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Allium sativum alters the Cyto-Architecture of the Medial Prefrontal Cortex and Neurobehaviour of Adult Wistar Rats

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ABSTRACT

Consumption of vegetables and fruits has been reported to protect humans against oxidative damage by inhibiting free radicals and reactive oxygen species. *Allium sativum* is a plant whose beneficial effects have been attributed to its role in oxidation. This study therefore investigated the effects of *Allium sativum* extract on the medial prefrontal cortex and neurobehaviour of male albino Wistar rats. Twenty-four adult male albino rats were divided into 4 groups of 6 rats each. The control received 1 ml of distilled water orally, while test groups received oral doses of 78 mg/kg (low dose), 156 mg/kg (medium dose) and 312 mg/kg (high dose) body weight of the *Allium sativum* extract respectively for 2 weeks. Thereafter, spontaneous alternation behavioural test was carried out, and immediately the rats were anaesthetized with 50 mg/kg of ketamine hydrochloride (i.p.), and perfusion-fixed with 10% buffered formalin. The whole brains were removed and the medial prefrontal cortex excised and processed for histological studies using haematoxylin and eosin and Cresyl fast violet stains. Neurobehavioral test revealed higher spontaneous alternation from the medium dose group compared with the other groups. The prefrontal cortical sections showed hypertrophy, hyperplasia, loss of brain cellular membranes and Nissl substance. In conclusion, *Allium sativum* modulates spontaneous alterations, and cause alterations in cellular integrity in the medial prefrontal cortex.

Keywords: *Allium sativum, Prefrontal cortex, Neurobehaviour, Histology, Wistar rat*

INTRODUCTION

Over the years medicinal plant extracts have been shown to contain substances of therapeutic significance (Valnet 1990). Epidemiological studies have shown that the consumption of vegetables and fruits can protect humans against oxidative damage by inhibiting free radicals and reactive oxygen species. Though these medicinal plants are effective in healing various disorders, continued use of these plants can in turn lead to a plethora of side effects (Ames et al. 1993).

Allium sativum, commonly known as garlic, is a species of the onion genus, *Allium*, and its close relatives include the onion, shallot, leek, chive, and rakkyo. In Nigeria languages, it is known as *ayuu* in Igbo, *ayo* in Yoruba, and *ayim mbakara* in Ibibio. It is commonly consumed in foods while some times also eaten in its raw form. Most of its benefits have been attributed to its protective effect against oxidative stress. However, reports have shown that high consumption of the plant can lead to various side effects (Ambati 2013).

Different effects of *Allium sativum* extracts have been investigated in various organs including the hippocampus, frontal lobe and heart among others

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hippocampus, frontal lobe and heart among others (Arabinda et al. 2007). Several beneficial, as well as adverse effects of this *Allium sativum* have also been reported. These include; neuroprotection against different tumours (Arabinda et al. 2007), improve memory retention (Haider et al. 2008), learning and cognition (Moriguchi et al. 1997), and prevention of neuronal death in the hippocampus (Chun et al. 2003). Prevention of degeneration of the brain's frontal lobe (Moriguchi et al. 1997) and reduction in diabetic symptoms have also been reported (Augusti and Sheela 1996). Adverse effects of *Allium sativum* include loss of body weight, lyses of red blood cells (Augusti 1996), changes in intestinal flora (Ackermann et al. 2001), destruction of the stomach mucosa (Hoshino et al. 2001) and loss of normal cellular architecture of heart, liver and kidneys (Banerjee et al. 2002).

Despite the various reports on the effects of *Allium sativum* on various organs of the body, there is limited information on the effect of garlic on the brain, and on the prefrontal cortex. The prefrontal cortex, an area of the cerebral cortex of the frontal lobe includes Brodmann areas 9, 10, 11, 12, 46, and 47 (Singh 2009). This brain region has been implicated in planning complex cognitive behaviour, personality expression, decision making, and moderating social behaviour (Yang and Raine 2009). Thus, this study was to investigate the effects of *Allium sativum* on the prefrontal cortex of adult Wistar rats and its neurobehaviour.

MATERIALS AND METHODS

Care and Grouping of the Experimental Animals

24 adult male albino Wistar rats of body weight 150-180 g were obtained and housed in cages (40 cm x 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 12 hours dark cycles at 27°C - 30°C room temperature. They were fed standard rat pelletized diet (Grand Cereals Ltd, Nigeria) and water *ad libitum*. The research was approved by the Ethics committee of University of Uyo, Nigeria, and all the animals were cared for following the guidelines of the National Institute of Health of the United States. The animals were randomly divided into groups 1, 2, 3 and 4, of 6 rats each. Group 1 was the control and groups 2, 3 and 4 were the test group.

Preparation and Administration 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. Each fresh garlic cloves was 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.

Control group was given 1 ml of distilled water, while groups 2, 3 and 4 received 78 mg/kg (low dose), 156 mg/kg (medium dose) and 312 mg/kg (high dose) of the extract of *Allium sativum* respectively for 2 weeks. The garlic extract and water was administered orally for 2 weeks in the morning (8-9am).

Neurobehavioral Test

On the 15th 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 cm (Deacon and Rawlins 2006). This test is based on the willingness of rodents to explore a new environment. 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 chooses 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 intraperitoneally with 50

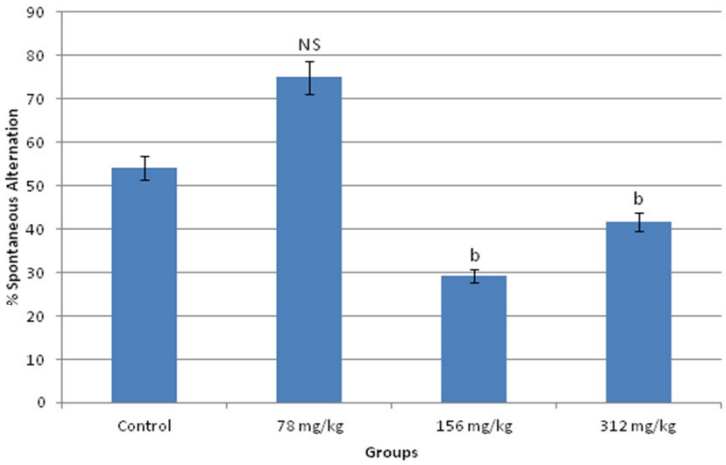

Fig. 1: Spontaneous alternation behaviour after 2 weeks exposure to *Allium sativum*
b- Significantly different from the low dose group at $p < 0.05$
NS- Not significantly different from the control group at $p < 0.05$

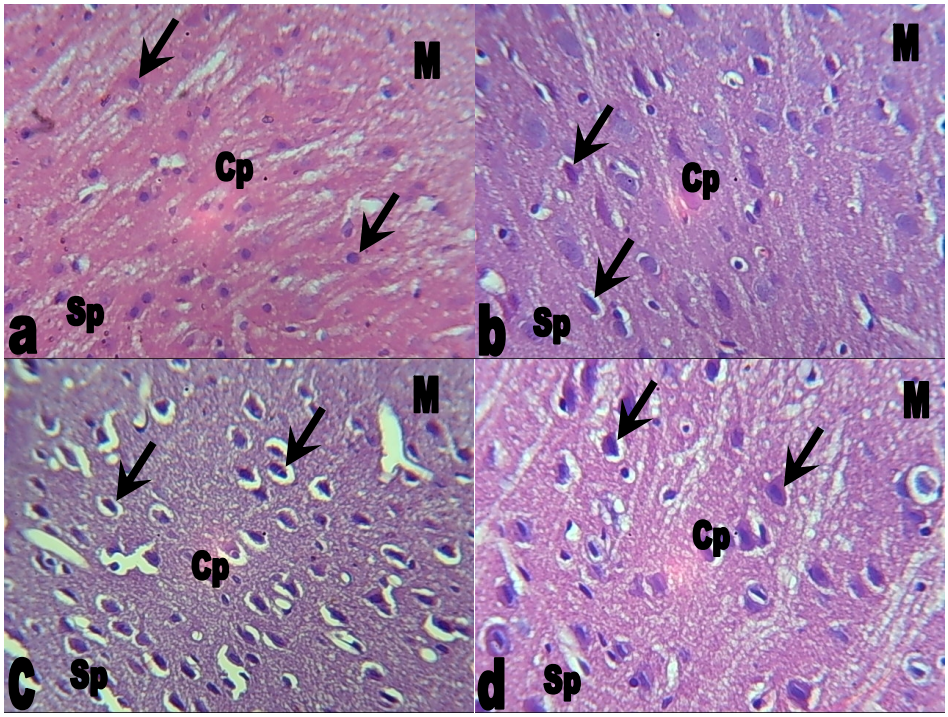

Fig. 2: Photomicrographs of the sections of the medial prefrontal cortex of the control and test group animals. Marginal layer (M); cortical plate (Cp); subcortical plate (Sp), (H&E, ×400)
a. The medial prefrontal cortex of the control group animals shows the marginal layer with few scattered cells. 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 low dose group shows hypertrophy and hyperplasia of cells especially in the cortical plate. The cortical cells also show loss of cell membranes (arrows). c. The medial prefrontal cortex of the medium dose group shows hypertrophy and hyperplasia of cells. The cortical cells also show loss of cell membranes (arrows). d. The medial prefrontal cortex of the high dose group shows much hypertrophy and hyperplasia of cells. The cortical cells also show loss of cell membranes (arrows).

mg/kg of ketamine hydrochloride (Rotex Medica, Germany). Their thoraco-abdominal walls were dissected and intracardially perfused with phosphate-buffered saline (0.1 Mol/l PBS, pH 7.4), and then 10 % buffered formalin. The brains of all the animals were removed and fixed in 10 % buffered formalin for 48 hours. Each prefrontal cortex was excised, processed routinely and stained with the Haematoxylin and Eosin and Cresyl fast violet stains for histological and Nissl substance studies, respectively.

RESULTS

Spontaneous Alternation Neurobehaviour Test

The spontaneous alternation neurobehaviour test result showed that the low dose (78 mg/kg) *Allium sativum* extract group had significantly ($P < 0.05$) higher spontaneous alternation compared with

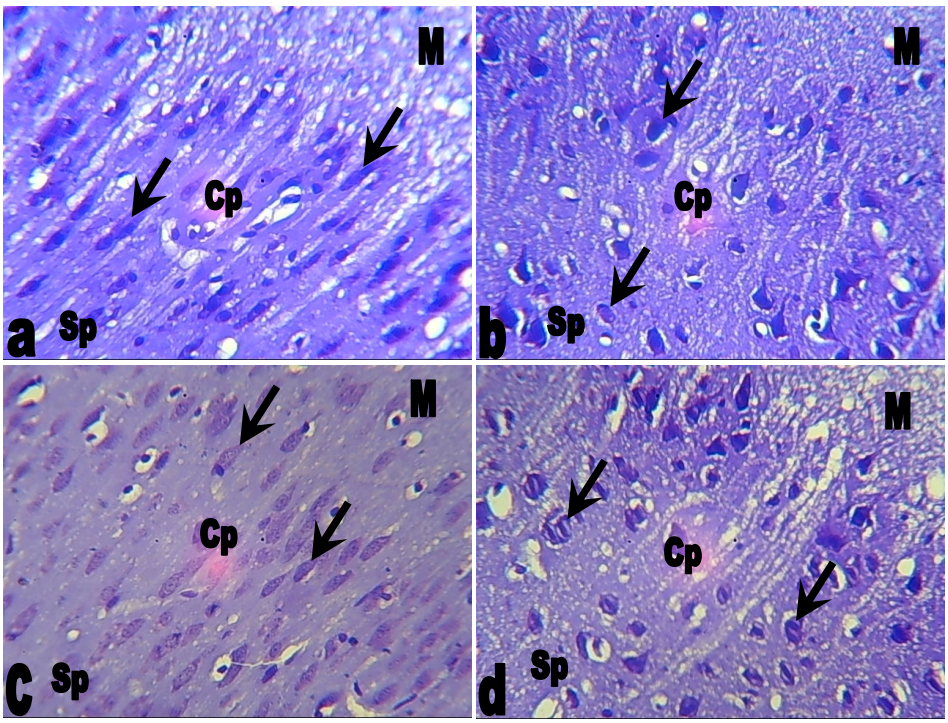

Fig. 3: Photomicrographs of the sections of the medial prefrontal cortex of the control and test groups. Marginal layer (M); cortical plate (Cp); subcortical plate (Sp), Cresyl fast violet, ×400):
a. The medial prefrontal cortex of the control group animals showing deeply stained Nissl substance (arrow) throughout the cortical layers. b. The medial prefrontal cortex of the low dose group showing deeply stained Nissl substance (arrow) throughout the cortical layers. c. The medial prefrontal cortex of the medium dose group showing loss of Nissl substance stain (arrow) in some of the neurons throughout the cortical layers. d. The medial prefrontal cortex of the high dose group showing loss of Nissl substance stain (arrow) in some of the neurons throughout the cortical layers.

the medium (156 mg/kg) and high (312 mg/kg) *Allium sativum* extract group. However, no difference was observed between these test groups and the control group (Figure 1).

Histological Observations

The medial prefrontal cortex of the control group 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. No obvious histopathology was observed (Figure 2a). The medial prefrontal cortex of the low dose *Allium sativum* extract 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 medium dose *Allium sativum* extract group showed hypertrophy and hyperplasia of cells. The cortical cells also show loss of cell membranes leaving hollows around them compared with the control group (Figure 2c). The prefrontal cortex of the high dose *Allium sativum* extract group showed much hypertrophy and hyperplasia of cells. The cortical cells also showed loss of cell membranes leaving hollows around them compared with the control group (Figure 2d).

The medial prefrontal cortex of the control group showed deeply stained Nissl substance throughout the cortical layers (Figure 3a). The medial prefrontal cortex of the low dose *Allium sativum* extract group showed deeply stained Nissl substance throughout the cortical layers compared with the control group (Figure 3b). The medial prefrontal cortex of the medium dose *Allium sativum* extract group showed loss of Nissl substance stain in some of the neurons throughout the cortical layers compared with the control group (Figure 3c). The section of the medial prefrontal cortex of the high dose *Allium sativum* extract group showed loss of Nissl substance stain in some of the neurons throughout the cortical layers compared with the control group (Figure 3d).

DISCUSSION

In the present study, the effects of varied low, medium and high doses (78 mg/kg, 156 mg/kg and 312 mg/kg) of *Allium sativum* extract on the medial prefrontal cortex histology and neurobehaviour was assessed in adult male Albino Wistar rats. The spontaneous alternation test showed that the low dose *Allium sativum* extract group had a higher spontaneous alternation compared with the medium and high doses groups, though not with the control group. Reports reveal that treatment with garlic extract or its component S-allylcysteine improved learning and memory retention which can be linked to unusual ability of neurons isolated from the hippocampus to grow and branch (Moriguchi et al. 1997). The spatial memory is responsible for recording information about the rat's environment, as well as its spatial orientation (O'Keefe & Dostrovsky 1971). It is this spatial memory that allows the rat to navigate its way through the various types of mazes and challenges presented to it. The results of this study shows that a low dose of *Allium sativum* extract may enhance spontaneous alteration, while at increased doses, spontaneous alteration may be reduced.

Histological results revealed that in all test groups there was hypertrophy and hyperplasia of cells of the medial prefrontal cortex. Hypertrophy and hyperplasia may be a physiologic or pathologic condition, and usually result from increase demand, chronic inflammatory response, hormonal dysfunction or compensation for damage or disease elsewhere (Goldstein et al. 1987). Hypertrophy mostly involves an increase in intracellular protein (Goldstein et al. 1987). However, the test groups also showed loss of cortical cellular membranes and adjoining cortical matrix which signifies degeneration, an indication that the hypertrophy and hyperplasia already reported in this study may be a pathologic condition.

Allium sativum has been reported to cause severe damage to cellular arrangement of organs such as the liver, kidney and heart (Banerjee et al. 2002), changes in the intestinal flora (Ackermann et al. 2001), as well as induction of cell apoptosis (Izzo et al. 2004), which supports our results. In contrast however, Moriguchi et al. (1997) and Chun et al. (1997) reported that garlic prevents degeneration of the brain's frontal lobe and neuronal death in the hippocampus, respectively.

Nissl substance was reduced in the medium dose and high dose *Allium sativum* extract groups, though the low dose *Allium sativum* group appeared not affected. The result of the present study showed that high dose of *Allium sativum* has a deleterious effect on Nissl bodies. Loss of Nissl substance may lead to chromatolysis which may have negative effects on the neurons. Chromatolysis is due to insufficient amount of protein in the neuron which will further lead to progressive loss of structure and function (neurodegeneration) (Scarborough 1938).

Nissl substances are granular substances found in neurons, and function to manufacture and release proteins for intra- and inter-cellular use. Nissl substance show changes under various physiological and pathological conditions, and may dissolve and disappear (chromatolysis) (Thompson 2000). 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. Phosphorylation of neurofilament has been implicated in radial axonal growth, neurofilament bundling and neurofilament axonal growth, however, hyperphosphorylation is a hallmark

of several neurodegenerative diseases (Holmgren et al. 2012). It is reported that increase in phosphorylated neurofilament proteins and cytoskeletal components, tubulin and actin occur in neurons undergoing chromatolysis (Goldstein et al. 1987). In the present study, chromatolysis could have resulted from toxicity induced by *Allium sativum* resulting in the degradation of the Nissl proteins.

The prefrontal cortex is the part of the brain concerned with reward, attention, short-term memory tasks, planning, and motivation via the presence of dopamine-sensitive neurons (Goldman-Rakic et al. 1996), however other neuronal types may also be involved. Degeneration of the prefrontal cortex leads to difficulty in planning and organization of activities, inappropriate behaviour in social and work activities, interaction with others and care for one self. Reduction of dopamine activity in the prefrontal cortex is related to poorer performance and inefficient functioning of that brain region during working memory tasks, and to slightly increased risk for schizophrenia (Goldman-Rakic et al. 1996). Hence, *Allium sativum* extract may have deleterious effects on the functioning of the prefrontal cortex.

CONCLUSION

Allium sativum alters spontaneous alternation depending on the dosage, and causes cellular pathologic changes in the medial prefrontal cortex. These effects were dose dependent.

Conflict of Interest

None declared.

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