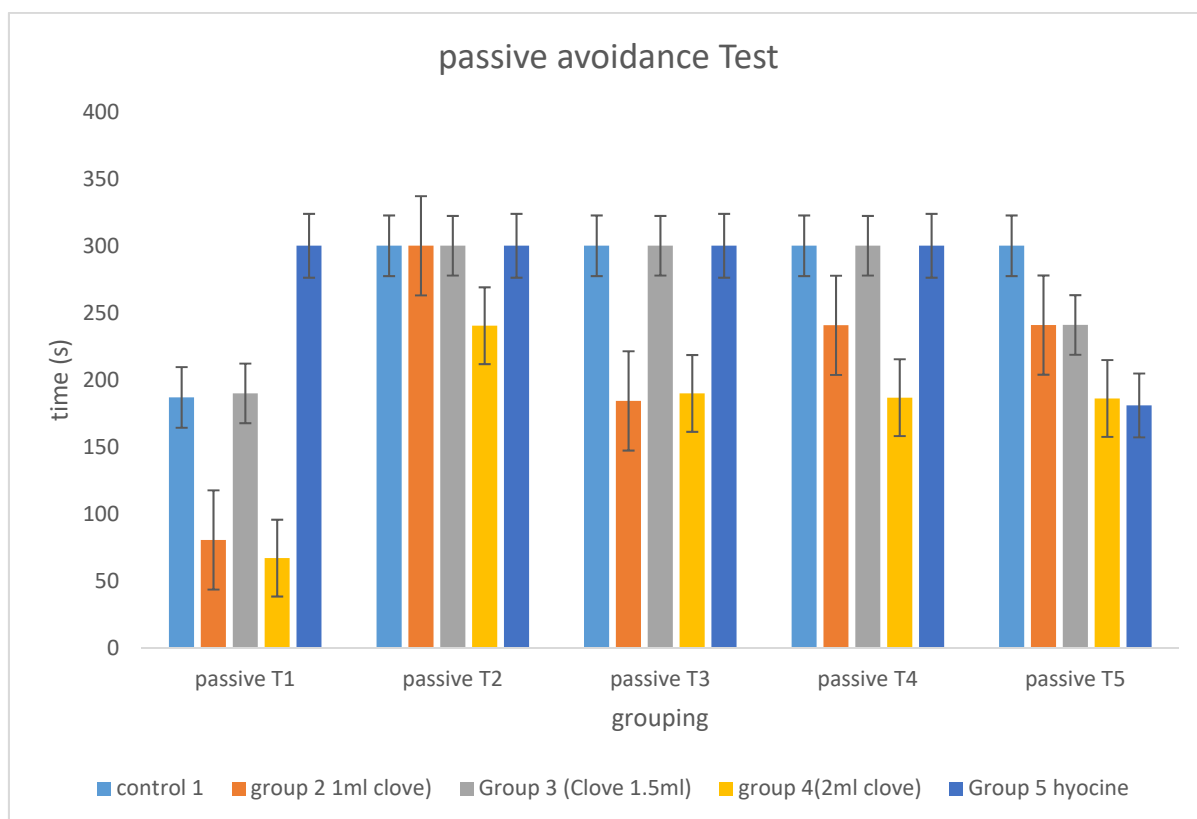


Fig 4. Comparative study of passive avoidance test across the groups

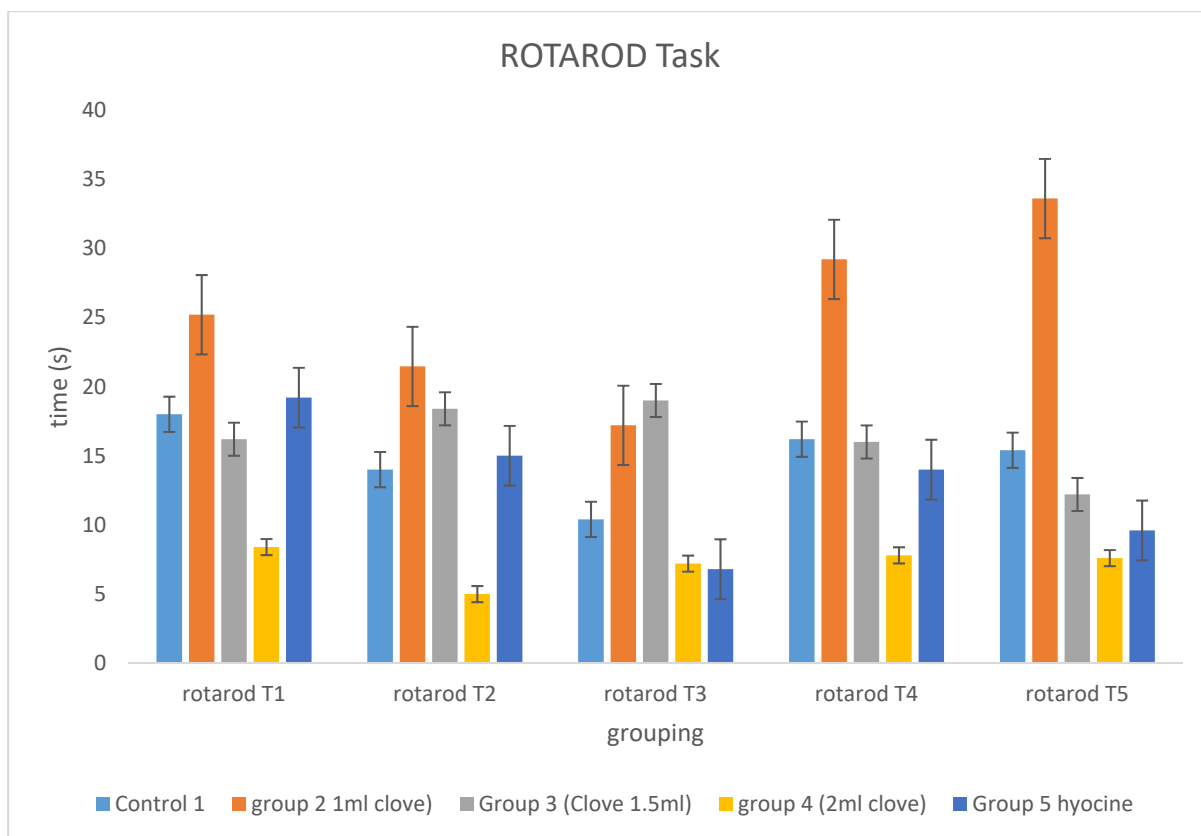


Fig 5. Comparative study of passive avoidance test across the groups

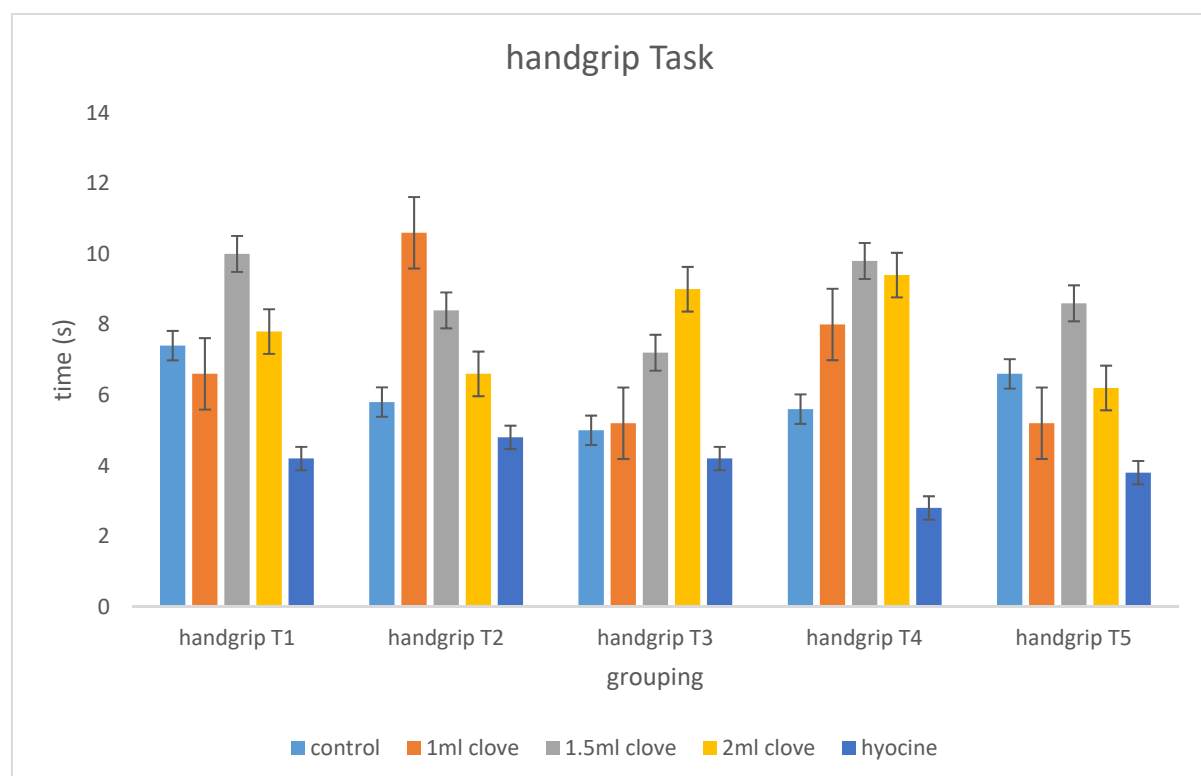


Fig 6. Comparative study of hand grip test across the groups

RESULTS

The investigations from the clove potency on anxiety-like and depression-like behaviours and motor deficits using navigation task model revealed that in week 1, the pattern of locomotion and the rapidity of task performance were best seen in group 3 treated with 1.5ml of clove. The summary of the observations recorded under this section was in accordance with that obtained from previous studies. Navigation is so vital to survival that it is conserved in phyla as basic as insects, including ants (7) and bees (8,9), avians, fish, bats (10), and all terrestrial mammals (11). In birds, bats, fish, and marine mammals, navigation has evolved to the extent of these animals being able to navigate in three dimensions rather than two.

Barnes maze task results from week 1 demonstrated that memory deficit was mostly expressed in the drug group while groups treated with various doses of clove lower level of motor decline in action. The results from the clove groups were comparable to the control group results. The results obtained were similar to that reportedly by Miykawa & co. (12). And (13,14,15). Inverted Screen task results presented a pattern of results that showed that anxiety expression and behavior were observed significantly less in groups 3 and 4 more and that accounted for better steadiness of the paw of the rats in task performance. The more the expression of anxiety-like behavior the more the poverty of steadiness. Various studies reported that certain rotenone administration in rats caused the selective degeneration of dopaminergic neurons and resulting in movement disorders (16,17,18). Therefore, in our study the motor deficits may be attributed to the observed DA deficiency produced by rotenone (19). Using Passive Avoidance Box task, memory and motor deficits were investigated with the task. Week 1 set of observations showed that cognition and motor activities were enhanced across board except in the control group as expected. There was also a decline in these activities in the drug group too as expected. The degree of awareness and memory recall was positively up-regulated by the clove administration but worsened by the drug. These observations were consistent in all the trials in this week irrespective of the dose administered. Previous studies reported that post-training morphine induced impairment on the retrieval process in a passive avoidance paradigm (20). Rotarod Task results were obtained and presented in table 4.5. From the results, the degree of stability and motor coordination in static state was observed to be significantly enhanced in 2ml clove group and the drug group. The two results were comparable. The depression-like behavior exhibited by these group only occurred in the earlier sets of trials but not seen towards the end in week 1. The beam test can detect motor deficits due to age, central nervous system lesions, and genetic and pharmacological manipulations in young and older rodents as reported previously by Carter et al, (21); Wallace et al, (22); Brooks & Dunnett, (23).

The neurogenic effects of clove extract were investigated in the study especially as it affects neurobehaviour. Graded doses of the extract were used to interfere with the neuraxis of the diencephalon. The study was designed to probe disruptions to the motor pathways and anxiety-like and depression-like behavioral patterns and centers. The study showed that the clove extract was efficacious in the maintenance of steady pattern and smooth execution of motor activities around the final common paths as indicated in the study. The extrapolations showed up-regulation in motor performance, significant abolition of resting tremor and end action coordination of the muscles. The improvement in adaptive locomotive activity using Navigation maze convincingly prove the speculated efficacy of the clove extract in smooth motor coordination.

It was further reported in the study that the clove extract also significantly ameliorated interference in memory circuits by internal stressors and there was apt memory recall and spatial tenacity at a dose-dependent and time-dependent fashion. These observations were consistent throughout the trial periods in the weeks under study. Patterns of results from the use of rotarod task showed a huge consistency in apt motor coordination and balance at a dose-dependent manner while results from inverted screen and handgrip task showed enhanced grasping and significant increase in endurance threshold and muscular tensility. Memory traces could be seen to be significantly excited from the observations from the Barnes maze task which could be attributable to the clove extract especially at higher doses in the study. The overall observations revealed that clove extract could possess strong anti-stress factors that tend to down regulate stressors at cellular levels and that neurogenic properties of the extract could significantly influence though positively the psychic centers in the diencephalon and other brain centers. Moreover, the study showed that prolongation of administration of the extract could offer different scenarios under the same conditions in the animals.

Conclusion

The clove extract could possess important neurogenic potential targeted significantly towards the psychic centers of the brain rather than the cortical centers in the frontal lobe. The results from the study suggested that with appropriate doses of the clove extract, appreciable level of enhancement in memory, motor, and cognito-motor activities could be achieved and sustained. The study further concluded that rapidity in motor task performance showed that clove extract could equally influence the motor segments as much as the motor pathways positively. There is no doubt the clove extract in the study has demonstrated beyond speculation its potency in memory up-regulation and motor activity smoothing coupled with abolition of resting tremor.

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