



Original Work

Chronic *Allium sativum* administration alters spontaneous alternation and cyto-architecture of medial prefrontal cortex of adult Wistar rats

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ABSTRACT: Intake of herbs and some of their constituents is reported to reduce risks of some diseases, with *Allium sativum* being one of such. This study was conducted to investigate the effects of *Allium sativum* extract on the medial prefrontal cortex and neurobehaviour of adult Wistar rats. Twenty-four male Wistar rats were divided into 4 groups (n = 6). Group 1 was the control and received distilled water placebo orally, while groups 2-4 received oral doses of 78 mg/kg, 156 mg/kg and 312 mg/kg *Allium sativum* extract for 28 days. On day 29, spontaneous alternation behavioural test was carried out, and immediately the rats were anaesthetized (50 mg/kg ketamine hydrochloride IP.) and perfusion-fixed with 10% buffered formalin. The whole brains were removed and the medial prefrontal cortex excised and processed for histomorphologic studies by haematoxylin and eosin, and cresyl fast violet techniques. Neurobehavioural test revealed less spontaneous alternation in 156 mg/kg *Allium sativum* group, while the prefrontal cortices of the test groups showed hypertrophy, hyperplasia, pyknosis, karyorrhexis, chromatolysis, loss of brain cellular membranes and Nissl substance. The 156 mg/kg *Allium sativum* group showed altered spontaneous alternation, while cellular pathologic changes were observed in the medial prefrontal cortex of these test groups in a dose dependent sequence.

KEY WORDS: *Allium sativum*; Medial prefrontal cortex; Neurobehaviour; Histomorphology; Wistar rat

INTRODUCTION

In recent years, plant-based chemicals have been extensively explored for their potentials as nutraceuticals in the treatment of various health conditions. Many plants including fruits and vegetables are recognized as sources of natural antioxidants that can protect against oxidative stress and thus play an important role in the chemoprevention of diseases that have their aetiology and pathophysiology in reactive oxygen species¹. One of such plants, garlic has been reported to utilize this antioxidant property due to its allyl cysteine, alliin, allicin, and allyl disulphide constituents².

Garlic is a member of the *Allium* (onion) family, and is closely related to onions, shallots and leeks.

It grows in many parts of the world and is a popular ingredient in cooking due to its strong smell and delicious taste. In local Nigerian languages, it is called *ayim mbakara* in Ibibio, *ayuu* in Igbo and *ayo* in Yoruba. The beneficial effects of garlic have been reported to be due to its antioxidant properties. Reported beneficial effects of *Allium sativum* include prevention and reduction of the severity of common illnesses such as flu and common cold, improvement of blood pressure level in cases of hypertension, reduction of low density lipoprotein cholesterol, prevention of degeneration of the brain's frontal lobe and in the hippocampus^{3,4}, improvement of memory retention, learning and cognition^{4,5}. However, high consumption of garlic leads to plethora of diseases such as loss of body weight, lysis of red blood cells, changes in intestinal flora, destruction of the stomach mucosa and loss of normal cellular architecture of heart, liver and kidneys⁶⁻¹⁰. There is limited report on the effect of garlic on brain areas including the prefrontal cortex, an area of the

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cerebral cortex of the frontal lobe involved in the planning of complex cognitive behavior, personality expression, decision making, and moderating social behaviour¹¹. The widespread culinary use of *Allium sativum* and the anticipated effect it would portend to cognitive behaviour, as well as a recent report on its adverse effect of neurobehaviour and frontal cortex histomorphology¹² motivated this research. Thus, the aim of this study was to investigate the effects of *Allium sativum* on the medial prefrontal cortex of adult Wistar rats.

METHODOLOGY

Care and grouping of the experimental animals

24 adult male albino Wistar rats of body weight 150-180 g were obtained and housed in 8 cages (40 cm × 35 cm) of 3 rats each in the animal house of the College of Health Sciences, University of Uyo, Uyo, Nigeria. The animals were allowed 12 hours light and dark cycles at 27°C-30°C room temperature. They were fed standard rat pelletized diet (Grand Cereals Ltd, Nigeria) and water *ad libitum*. All the animals were cared for following the guidelines of the National Institute of Health of the United States, and randomly divided into groups 1, 2, 3 and 4, of (n = 6). Group 1 was designated the control and groups 2, 3 and 4 the test groups.

Preparation of the *Allium sativum* extract

Fresh garlic bulbs (*Allium sativum*) were obtained from Itam market in Uyo, Akwa Ibom State of Nigeria, and were identified and authenticated by the Curator of the University of Uyo Herbarium. The fresh garlic cloves were peeled, pounded with mortar and pestle and blended with a blender (Century, China) to form a pulp. The pulp was extracted in 60 % ethanol for 30 minutes after which it was evaporated in a water bath at 40°C. The extract was further reconstituted in distilled water and stored in a refrigerator at 4°C.

Administration of the *Allium sativum* extract

Group 1 was given 5 ml/kg of distilled water as placebo, while groups 2, 3 and 4 received 78 mg/kg, 156 mg/kg and 312 mg/kg dose of the extract of *Allium sativum* respectively for 28 days. The garlic extract and water was administered once orally in the mornings (8-9am).

Neurobehavioural test

On the 29th day of the experiment, animals in all groups were subjected to spontaneous alternation neurobehavioural test using the T-maze. The T-

maze is shaped like a 'T' with two goal arms and a start arm. The dimensions of the goal arms are 50 cm x 10 cm, while the start arm is 50 cm x 16 cm. The central partition, which extends into the start arm, is 10 cm. The height of the wall is 3 cm¹³. This test is based on the willingness of rodents to explore a new environment.

Briefly, the animals were first placed in the long arm of the T-maze making it the start arm. Upon leaving the start arm, the rat chose between entering either of the short arms (the left or the right goal arm). After 5 trials, the percentage of alternation (number of turns in each goal arm) and total trial duration were recorded.

Termination of the experiment

Immediately after the neurobehavioural test the animals were anaesthetized with 50 mg/kg of ketamine hydrochloride (Rotex Medica, Germany) intraperitoneally, and their thoraco-abdominal walls were dissected and intracardially perfused with phosphate-buffered saline, and then perfusion-fixed with 10 % buffered formalin. The brains of all the animals were removed and fixed in 10 % buffered formalin for 48 hours. Each medial prefrontal cortex was excised, processed routinely and stained with the haematoxylin and eosin, and cresyl fast violet stains. Processed slides were viewed under the light microscope and photomicrographs obtained using a computer assisted digital microscope camera. Cellular population was determined with ImageJ software.

Statistically analysis

One-way analysis of variance was applied for statistical analysis followed by post hoc Tukey test. All data are presented as mean ± standard error of mean. Data with probability level $p < 0.05$ was regarded as significant.

RESULT

Spontaneous alternation neurobehavioural test

The spontaneous alternation neurobehavioural test result showed that the 156 mg/kg *Allium sativum* group had a significantly ($P < 0.05$) lower spontaneous alternation compared with the control group. However, there was no difference in spontaneous alternation between the 78 mg/kg *Allium sativum* group and 312 mg/kg *Allium sativum* group compared with the control group (Figure 1).

Histological observations

The medial prefrontal cortex of the control group at ×400 magnification showed the marginal layer with

few scattered cells. The cortical and subcortical plates had more cellular density. These cortical layers contained cells of different sizes and shapes.

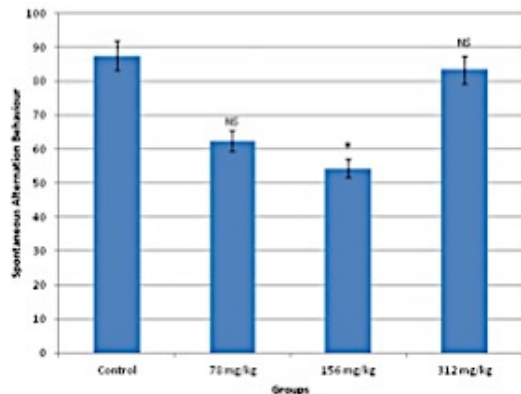


Figure 1: Spontaneous alternation behavior test
 * - Significantly different from the control group at $p < 0.05$; NS - Not significantly different from the control group at $p < 0.05$

No obvious histopathology was observed (**Figure 2a**). The medial prefrontal cortex of the 78 mg/kg *Allium sativum* group showed hypertrophy and hyperplasia of cells especially in the cortical plate. The cortical cells also showed loss of cell membranes leaving hollows around them compared with the control group (**Figure 2b**)

The medial prefrontal cortex of the 156 mg/kg *Allium sativum* group showed hypertrophy and hyperplasia especially in the cortical plate. The cortical cells also showed loss of cell membranes leaving hollows around them. Some of their nuclei appeared pyknotic and karyorrhectic compared with the control group (**Figure 2c**). The medial prefrontal cortex of the 312 mg/kg *Allium sativum* group showed hypertrophy especially in the cortical plate. The cortical cells also showed loss of cell membranes leaving hollows around them. Some of their nuclei appeared pyknotic and karyorrhectic compared with the control group (**Figure 2d**).

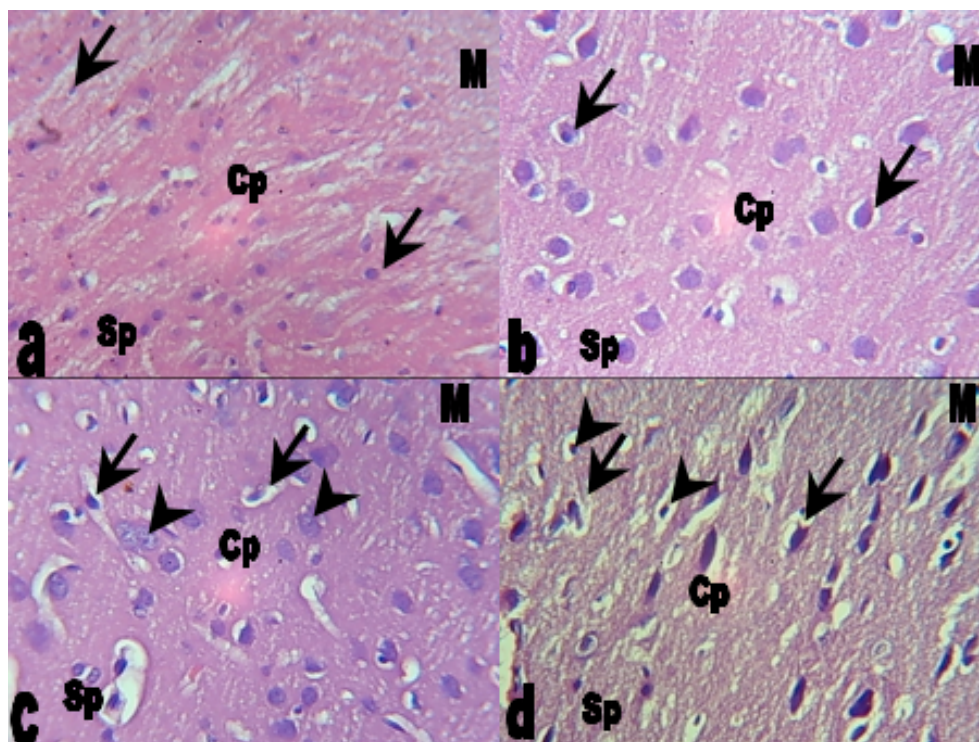


Figure 2: Photomicrographs of the sections of the medial prefrontal cortex of the control and test group animals, Marginal layer (M); cortical plate (Cp); subcortical plate (Sp), H&E, Mag×400. a. The medial prefrontal cortex of the control group shows the marginal layer with few scattered cells. The cortical and subcortical plates have more cellular density (arrows). These cortical layers contain cells of different sizes and shapes; b. The medial prefrontal cortex of the 78 mg/kg *Allium sativum* group shows hypertrophy and hyperplasia of cells especially in the cortical plate. The cortical cells also show loss of cell membranes (arrows) leaving hollows around them (arrow); c. The medial prefrontal cortex of the 156 mg/kg *Allium sativum* group shows hypertrophy and hyperplasia of cells with pyknotic and karyorrhectic (arrow heads) appearance of the nuclei. The cortical cells also show loss of cell membranes leaving hollows around them (arrows); d. The medial prefrontal cortex of the 312 mg/kg *Allium sativum* group shows much hypertrophy of cells with pyknotic (arrow heads) and karyorrhectic appearance of the nuclei. The cortical cells also show loss of cell membranes leaving hollows around them (arrows).

There was significant ($p < 0.05$) cellular population increase between all the test groups and the control. However, the 78 mg/kg *Allium sativum* group had a significantly ($p < 0.05$) higher cellular population compared with the 156 mg/kg and 312 mg/kg *Allium sativum* groups (Figure 3).

The section of the medial prefrontal cortex of the control group showed deeply stained Nissl substance throughout the cortical layers (Figure 4a). The section of the medial prefrontal cortex of the 78 mg/kg *Allium sativum* group showed loss of Nissl substance stain in most of the neurons with chromatolytic appearance throughout the cortical layers compared with the control group (Figure 4b). The section of the medial prefrontal cortex of the 156 mg/kg *Allium sativum* group showed loss of Nissl substance stain in most of the neurons with chromatolytic appearance throughout the cortical layers compared with the control group (Figure 4c). The section of the medial prefrontal cortex of the 312 mg/kg *Allium sativum* group showed loss of Nissl substance stain in most of the neurons with chromatolytic appearance throughout the cortical

layers compared with the control group (Figure 4d).

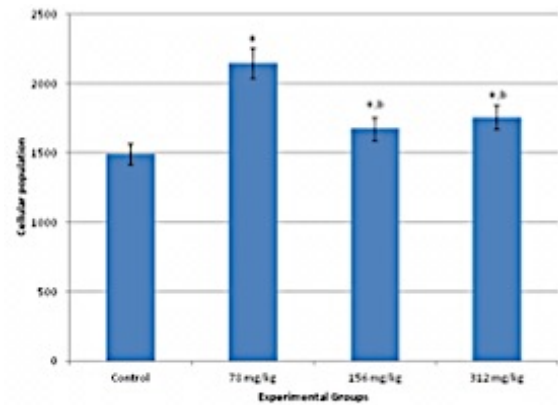


Figure 3: Cellular population count

* - Significantly different from the control group at $p < 0.05$; b - Significantly different from 78 mg/kg *Allium sativum* group at $p < 0.05$

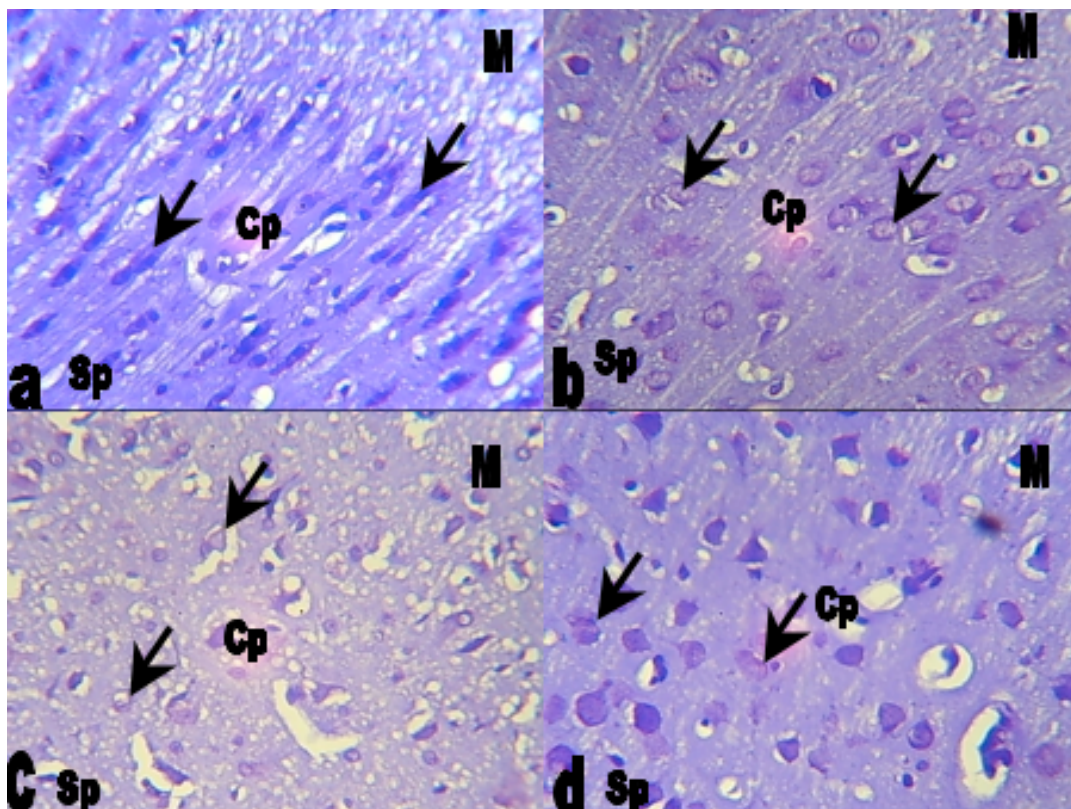


Figure 4: Photomicrographs of the sections of the medial prefrontal cortex of the control and the test groups, Marginal layer (M); cortical plate (Cp); subcortical plate (Sp), (cresyl fast violet, Mag. $\times 400$). a. The medial prefrontal cortex of the control group animals showing deeply stained Nissl substance (arrows) throughout the cortical layers; b. The medial prefrontal cortex of the 78 mg/kg *Allium sativum* group showing loss of Nissl substance stain in most of the neurons with chromatolytic appearance (arrows) throughout the cortical layers; c. The medial prefrontal cortex of the 156 mg/kg *Allium sativum* group showing loss of Nissl substance stain in most of the neurons with chromatolytic appearance (arrows) throughout the cortical layers; d. The medial prefrontal cortex of the 312 mg/kg *Allium sativum* group showing loss of Nissl substance stain in most of the neurons with chromatolytic appearance (arrows) throughout the cortical layers.

DISCUSSION

In the present study, the effects of *Allium sativum* extract on spontaneous alternation and the histology of the medial prefrontal cortex was studied in adult male albino Wistar rats. The results of the T-maze spontaneous alternation test showed a significantly lower spontaneous alternation in the 156 mg/kg *Allium sativum* group, though no differences was observed in the 78 mg/kg and 312 mg/kg *Allium sativum* groups. Spontaneous alternation allows an animal when given two consecutive trials in quick succession to choose a different arm from its initial choice thereby reflecting memory of the first choice¹³. T-maze alternation is applied in detecting cognitive dysfunction¹³. Spatial memory is one of the cognitive behaviors responsible for recording information about the rodents' environment, as well as its spatial orientation¹⁴. It is this spatial memory that allows the rodent to navigate its way through the various types of mazes. Naturally, in T-maze test, rats tend to alternate the goal arm they choose and this involves the use of 'working memory'¹⁵. This alternation of the goal arm chosen reflects the motivation of the animal to explore its environment¹³. It has been reported that treatment with garlic extract or its component S-allylcysteine improved learning and memory retention probably due to stimulation of nitric oxide secretion¹⁶, required for physiological activities¹⁷, including learning and memory stimulation¹⁸, as well as the unusual ability of neurons to grow and branch⁴. However, its role in the present results of spontaneous alternation cannot be explained.

The present results indicate that the 78 mg/kg *Allium sativum* group did not affect spontaneous alternation. On the other hand, the 156 mg/kg *Allium sativum* group resulted in reduced spontaneous alternation, while the 312 mg/kg *Allium sativum* group may reverse such alteration. This is at variance with a previous report where the *Allium sativum* groups did not affect spontaneous alternation except among the test groups¹², which may be attributed to the dose duration period of 14 days.

Histological results showed hypertrophy and hyperplasia of cells of the medial prefrontal cortex in the test groups. Hyperplasia is indicated by an abnormal increase in the number of organic tissue, while hypertrophy is the increase in cell size¹⁹. These changes may be physiologic or pathologic, and usually result from increase demand, chronic inflammatory response, hormonal dysfunction or compensation for damage or disease elsewhere²⁰, which may affect the function of the medial prefrontal cortex. Loss of cortical cellular membranes was also observed in the test groups indicating delipidization, which is also a prelude to degeneration and this may imply that the

hyperplasia and hypertrophic changes may be pathological. It was observed that administration of 156 mg/kg and 312 mg/kg of the extract caused pyknosis of the nuclei. This points to a degenerative state of the cell nucleus and it is indicated by an irreversible condensation of chromatin in the nucleus of a cell undergoing necrosis²¹. However the group that received 156 mg/kg of the extract also showed evidences of karyorrhexis. This is the fragmentation of the nucleus²². This result is similar to a previous report¹².

Several investigations have shown that *Allium sativum* has deleterious effects on the cellular arrangement of various body parts including the prefrontal cortex, liver, kidney and heart^{9,12}, as well as changes in the intestinal flora⁷, and induction of cell apoptosis²². However, it has also been reported that garlic prevents degeneration of the brain's frontal lobe and neuronal death in the hippocampus^{3,4}, which may not be the case in the present study.

There was reduction of Nissl substance in all the test groups with chromatolytic appearance throughout the cortical layers. Chromatolysis indicates neurodegeneration due to insufficient amount of protein in the neuron²³. Nissl substances help in the manufacture and release of proteins for intra- and inter-cellular use. Under pathologic conditions, it may dissolve and disappear leading to chromatolysis²⁴. Chromatolysis can be triggered by axotomy, ischemia and toxicity to the cell, as well as cell exhaustion or virus infections leading to disintegration of Nissl substances. Loss of Nissl substances as recorded in the test groups of the present study may be an apoptotic reaction that may eventually lead to neurodegeneration of the neurons. This is similar to a previous report, where Nissl loss especially in the high dose groups was also recorded¹².

The prefrontal cortex of the brain plays a major role in moderating social behaviour, working memory, attention, reward, planning and motivation; degeneration of which can lead to abnormal social behaviour, inappropriate coordination of activities, and poor performance during working memory tasks²⁵.

CONCLUSION

Allium sativum extract appears not to alter spontaneous alternation at low and high doses, but do so at medium dose. Cellular pathologic changes were also observed in the medial prefrontal cortex of these test groups though in a dose dependent sequence.

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