

Effect of Acute Administration of Ethanol Extract of Wild Fig (*Ficus thonningii*) on Spatial Memory and Learning in CD-1 Mice

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ABSTRACT

Background and Aim: Since there is an increasing demand by people for means of enhancing the neuro-cognitive functions of the brain and the side effects of many neuro-cognitive drugs which have left many persons with irreversible neurological damage. This situation has lead to our investigation of the neuro-cognitive properties (learning and memory) of the plant, *Ficus thonningii* (wild fig). **Methods:** 30 Adult Swiss mice weighing between 16 -21g, were divided into 3 groups, 10 mice per group. Before the neurobehavioral parameters were assessed, the LD₅₀; acute toxicological screenings of the plant were determined. Group A being the control, received rat feed with normal saline, group B being the low dose were administered with *Ficus thonningii* extract at a dose of 10mg/kg and group C being the high dose were administered with *Ficus thonningii* extract at a dose of 20mg/kg, this administration lasted for 14 days. All animals were allowed clean drinking water. **Results:** The data's were analyzed and the results showed that on days 1, 2 and 3 of acquisition training mice from low dose and high dose learned equally when compared to the control. In the reversal training, memory was improved in the low dose and high dose treated mice when compared to control at ($p<0.05$, $p<0.01$ and $p<0.001$). During the probe trial, the swim duration in the South-East quadrant was significantly higher for high dose and low dose compared to control ($p<0.01$ and at $p<0.001$). However, during the visible platform task, the swim latencies for the low dose and high dose group were also significantly lower compared to control ($p<0.001$). **Conclusion:** The results suggest that consumption of *Ficus thonningii* extracts enhances learning and memory in mice, thus *Ficus thonningii* containing diet may be beneficial in the improvement of learning and memory.

Key words: Wild fig, Morris water maze, Memory, Mice.

INTRODUCTION

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A lot of synthetic drugs have been produced by man and tested over the years; these drugs have been made due to the increase in mortality and diseases. Some have been produced to enhance certain abilities of the body system. Most of these drugs due to their synthetic nature (although they are produced from natural extracts) are not as effective as unsynthesized herbal extracts. This has lead man into the discovery of various herbal extracts, an example being the *Ficus thonningii* plant. Local names include: Africaans (gewonewurgvy), Arabic (jammeiz al abiad), English (strangler fig, bark-cloth fig and common wild fig), French (india-laurel fig), Fulani (bikeshi), Hausa (chediya), Yoruba (odan) etc.^[1-4] *Ficus thonningii* is an evergreen tree, 6-21m high; the leaves are simple, glossy, dark green, thin and papery.^[5] The species is widely distributed in upland forest, open grass land, riverine and rocky areas and sometimes in savannah. It occurs naturally from the democratic republic of Congo and Tanzania in the North to Eastern Cape of South Africa. The trees are relatively drought resistant. Products such as, jam, fibre, timber, latex and medicinal extracts are gotten from the *thonningii* plant.^[1] *Ficus thonningii* is extensively used by ethnomedical practitioners for treating various ailments.^[6-10] The pharmacodynamic basis supporting the use of *F. thonningii* extracts in ethnomedicine. Ethnomedicinal systems has been established and pharmacological studies have demonstrated the anti-inflammatory, analgesic, antimicrobial, antihelmintic, antioxidant, cardioprotective, hypotensive and hypoglycaemic effects of the plant extracts.^[11-14] The remarkable therapeutic effects exhibited by *F. thonningii* are as a result of the presence of an array of phytochemicals which include flavonoids, alkaloids, tannins, stilbenes, terpenoids and other active proteins.^[15-16] However, human memory is not a unitary process. Research suggests that, at the psychological level, various types of memory are at work in human beings. It is likely that these various systems bring different parts of the brain into play. Depending on the criterion used, with duration as the criterion, at least three different types of memory can be distinguished: sensory memory, short-term memory and long-term memory. It is therefore conceivable that the plant, *Ficus thonningii* may have an effect on neurobehavioral parameters, such as in learning and memory.

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MATERIALS AND METHODS

Animals

30 adult Swiss white mice weighing between 16-21g were bought and used for this research work, from the animal house of the Department of Physiology, Abia state university, Uturu. These animals were kept in the animal house of the Department of Physiology, Abia State University Uturu and were housed in groups of 3 (control, low dose and high dose) in plastic cages, maintained under standard dark-light cycle. Food and water were available ad libitum. All rules applying to animal safety and care were observed.

Experimental design

Identification of animals was simply done by attaching identification cards on the cages. The mice were grouped into 3 groups consisting of 10 mice per group (control, low dose and high dose). In all, 30 mice were used for the experiment which lasted for 32 days in total, acclimatization lasted for 14 days, administration of extracts lasted another 14 days, the experiment proper lasted for 8 days. Animals in group A (Control group) received rat fed with normal saline, group B (low dose group) animals received a solution of *F. thonningii* at a dose of 10 mg/kg and group C, (High dose group) received extract of *F. thonningii* at 20 mg/kg. Extracts were administered via an oral cannula daily for a period of 14 days. However, the lethal dose (LD_{50}) of the *F. thonningii* was determined prior to administration which was about (5115mg/kg) using the method proposed by.^[17]

Apparatus and experimental protocols

Morris water maze

The Morris water maze is widely used to study spatial memory and learning. Animals are placed in a pool of water that is colored opaque with powdered non-fat milk or non-toxic tempera paint, where they must swim to a hidden escape platform. Because they are in opaque water, the animals cannot see the platform and cannot rely on scent to find the escape route. Instead, they must rely on external/extra-maze cues. As the animals become more familiar with the task, they can find the platform more quickly. Developed by^[18] this paradigm has become one of the "gold standards" of behavioral neuroscience.

Experimental procedure using the Morris water maze

Apparatus

A Morris Water Maze modified for mice was used.^[19] The water maze was constructed out of a circular polypropylene pool that measured 110 cm in diameter and 20 cm in depth. The pool was filled to a depth of 14 cm with room-temperature tap water. The water was made opaque with the addition of liquid milk to ensure camouflage of the white escape platform.

The pool was filled with water until it had reached a height of about 5 cm, until the platform is submerged by 1 cm of white water. The water was left to sit overnight to achieve room temperature (22 °C). The pool was divided into four quadrants: Northwest, Northeast, Southwest and Southeast. Boundaries of these quadrants were marked on the edges of the pool with masking tape and labeled: North, South, East and West. A wooden board was built as a box and used as the escape platform; a large stone was tied to the bottom part of the wooden box to weigh it down in the pool. There was furniture in the room that provided visual cues.

Procedure

Testing in the Morris Water Maze lasted eight days. The first three days were acquisition training with an invisible platform. Days 4 – 6 were reversal training, again with an invisible platform. On the seventh day, a

probe trial was conducted with no escape platform. On day eight, 4 trials were conducted using the visible platform.

Running Mice

During the test period, each mouse was placed in a clean empty cage (no bedding). Paper towel was torn and placed in the bottom of the cage to allow the mice to dry more quickly. This paper towel was replaced when it became completely wet. Mice were then run in squads of 5-6 with 10 minutes between each trial for each mouse.

Acquisition (for days 1, 2, 3)

During acquisition training, the water was adjusted appropriately such that the platform was covered by 1 cm of water (invisible platform). The platform was placed in the center of the Northwest quadrant. Each animal received 4 trials of 60 secs (max) per day. The starting positions of the animals were predetermined, which prevented any sequence of 2 trials to be repeated by the same animal during any other day.

Possible start positions were at the boundaries of the quadrants (e.g. West, North, East or South). Each mouse was removed from its holding cage using a small, clean 500 ml plastic container to minimize handling stress. The animal was then placed into the water at the appropriate start position, facing the center of the pool. The mouse was then permitted to explore the pool and to search for the hidden escape platform for 60 secs. When the animal located the platform, the timer was stopped and the animal was removed using the plastic container and placed in the holding cage. If the animal did not find the platform during the allotted time, the animal was guided onto the platform using the plastic container. Once on the platform, the mice were permitted to visually explore their surroundings for 20 secs at which point, the mouse was picked up in the plastic container and returned to the appropriate holding cage.

The next mouse was then placed in the pool and the same procedure was followed. Each animal completed 4 trials per day over 3 days, for 12 trials of acquisition training.

Reversal (for days 4, 5, 6)

Reversal training began on day 4. The invisible platform was moved to the opposite quadrant and mice were again assigned to appropriate start positions. The same procedures as in acquisition training were again carried out during reversal training. Each of the animals completed 4 trials per day for 3 days for a total of 12 trials of reversal training.

Probe (No platform, Day 7)

A probe trial was conducted on the day 7. Currently, there was no escape platform in the maze at all. Each animal completed one trial of 60 secs. Each mouse was placed in the maze from one of the four possible start positions and allowed to explore the pool.

Visible Platform (Day 8)

The visible platform task was conducted on day 8. The visible platform was placed in the Southwest quadrant of the pool. The same procedures as in acquisition and reversal training were carried out and mice completed 4 trials.

What is measured?

During acquisition and reversal training the following behaviors were measured:

- (1) Swim latency (time to find and mount the escape platform),
- (2) swim distance,
- (3) Average velocity and
- (4) Duration and frequency of thigmotaxic behavior
- (5) Swim path error
- (6) Proximity to platform

During the probe trial, the following measures were recorded:

1. Duration of time spent in each quadrant (Northeast, Southeast, Northwest and Southwest).
2. The number of times the mouse crossed the location of the platform during reversal training (annulus reversal crossing).
3. The number of times the mouse crossed the location of the platform during acquisition training (annulus acquisition crossing).

Statistical Analysis of Data

Data Obtained from the experiments were statistically analyzed using Microsoft excel, with factorial ANOVA/T-test in the statistics programme start view version for windows or Mac. Newman-keuls design. Post-hoc comparison was also done using the students' 't' test. Data were expressed as Mean± SEM and a "P" value less than 0.05 and 0.001 were considered as significant.

RESULTS

MORRIS WATER MAZE

Swim latencies during acquisition training in the Morris water maze

Figure 1 compares the swim latencies during the acquisition training in the Morris water maze task, between mice administered low and high dose of *F. thonningii* and control diet. The values for the swim latencies for the three days of acquisition training are show below. The swim latencies for the low and high dose groups were not significantly different compared to the control group during the three days of acquisition training.

	Control	Low dose	High dose
Day 1:	1:44.37±24.99	28.37±18.98	32.75± 20.71
DAY2:	44.75 ±24.59	28.00± 19.47	41.12 ±24.73
Day 3 :	36.62± 27.98	32.87 ±24.02	25.87± 27.61

Swim latencies during reversal training in the Morris water maze.

The reversal training in the Morris water maze task lasted for three days (days 4-6). The latencies which is the time spent by the mice to locate the hidden platform are shown below. Figure 2 shows the swim latencies for the three groups of mice been compared. The latencies for the high dose group was significantly lower ($P<0.01$) compared to control (day 4) while, the swim latencies for the low and high dose groups were significantly lower ($p<0.01$) compared to control at day 5. However, the swim latencies were also significantly lower for the low and high dose group of mice at day 6 compare to control at $p<0.01$ and at $p<0.001$. (Figure 2)

	Control	Low dose	High dose
Day4:	40.03±7.71	26.85±8.28	16.79± 1.58
Day5:	51.45 ±7.82	21.21± 13.24	17.49 ±2.75
Day6:	28.14± 6.97	13.81 ±1.36	11.02± 1.68

Quadrant duration during the probe trial task in the Morris water maze.

Figure 3 compares the quadrant duration during the probe trial in the Morris water maze between the three experimental groups of mice. During the trial, the three groups had more preference for the SE quadrant (that bears the platform during the reversal training). However, the low and high dose groups paid more preference to the South East (SE) quadrant at $p<0.01$ and at $p<0.001$ compared to control.

	Control	Low dose	High dose
NE:	13.41±6.16	16.56±7.40	18.49± 7.09
NW:	11.09 ±6.46	9.31± 4.07	12.67 ±3.24
SE:	16.75± 6.33	31.45 ±11.04	36.73± 10.05
SW:	13.48± 7.13	14.75 ±7.81	17.72± 5.63

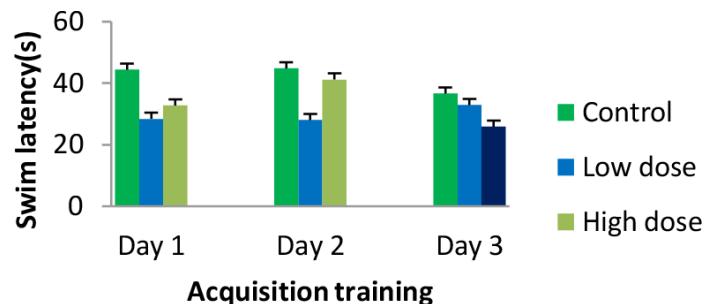


Figure 1: Comparison of the swim latencies during the acquisition training in the Morris water maze between mice administered low and high dose of *Ficus thonningii* and control.

**-Significant at $p<0.01$ compared to control

***-Significant at $p<0.001$ compared to control.

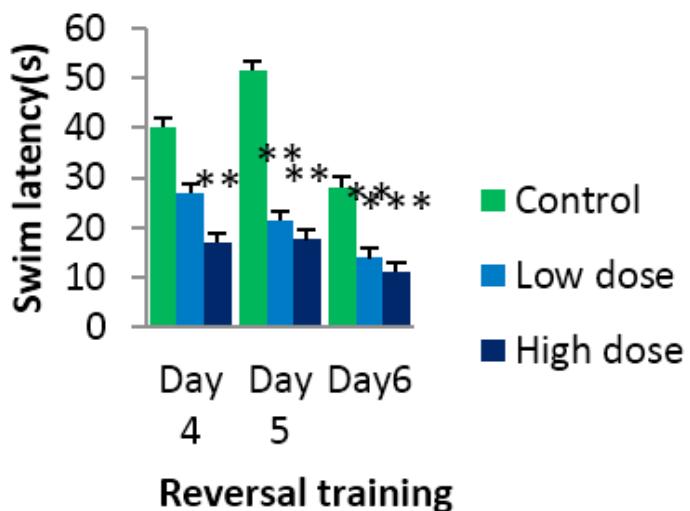


Figure 2: Comparison of the swim latencies during the reversal training in the Morris water maze between mice administered low and high dose *Ficus thonningii* and control.

**-Significant at $p<0.01$ compared to control

***-Significant at $p<0.001$ compared to control.

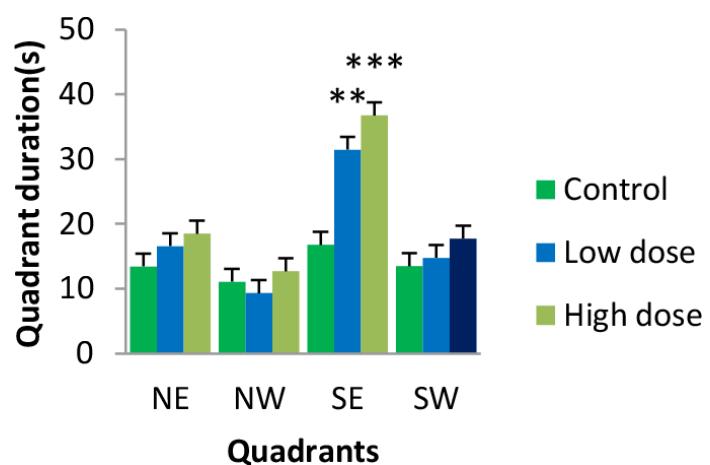


Figure 3: Comparison of quadrant during the probe trial in the Morris water maze between mice fed low and high dose *F. thonningii* and control diets.

***-Significant at $p<0.001$ compared to control

Annulus acquisition and annulus reversal crossings during the Morris water maze task

Figure 4 showed the annulus acquisition and reversal crossings during the Morris water maze task, between mice administered low and high dose of *F. thonningii* and control diet. The annulus acquisition crossing showed no significant difference among the groups. However, in the annulus reversal crossings, the low and high dose groups were significantly higher ($P<0.001$) compared to control.

	Control	Low dose	High dose
AAC:	11.66±9.01	10.33±4.50	11.00±1.00
ARC:	23.00±6.08	62.00±3.00	67.66 ±9.29

Swim latencies during the visible platform task in the Morris water maze.

Figure 5 compares the swim latencies between the mice administered low and high dose of *Ficus thonningii* and control diet. During the visible platform task, the swim latencies for the low and high dose groups administered *Ficus thonningii* were significantly lower ($P<0.001$) compared to control. However, there was no significant difference between the low and high dose groups when compared.

Control	Low dose	High dose
21.12±2.64	11.12±6.35	5.00±3.38

DISCUSSION

The parameter that was observed includes learning and memory using the Morris water maze apparatus. The principle of using this apparatus is to study spatial memory and learning. The Morris water maze is based

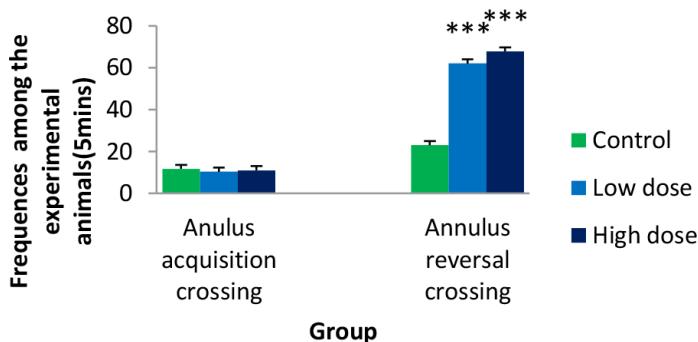


Figure 4: Comparison of the annulus acquisition and reversal training during the Morris water maze task between mice administered low and high dose of *F. thonningii* and control diet.

***-Significant at $p<0.001$ compared to control

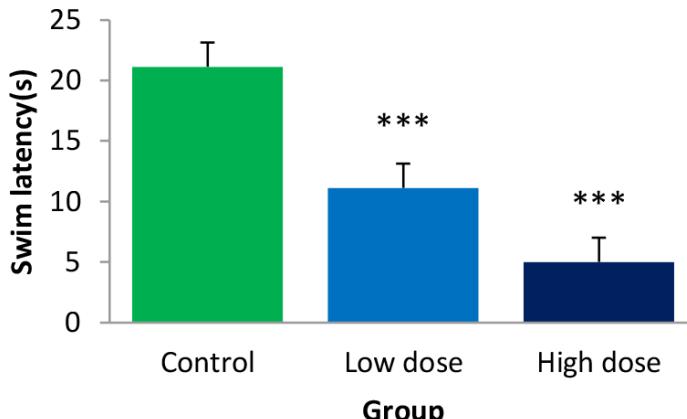


Figure 5: Comparison of the swim latencies during the visible platform task in the Morris water maze between mice administered low and high dose *Ficus thonningii* and control diet.

on the ability of the mice to be able to remember what was thought (how to locate a platform).^[18] A Morris Water Maze modified for mice was used.^[19] The water maze was constructed out of a circular polypropylene pool that measured 110 cm in diameter and 20 cm in depth. The pool was filled to a depth of 14 cm with room-temperature tap water. The water was made opaque with the addition of liquid milk to ensure camouflage of the white escape platform. The task exploits the ability of the mice to locate an escape platform after training under the influence of the extract *Ficus thonningii*. The swim or escape latency refers to the time taken for the mice to locate the escape platform. This measure is assessed by the swimming speed.^[20] In the Morris water maze experiment, during the acquisition training which lasted for the first 3 days, showed that the swim latencies for the mice in the control group to locate the platform compared to the group administered with low and high doses of the extract were not significantly different.

During the reversal training, the swim latencies for the high dose and low dose groups were significantly lower compared to the control group during the (4-6 days) that the reversal training was carried out, suggesting a better learning and memory in the low and high dose groups compared to control.

During the probe trial, the quadrants (NE, NW, SE and SW) were compared. The frequency of quadrant exploration in the 3 groups (i.e. NE, NW and SW) were both not significantly different compared to control, though the treated groups tend to have a higher frequency. However, the frequency of quadrant exploration in the three groups showed preference for the South-East quadrant (that bore the escape platform during the reversal training). Nevertheless, the frequency of quadrant exploration in the South-East were higher and as well significant for the low and high dose groups compared to the control. This showed that they had better memory than the control group. The annulus acquisition and reversal crossing refers to the number of times the mouse crossed the location of the platform during the acquisition and reversal training. The annulus acquisition crossing showed that there was no significant difference between the mice in the 3 groups. However, in the annulus reversal crossing, our results showed that the low and high dose groups of mice had better performance which showed better learning ability than the control.

During the visible platform task, it took lesser time for the mice treated with low and high dose groups to locate the platform compared to the control group. Shorter swim latency in the visible platform task indicates better cued learning and longer swim latency indicate poor cued learning. This means that the low and high dose groups, administered *F. thonningii* improved learning process and visual integrity in mice. It is possible therefore, that either a lower or higher dosage or otherwise of *F. thonningii* consumption could show a remarkable difference in learning and memory.

CONCLUSION

The results suggest that consumption of *Ficus thonningii* extract enhances learning and memory in mice. Thus, *F. thonningii* containing diet may be beneficial in the improvement of learning and memory.

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CONFLICT OF INTEREST

No conflict of interest in respect to the publication of this piece.

Authors' contribution

I performed most of the experiments and wrote the draft of the manuscript, designed the study and performed the statistical analysis. However, Miss Vivian managed the literature search.

ABBREVIATIONS

CD 1: Cluster of Differentiation 1; **AAC:** Annulus acquisition; **ARC:** Annulus reversal crossings; **SE:** South East; **SW:** South west; **NE:** North East; **NW:** North west.

SUMMARY

Since there is an increasing demand by people for means of enhancing the neuro-cognitive functions of the brain and the side effects of many neuro-cognitive drugs which have left many persons with irreversible neurological damage. This situation has lead to our investigation of the neuro-cognitive properties (learning and memory) of the plant, *Ficus thonningii* (wild fig). Thirty Adult Swiss mice weighing between 16 -21g, were divided into 3 groups, 10 mice per group. Before the neurobehavioral parameters were assessed, the LD₅₀, acute toxicological screenings of the plant were determined. Group A being the control, received rat feed with normal saline, group B being the low dose were administered with *Ficus thonningii* extract at a dose of 10mg/kg and group C being the high dose were administered with *Ficus thonningii* extract at a dose of 20mg/kg, this administration lasted for 14 days. All animals were allowed clean drinking water. The data's were analyzed and the results showed that on days 1, 2 and 3 of acquisition training mice from low dose and high dose learned equally when compared to the control. In the reversal training, memory was improved in the low dose and high dose treated mice when compared to control at ($p<0.05$, $p<0.01$ and $p<0.001$). During the probe trial, the swim duration in the South-East quadrant was significantly higher for high dose and low dose compared to control ($p<0.01$ and at $p<0.001$). However, during the visible platform task, the swim latencies for the low dose and high dose group were also significantly lower compared to control ($p<0.001$). The results suggest that consumption of *Ficus thonningii* extracts enhances learning and memory in mice, thus *Ficus thonningii* containing diet may be beneficial in the improvement of learning and memory.

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