Effects of Acetone Extract of *Cola nitida* on Memory and Brain Glutamate Receptors in Female Wistar Rats

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Abstract

Cola nitida is the seed of the Kola tree which is commonly grown in the tropical rainforest of Africa. It has been in vogue use for its caffeine and many other beneficial contents such as the obromine. This study aims to investigate the effects of acetone extract of *Cola nitida* on memory, plasma aspartate aminotransferase activity and metabotropic glutamate receptor 1a in female Wistar rats.

Fifteen female Wistar rats were used and grouped into three. Control was given 1.2ml normal saline,lowdosegroupwasadministered50mg/kgbody weight (bw) of *Cola nitida* extract and highdosegroupwasadministered100mg/kgbw. Memory and cognition tests were evaluated using Morris water maze, Y-maze and using novel object recognition test. Plasma lactate dehydrogenase and aspartate aminotransferase levels were estimated and immunohistochemical analysis of the hippocampus and amygdala was observed.

There was significant($p<0.05$)decreasein duration to get the escape platform in high dose group compared to control in the Morris water maze test. Also, there was a significant ($p<0.05$) increase in aspartate aminotrannsferase level in the group administered low dose of the extract of *Cola nitida.* Avivid stimulation of the glutamate receptors was observed following immunohistochemical analysis.

In conclusion, *Cola nitida* enhance memory in female Wistar rats through the stimulation of glutamate receptors and increase in plasma aspartate aminotrannsferase level.

Keywords: *Cola nitida*, glutamate receptors, immunohistochemistry, Wistar rats, hippocampus, amygdala.

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Introduction

Memory is the internal mental records that are maintained, which gives instant access to our past, complete with all the facts that we know, and the skills that have been cultivated. It is the retention of information over time for the purpose of influencing future action.[1] Encoding, storage and retrieval are the three (3) primary stages of human memory process.[2] During the encoding stage, information is sent to the brain, where it is dissected into its most significant composing elements. Different brain areas involved in the neuroanatomy of memory are thought to be involved in specific types of memory. The hippocampus is believed to be involved in spatial learning and declarative memory. The amygdala is part of a system that serves to regulate the strength of memories in relation to their emotional significance.[3]

Glutamate receptors are a kind of synaptic receptors that are located primarily on the membranes of neuronal cells. Metabotropic glutamate receptors are widely distributed in the amygdala and have been reported to affect learning, fear and extinction[4]mGlu1a are found mainly in hippocampal interneurons.[5]They are implicated in a number of neurological conditions and their central role in excitotoxicity and prevalence in the central nervous system has been speculated to be linked to many neurodegenerative diseases, also several other conditions have been further linked to glutamate receptor gene mutations or receptor auto antigen/antibody activity. These receptors have long been implicated in neurological processes underpinning learning and memory.[6]

Cola is an evergreen forest plant that is common in tropical region of Africa. One of its species, Cola acuminate is predominant in Angola, Ivory Coast, Liberia, Nigeria, Senegal and Togo. It has been reported to contain some stimulants that prevent fatigue and dispel sleepiness[7] and can be used to quench thirst and hunger, thereby facilitating weight loss.[8] Besides the ceremonial uses, many Africans consume kola nuts regularly, for the medicinal effects such as diabetes.[9] More recently, kola nut extracts have become popular in Europe and North America as a natural or alternative medicine for the treatment of diabetes.[9]

Imam-Fulani *et al.*[10]reports in a previous study that acetone extract of kola nut increased spatial memory of rats while Umoren *etal.*[11] studied the effects of chronic consumption of kolanut (*Colanitida*) and caffeine diets on exploration, anxiety and fear in Swiss white mice.

The aim and objectives of this study is to further investigate the effects of acetone extract of *Cola nitida* on memory and glutamate receptors in the hippocampus and amygdala of female Wistar rats.

Materials and methods

Drugs and Chemicals

Primary antibody (Rabbit polyclonal metabotropic glutamate receptor (mglutr1a) and secondary antibody (biotinylated goat antipolyvalent) were purchased from ABCAM U.S.A. Acetone, PBS, paraformaldehyde, ketamine, sucrose were purchased from Dennis & Dennis at Ibrahim Taiwo road, Ilorin, Kwara State**.**

Animals

Fifteen (15) female Wistar rats with an average weight of 100g-160g were procured from a private animal breeder around Oke-Ose in Ilorin and were used for this experiment. They were kept in well ventilated wooden cage sand housed in the animal house of faculty of basic medical sciences, University of Ilorin. The animals acclimatized for two (2) weeks with adequate food and water made available for them. The study was conducted in accordance with the care and use of animals of the Ethical committee of the Faculty of Basic Medical Sciences, University of Ilorin.

Plant material

Fresh seeds of *Cola nitida* were collected in Oja-Oba market, Ilorin, Kwara State. The identification and authentication of the seeds was carried out at the Department of Plant Biology, University of Ilorin, Ilorin, Nigeria.

Preparation of extracts

The seeds were chopped into smaller pieces and air-dried in the Physiology laboratory for 3 days. The dried seeds were then grinded into powdery form and sieved. About 200g of the powdered seed was soaked in 80% acetone using 10g to 100ml of acetone. It was soaked for 72hours to get the extract. Extract was filtered (using Whatman No. 2 filter paper). The filtrate was then placed in a water bath for evaporation, at 45°C until about 90% of the filtrate had been evaporated. Extract was kept in the refrigerator and stored for subsequent use.

Experimental design

The animals were randomly divided into three (3) groups with each groups consisting of five rats. The three groups of rats were subjected to the following oral treatments once a day for 6weeks:

Group 1: Control, received 1.2ml of normal saline.
Group 2: low dose, received 50mg/kg bw of AECN
Group 3: high dose, received 100mg/kg bw of AECN

At the end of the experiment, the rats were fasted for 12hours and were sedated using ketamine administered intramuscularly. The rats then underwent perfusion followed by removal of the brain.

Determination of Body weight

Experimental animals were weighed before commencement of administration twice a week throughout the period of the experiment.

Behavioural studies

Animals were subjected to various behavioural tests at the end of administration which include memory and cognition.

Morris water maze test

The Morris water maze procedure was carried out as described and designed by Richard Morris.[12] Animals were trained in the Morris water maze for 3days. They were allowed to swim until they found the escape platform and allowed to stay there for 20 seconds before they were picked up. Animals that failed to find the hidden platform within the time frame were guided to it.

During the memory test, the animals were allowed swim for 60 seconds, this procedure was recorded with a video camera mounted over the maze and the time spent to reach the escape platform was gotten using a stopwatch.

Object recognition test

The novel object recognition task is used to evaluate cognition, particularly recognition memory. The test is based on the spontaneous tendency of rodents to spend more time exploring a novel object than a familiar one. The choice to explore the novel object reflects the use of learning and recognition memory. The task was conducted in an open field arena with two different kinds of objects. During familiarization period, the animal was exposed to two identical objects placed at an equal distance and during the testing period, one of the objects is replaced with a new object to test recognition memory.

Each session was carried out for 5minutes, the arena and objects were cleaned after each session. The time spent to explore the novel object was recorded through a video camera mounted above the arena.

Y-maze test

Y-maze spontaneous alternation is a behavioral test for measuring the willingness of rodents to explore new environments. Testing occurs in a Y-shaped maze with three opaque arms at a 120° angle from each other. The number of arm entries and the number of trials are recorded in order to calculate the percentage alternation. An entry occurs when all four limbs are

within the arm. Y-maze testing was carried out for duration of 5minutes and the maze was cleaned with cotton wool soaked in methylated spirit. Animal was placed in the centre of the maze and trained to explore the arms of the maze in an orderly sequence, from A to B then to C.

During the testing, the animals were allowed to freely explore all arms, and the number of entries into arms and correct alternations between arms was recorded to determine the percentage alternation.

Determination of aspartate aminotransferase and lactate dehydrogenaselevel

Blood samples were collected through cardiac puncture into EDTA bottles. The samples were centrifuged for 10minutes at 4000rpm to get the supernatant plasma to be used for the determination of aspartate aminotransferase activity. The determination was done in the haematology laboratory of the University of Ilorin teaching hospital.

Immunohistological analysis

Brain tissues were harvested and fixed in 4% paraformaldehyde solution and later embedded in agarose. The tissues were then cut into sections using a vibratome and were subsequently stained. Binocular light microscope was used to examine and capture photomicrographs of the brain slices at magnification x400.

Statistical analysis

All data from this study were statistically analysed using one-way analysis of variance (ANOVA) performed on IBM Statistical Packages for Social Science, version 22. The mean and standard error of mean were calculated for all values. Differences were considered statistically significant at $p<0.05$.

Results

Effect of administration of AECN on body weight in female Wistar rats

Table 1 shows there was no significant ($p<0.05$) difference in body weight in groups administered low

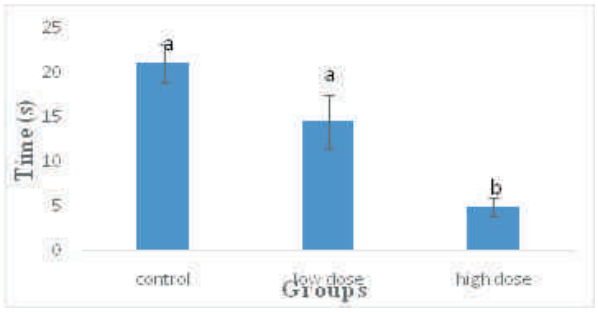
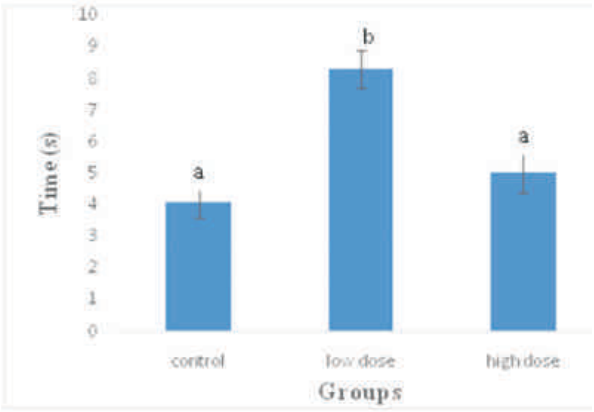


Fig. 1. Effect of administration of acetone extract of *Cola nitida* on short term memory in female Wistar rats. Bars with different letters are significantly ($p<0.05$) different.



. 2. Effect of administration of acetone extract of *Cola nitida* on novel object recognition in female Wistar rats. Bars with different letters are significantly ($p<0.05$) different.

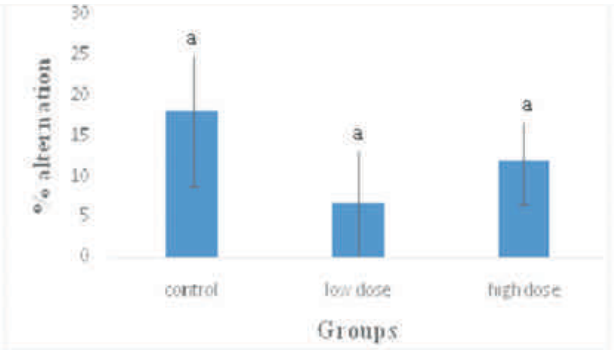


Fig. 3. Effect of administration of acetone extract of *Cola nitida* on spatial memory in female Wistar rats.

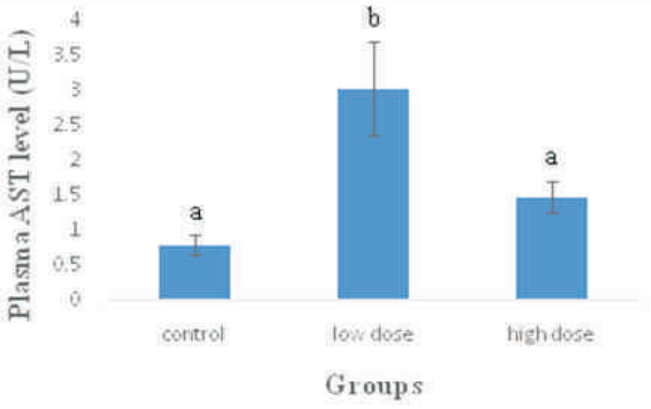


Fig. 4. Effect of administration of acetone extract of *Cola nitida* on plasma aspartate aminotransferase level in female Wistar rats. Bars with different letters are significantly ($p<0.05$) different.

Table 1: Effect of administration of AECN on body weight in female Wistar rats.

GROUPS	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
Control	171.80±5.05[a]	176.00±4.53[a]	181.60±5.90[a]	184.60±6.67[a]	190.80±7.69[a]	190.60±7.83[a]
Low dose	164.00±9.90[a]	170.00±9.09[a]	167.40±15.2[a]	179.00±8.76[a]	184.60±9.35[a]	177.00±7.16[a]
High dose	151.60±5.58[a]	156.00±7.42[a]	164.00±5.15[a]	169.20±5.58[a]	171.00±6.20[a]	161.40±3.57[b]

Values are expressed as mean±S.E.M (standard error of mean). Columns with different letters are significantly ($P < 0.05$) different when compared with control. n=5

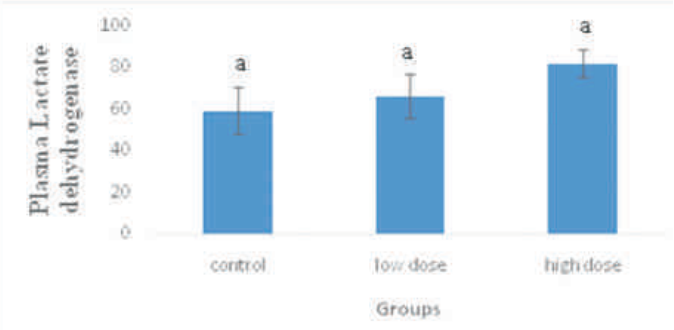


Fig. 5. Effect of administration of acetone extract of *Cola nitida* on plasma lactate dehydrogenase level in female Wistar rats.

dose and high dose of *AECN* when compared to control from week 1 to week 6. However, at week 6, there was a significant ($p<0.05$) decrease in the group administered high dose of AECN compared to control.

Effect of administration of AECN on short term memory in female Wistar rats

The result showed that there was a significant ($p<0.05$) decrease in time spent to get the Morris water maze escape platform in the group administered high dose of AECN when compared to control and no significant ($p<0.05$) difference in groups administered low dose of AECN when compared to control. Also, there was significant difference between groups administered low and high dose of AECN as shown in figure 1.

Effect of administration of AECN on novel object recognition in female Wistar rats

The result of this experiment showed that there was a significant ($p<0.05$) increase in time spent probing the new object in the group administered low dose of AECN when compared to control group and no significant ($p<0.05$) difference in the group administered high dose of AECN when compared to control. There was also a significant ($p<0.05$) difference between the groups administered low and high dose of acetone extract of *Cola nitida*as shown in figure 2.

Effect of administration of AECN on spatial memory in female Wistar rats

Figure 3 shows there was no significant ($p<0.05$) difference in percentage alternation in all groups.

Effect of administration of AECN on plasma aspartate amino transferase level in female Wistar rats

The result showed that there was a significant ($p<0.05$) increase in AST level in the group administered low dose of acetone extract of *Cola nitida* when compared to control while the group administered high dose of acetone extract of *Cola nitida* showed no significant ($p<0.05$) difference when compared to control group. Also there was no significant ($p<0.05$) difference between the groups administered low and high dose of acetone extract of *Cola nitida* as shown in figure 4.

Effect of administration of acetone extract of Cola nitida on plasma lactate dehydrogenase level (LDH) in female Wistar rats

Figure 5 shows that there was no significant ($p<0.05$) difference in LDH level of the groups administered the extract when compared to control.

Metabotropic glutamate receptor-1a immunohistochemistry analysis of brain slices

The immunohistochemistry analysis showed that there was no alternation to glutamate receptors in the control groups in different part of the brain such as amygdala and the hippocampus. Groups administered low dose AECN showed stimulation of the receptors in the hippocampus and amygdala while high dose of AECN showed scanty distribution of the neuronal cells as shown in figure 6 and 7.

Discussion

This study was carried out to investigate the

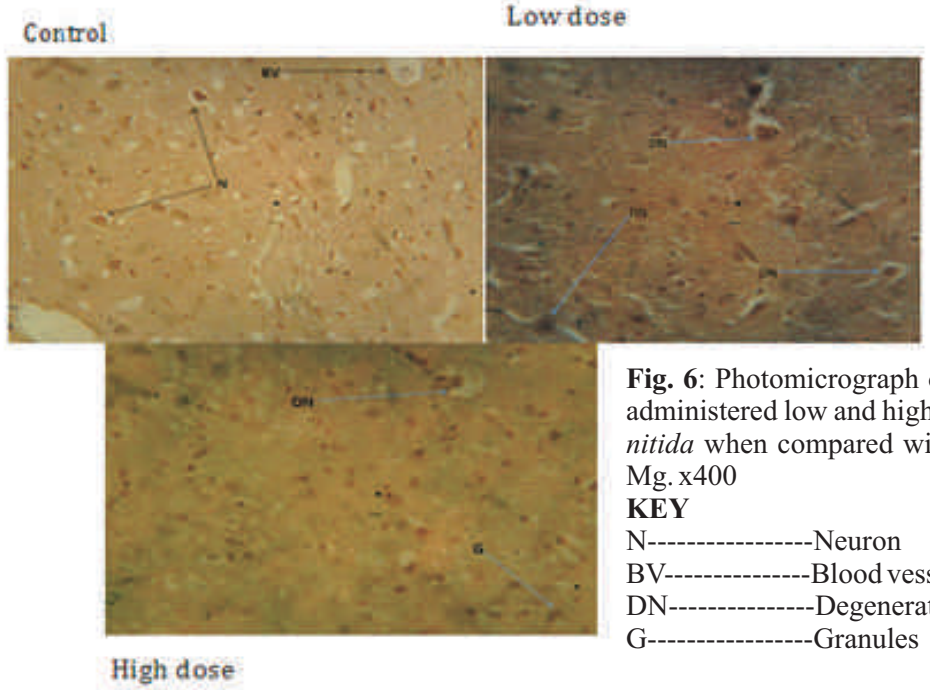


Fig. 6: Photomicrograph of section of Amygdala of rats administered low and high dose of acetone extract of *cola nitida* when compared with control. Glutamate receptor Mg. x400

KEY

N----------Neuron
BV----------Blood vessel
DN----------Degenerating neuron
G----------Granules

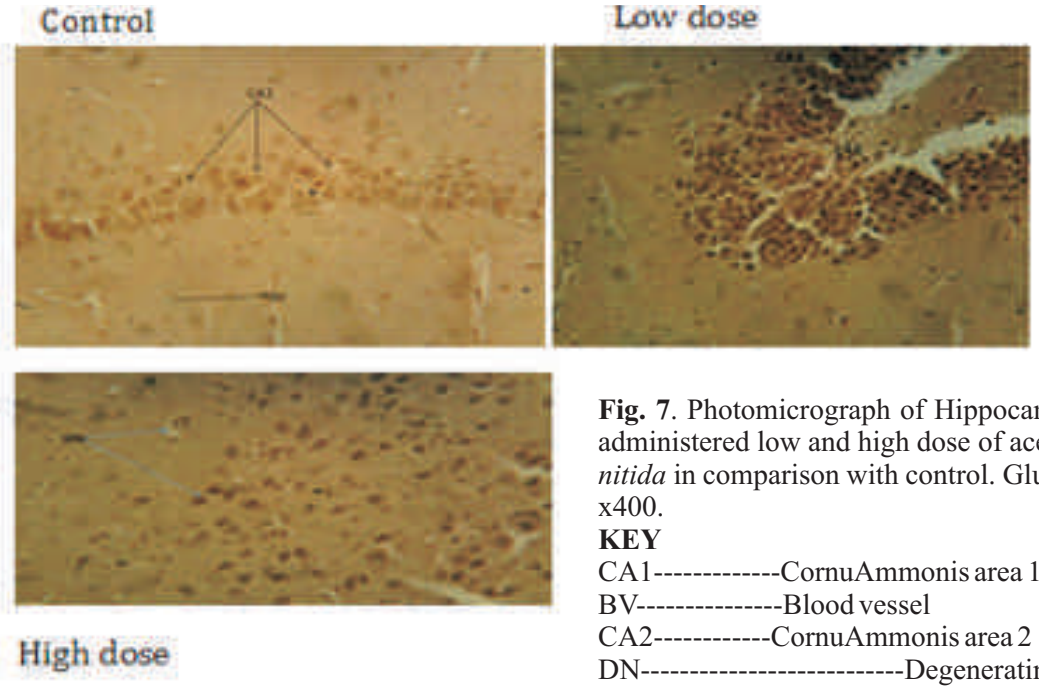


Fig. 7. Photomicrograph of Hippocampus section of rats administered low and high dose of acetone extract of *cola nitida* in comparison with control. Glutamate receptor Mg x400.

KEY

CA1----------CornuAmmonis area 1
BV----------Blood vessel
CA2----------CornuAmmonis area 2
DN----------Degenerating neuron

effect of AECNon memory using Wistar rats. The results obtained from the study shows there was significant ($p<0.05$) increase in the short term memory of experimental animals using the Morris water maze as the time taken for the group administered *cola nitida* extract (especially high dose) to get the escape platform was reduced compared to the control group. However, using the Y-maze, there was no significant ($p<0.05$) difference in spatial memory of experimental animals.

There was also a significant ($p<0.05$) increase in time spent probing the new object time in the group administered low dose of the extract compared to the control. This is in consistence with the study by Duarte *et al.*[13] which indicates that chronic consumption of caffeine can prevent neurodegeneration and memory impairment. It was also observed in this study that there was an enhancement of memory which could be as a result of the high content of caffeine present in *Cola nitida.*

According to the research conducted by Umoren*et al.*[11], it was concluded that kola nuts could help suppress appetite. Suppression of appetite can lead to weight loss and as shown in this study there was reduction in weight change in groups administered both high and low dose, though not significant, but there was a significant ($p<0.05$) decrease in weight in groups

administered high dose of acetone extract of *Cola nitida* when compared to the control group at the end of administration at week 6.

Aspartate aminotransferase is the most frequent reliable biomarker of liver injury, although it plays a central role in glutamate production[14] which is a principal excitatory neurotransmitter that affects memory functions in the brain because the brain needs glutamate to form memory.[15] There was a significant ($p<0.05$) increase in AST activity in groups administered low dose of acetone extract of *Cola nitida* when compared to the control group. This shows that there is a slight increase in AST level which can increase the production of glutamate. In contrast to AST, there was no significant ($p<0.05$) difference in lactate dehydrogenase level of the other groups when compared to control.

The immunohistochemical analysis of the hippocampus and amygdala carried out in this study showed that the extract did not alter the glutamate receptors. A stimulation of glutamate receptors was observed in the group administered low dose of the extract showing enhancement of the receptors. The group administered high dose of the extract showed a disruption in the neuronal cells. There is a clear involvement of metabotropic glutamate receptor1 in cognitive processes and nociceptive mechanisms.[16]

Conclusions

It can be concluded from this experiment that *Cola nitida* has a positive effect on memory. The less time used to get the escape platform by the animals administered the extract, and more time spent probing the new object is considered a measure of enhanced memory. The observed memory enhancement may be due to increased plasma aspartate aminotransferase level and through stimulation of the glutamate receptors.

We recommend that further research should be carried out on kola nuts to determine the active constituents that are responsible for the outcome of this study, and also to find out its other effects on the human system either positive or negative.

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