Ameliorative Effects of Enriched Environment and Chronic Administration of Aqueous-methanol Extract of Garlic (*Allium sativum*) on Mice Models of Depression

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Authors' contributions

This work was carried out in collaboration among all authors. Author PPM and MIAS designed the experiment, carried it out, analyzed the data and write the manuscript; author OOO was involved with experimentation and literature search while author AAM also was involved in experimentation. All authors approved the final manuscript.

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ABSTRACT

Depression is a state of mood or energy level that includes lack of motivation, a sense of hopelessness and a loss of physical energy. The World Health Organization revealed that depression is one of the leading causes of ill health and disability worldwide. More than 300 million people are living with depression. Many of the currently available antidepressant drugs have proven to be effective but they are burdened with some disadvantages such as various adverse effects, problematic interactions and relatively low response. Therefore the need to utilize a natural agent in the management of depression is paramount. The aim of this study was to investigate the effects of chronic administration of extract of *Allium sativum* and Enriched environment in depression. 42 albino mice were used and divided into seven groups of five mice each. Group 1 was given distilled water; groups 2 and 3 received 200 mg/kg and 400 mg/kg of aqueous extract of *Allium sativum* respectively; while groups 4 and 5 were in addition to receiving 200 mg/kg and 400

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mg/kg of the extract, housed in an enriched cage. Group 6 was only housed in an enriched cage and group 7 receives 10 mg/kg of imipramine. The experiment lasted for six weeks after which Force Swimming Test, Tail Suspension Test and Sucrose Preference test were conducted. The mice were sacrificed and their brain isolated, homogenized and centrifuged. The supernatant was used for biochemical assays (MDA, SOD, GPx, BDNF & TNF- α). The results showed that *Allium sativum* and Enriched Environment helped in mitigating depressive disorders. Therefore, conducive environment and garlic extract could be used in the management of depression.

Keywords: Depression; *Allium sativum*; environmental enrichment; mice.

1. INTRODUCTION

The World Health Organization (WHO) revealed that depression is one of the leading causes of ill health and disability worldwide. More than 300 million people are living with depression, an increase of more than 18% between 2005 and 2015. It is exceeded by lower respiratory infections, perinatal conditions and HIV/AIDS [1,2]. Two-third of depressed patients experience suicidal thoughts and 10-15% of them attempt suicide [3]. At its worst, depression can lead to suicide [1]. It is a state of mood or energy level that includes lack of motivation, a sense of hopelessness and a loss of physical energy. It is an emotional status that can result from many parts of our life. It is a debilitating disease that affects a person's work, family, sleeping and ability to assess life [4,5]. A state of constant depression may suggest a biochemical imbalance or continual stress [4]. Depression is different from usual mood fluctuations and short-lived emotional responses to challenges in everyday life [1].

The main symptoms of depression are due to functional deficiency in the levels of monoaminergic transmitters, noradrenalin, 5-hydroxytryptamine and dopamine in the brain [6]. Drugs that increase the level of these neurotransmitters in the Central Nervous System (CNS) show antidepressant activity [7]. Noradrenergic and dopaminergic systems (monoaminergic neurotransmission) are precisely associated with pathogenesis of depression, and should be therefore considered as valuable targets in patients' treatment. Therefore the major antidepressant therapies aim for an enhancement in the transmitters levels in the neurons and thus normalize the neurotransmission [8, 9].

Depression is a common disorder with increasing lifetime rates [10]. It is often a debilitating disease that affects a person's work, family, sleeping and ability to assess life. Despite the advances in the

treatment of depression with selective serotonin reuptake inhibitors (SSRIs) and serotonin and norepinephrine reuptake inhibitors (SNRIs), there continue to be many unmet clinical needs with respect to both efficacy and side effects [4].

Many of the currently available antidepressant drugs have proven to be effective but they are burdened with some disadvantages such as various adverse effects, problematic interactions and relatively low response [11]. In addition, it is also reported that only two out of three patients respond to any given treatment and, of these, one would probably have responded to placebo alone [12]. On the other hand, drugs obtained from natural sources have good efficacy, least risk and low side effects profile. Recently, the search for novel pharmacotherapy from medicinal plants for psychiatric illnesses has progressed significantly. Therefore, herbal therapies should be considered as alternative or complementary medicines [13]. Hence there is an increasing interest in total medicinal plant extracts, the largest value of which may be due to its constituents that subscribe to the modulation of the oxidative balance in vivo. Additionally, the special importance of total plant extracts is that they are easily available products, without purification to apply them in possible prevention of diseases [14].

Oxidative stress is a condition of imbalances between oxidants and antioxidants. Numbers of evidences are supporting the involvement of oxidative and nitrosative stress in the pathophysiology of Major Depressive Disorder. Many of the negative effects of oxidative stress are decreased after supplementation with dietary antioxidants [15,16,17,18]. Medicinal plants have been utilized in the treatment of ailments for many years in different aboriginal medicine as well as folk medicine [19]. Over the centuries, garlic has acquired a special position in the folklore of many cultures as a formidable prophylactic and therapeutic medicinal agent [20]. Garlic and its preparations have been

widely recognized as agents for prevention and treatment of cardiovascular and other metabolic diseases, atherosclerosis, hyperlipidemia, thrombosis, hypertension, dementia, cancer and diabetes [19,21]. It has been reported to possess anti-stress, anti-ageing, memory improving properties and has the potential for preventing the progression of Alzheimer's disease [22,23,24]. Garlic has antibiotic, anti-cancer, antioxidant and anti-inflammatory properties; it can lower blood sugar levels and has protective effects on the cardiovascular. Furthermore, it is cheaper than many chemical drugs and easily accessible; therefore, it is generally better accepted by the patients resulting in a higher compliance rate. Garlic has neuroprotective effects attributed to its three interrelated antiatherogenic, antioxidant and anti apoptotic properties [25,22,26].

Neurotrophic factors are critical regulators of the formation and plasticity of neuronal networks. Brain-derived neurotrophic factor (BDNF) is abundant in the brain and periphery, and is found in both human serum and plasma. Studies have indicated that serum or plasma BDNF levels are decreased in untreated major depressive patients. Antidepressant treatment for at least four weeks can restore the decreased BDNF function up to the normal value. Antidepressant treatment promotes increased BDNF activity as well as several forms of neuronal plasticity, including neurogenesis, synaptogenesis and neuronal maturation [27]. Tumor necrosis Factor (TNF- α), a pro-inflammatory cytokine, increases in chronic disease conditions leading to depression. Therapy inhibiting TNF- α reduces depression in people with chronic disease [28].

The common animal model of depression is Forced swimming test (FST) because it induces a depressive-like manner to conceal antidepressant effect of chemicals and can conclude depressive-like behaviour in rats after exposure to other stressors. FST explains a stressing situation which capable of creating a state of lower habit, reflected in the motionlessness induced in animals. The suggested mechanism by which stress causes its effects is via the hypothalamic–pituitary–adrenal axis and/or via the sympathoadrenomedullary system [29].

Environmental enrichment (EE) influences the frequency and diversity of positive natural behaviour, decreasing the occurrence of abnormal behaviour [30]. Enriched rats usually show a superior ability to adapt or cope, when a

situation is highly conflicting or stressful and has to be solved by using complex strategies [31,32]. Enriched housing has a positive effect on the immune system and can be hypothesized to act directly to enhance, or indirectly to buffer, the immune system upon exposure to an acute environmental or pharmacological challenge [33,34]. Also EE has been shown to promote plasticity and to protect against CNS insult. Neurogenesis by enrichment is promoted in adult rat hippocampus [35]. Some studies have demonstrated that EE can ameliorate stress-induced depressive-like behaviours [36].

2. METHODOLOGY

2.1 Extract Preparation

Fresh garlic (*Allium sativum*), was purchased from a local grocery store. The fresh garlic was air-dried, grinded into powder. Extraction was carried out using cold maceration with occasional shaking for 72 hr using 500 ml of 70% aqueous methanol for each 100 g of powdered material. Total of 300 g of garlic powder was used in 1.5 L of aqueous methanol. The extracts were concentrated in vacuo and subsequently referred to as aqueous methanol extract. The ratio of methanol (70%) to aqueous (30%) was 2:1 [37].

2.2 Acute Toxicity Study

Locke's method as described by [38] was used. It has two phases. In phase one, nine mice were divided into three groups of three mice each and administered with different doses (10, 100 and 1000 mg/kg) of the aqueous methanol extract of the garlic. They were observed for 24 hours to monitor their behaviours as well as mortality. While in phase two, three mice were divided into three groups of one mice each and administered higher doses (1600, 2900 and 5000 mg/kg) of the garlic extract and observed again for 24 hours.

Then the LD₅₀ was calculated by the formula:

$$LD_{50} = \sqrt{(D_0 \times D_{100})}$$

D_0 = Highest dose that gave no mortality,
 D_{100} = Lowest dose that produced mortality.

2.3 Phytochemical Screening

The extract was tested for the presence of the phytochemicals, Saponin, Tannins, Flavonoid, Alkaloid, Steroid, Terpenoid and Anthraquinone using the method described by [39].

2.4 Experimental Protocols

Forty two (42) male albino mice was purchased at the Gombe State University Department of Physiology Animal House, transported and housed in plastic cages at the animal house of the Department of Biological Sciences, Abubakar Tafawa Balewa University, Bauchi. After acclimatization, they were divided into seven groups of six mice each. Group 1 was given distilled water; groups 2 and 3 received 200 mg/kg and 400 mg/kg of aqueous methanolic extract of *Allium sativum* respectively; while groups 4 and 5 were in addition to receiving 200 mg/kg and 400 mg/kg of the extract, housed in an EE cage. Group 6 was only housed in an EE (enriched cage) and group 7 receives 20 mg/kg of imipramine hydrochloride (Kayhelt Pharmaceutical Ltd, Jos, Nigeria). The experiment lasted for six weeks after which the mice were subjected to behavioural tests and biochemical assays.

2.5 Enriched Environment

Ferplast rodent cage was purchased from Amazon, USA used for the test as an EE. Enrichment was defined in terms of physical environment and not social housing [40]. The cage consisted of coloured platforms, rodent tunnels, a ball, toys and a running wheel. A water bottle was suspended above the ceiling of the cage and food pellets were provided on the floor. For housing conditions the sawdust of the cage was changed once a week [41].

2.6 Force Swimming Test

The forced swimming test (FST) was for a period of two days and was carried out as a slight modification to the method of [42]. Rectangular glass cylinder of height 45cm and diameter 20cm was used. It was filled with water to the 15cm mark (maintained at 21-23 °C). On the first day, the animals were introduced into the glass cylinder and forced to swim for period of 15min until they became exhausted, then they were removed, dried and returned to their cages. On the second day, the animals were subjected to the same test 30 minutes after administration of the garlic extract (200 mg/kg and 400 mg/kg), (20 mg/kg) Imipramine was used as the standard control and (10 ml/kg) distilled water as the negative control. The animals were observed for a period of 6 min (2 min initial adjustment and 4 min for immobility observation) and immobility

time (period without swimming or moving any of its limbs) taken.

2.7 Tail Suspension Test

The tail suspension test (TST) was performed according to the method described by [43]. The principle of this test is that suspending mice upside down leads to characteristic behaviour immobility which resembles human depression. Mice were considered immobile when they hanged passively and completely motionless [44]. Each mouse was suspended by an adhesive tape attached 1-2cm from the tip of their tail to a horizontal bar for 30 minutes after the administration of the test substances. The bar was raised 50cm from the floor, the mice were observed for a period of 6 minutes (2 min initial adjustment and 4 min for immobility observation) and the immobility time (Total exposure time - Mobility time) was recorded with the aid of a stop watch. At the end of the experiment the tape was removed and the mice were returned to their cages.

2.8 Sucrose Preference Test

The sucrose preference test (SPT) as described by [45] was employed. This is a reward-based test, used as an indicator of anhedonia or the decreased ability to experience pleasure, which represents one of the core symptoms of depression. Rodents are born with an interest in sweet foods or solutions. Reduced preference for sweet solution in SPT represents anhedonia, while this reduction can be reversed by treatment with antidepressants. SPT was carried out in the animal's home cage. For the SPT, mice were presented with 2 dual bearing sipper tubes. One tube contained plain drinking water, and the second contained a sucrose solution. Water and sucrose solution intake was measured daily. Sucrose preference was calculated as a percentage of the volume of sucrose intake over the total volume of fluid intake and averaged over testing period.

2.9 Biochemical Analysis

2.9.1 Animal sacrifice and tissue processing

At the end of the behavioural studies, the mice were fasted overnight and subsequently anaesthetised with combination of ketamine (75 mg/kg) and diazepam (5 mg/kg) administered intramuscularly (im). They were dissected and the brain removed and washed in phosphate-

buffered solution (pH 7.4). Some brain tissues were homogenised with phosphate-buffered solution, centrifuged at 447 x g for approximately 20 min. The supernatant was carefully collected and used for the ELISA kit assays.

2.9.2 ELISA kits techniques

Malondialdehyde (MDA) ELISA kits, glutathione peroxidase (GPx) assay ELISA kits, Superoxide dismutase (SOD) ELISA kits, Tumour necrosis factor (TNF α) ELISA kits and Brain derived neurotrophic factor (BDNF) ELISA kits was purchased from FineTest Biotech company limited, Wuhan, Hubei, China. The assay was done following the manufacturer's manual based on the principles of each of the test. ELISA microplate reader was used to read the absorbance and the concentration of the solutions.

2.10 Data Analysis

All data obtained was analysed with SPSS version 23 using one way ANOVA followed by Tukey post hoc test and expressed as Mean $\pm$ SEM. Values with $P < 0.05$ was considered statistically significant.

3. RESULTS AND DISCUSSION

The result of the phytochemical screening of aqueous methanol extract of garlic showed the presence of Saponin, Tannins, Flavonoid, Terpenoid and Anthraquinone. And for the acute toxicity testing, the result achieved showed that the garlic extract is relatively safe without mortality even at 5000 mg/kg of animal. However the dosage of 200 mg/kg and 400 mg/kg were used.

The FST result (Fig. 1) showed that aqueous methanol extract of *Allium sativum* significantly reduced the immobility time in all the treated groups but more significantly in the 400 mg/kg group. There was also significant reduction in immobility time in the group kept in Enriched cage and received 400 mg/kg of the extract and the positive control (imipramine) group when compared with the negative control (distilled water) group. The group that received no intervention and housed in an enriched cage had significant reduction in immobility time when compared to the negative control (distilled water) group. Similarly there was significant reduction in immobility time in all treated groups but more significantly in the 400 mg/kg garlic extract and

EE group. The immobility displayed reflects a state of despair, lowered mood which reflects depressive disorders in humans but significantly reduced by treatment [46]. This result collaborated the findings of [47] which stated that garlic extract significantly decreased immobility time in dose-dependent manner in both TST and FST and is indicating of significant antidepressant activity (Figs. 1 & 2). And since the antidepressant - like action of the extract was comparable to imipramine (standard antidepressant), garlic extract may have therapeutic value for the management of depression probably through the inhibition of MAO-A and MAO-B levels and interacting with adrenergic, dopaminergic, serotonergic and GABAergic systems as postulated by [47]. However, this result doesn't agree with [24] who stated that processed garlic powder show no effect against stress. EE protects against the effects of chronic stress [48,49].

The effect of the extract was noticeable in the SPT as it showed that mice in the distilled water group had less preference for sucrose (anhedonia) as compared to the other groups that received garlic extract and/or housed in an EE and received imipramine (Table 1). Garlic and EE are factors for the reduction of anhedonia in the mice collaborating the finding of [50] that it significantly reversed sucrose preference index.

The increase in MDA concentration (Fig. 3) in negative control group (distilled water) could be due to the chronic mild stress the mice went through. The MDA concentration significantly reduced in all the garlic extract and EE groups. This is in agreement with the finding of [51] who stated that MDA level increases in predictable chronic stress. Elevated reactive oxygen species is an important mechanism in the pathophysiology of depression in rodents and humans with increase activity of SOD [52], though our finding showed no increase in SOD. There was a significant increase in antioxidant enzyme, GPx, in all treated group when compared to the distilled water group (Fig. 5). But the SOD increase wasn't statistically significant (Fig. 4). This correlate with the work of [53], who looked at the effects of chronic mild stress and imipramine on markers of oxidative stress and antioxidant system in rat liver and found no changes in SOD activity in any of the studied groups but noticed increased GPx activities [54] and also found that individual treatment with EE tends to up-regulate the activities of GPx. The GPx scavenges primarily

lipid peroxides and the increase in its activity is as a compensatory response to the lipid peroxidation that might have taken place due to the chronic stress.

Fig. 1. Effect of chronic administration of aqueous methanol extract of garlic (*Allium sativum*) on the behaviour of mice in the forced swimming test (FST): data presented as mean $\pm$ SEM; $p=0.05$ (compared with control) En = environment; a = statistical significance when compared to control (distilled water) group; b = statistical significance when compared to imipramine (standard drug); n = 6

Fig. 2. Effect of chronic administration of aqueous methanol extract of garlic (*Allium sativum*) on the behaviour of mice in the tail suspension test (TST): data presented as mean $\pm$ SEM; $p=0.05$ (compared with control) En = environment; a = statistical significance when compared to control (distilled water) group; b = statistical significance when compared to imipramine (standard drug); n = 6

Table 1. Effect of chronic administration of aqueous methanol extract of garlic (*Allium sativum*) on the behaviour of mice in sucrose preference test (SPT): data presented as Mean $\pm$ SEM; $p=0.05$ (compared with control), a = statistical significance when compared to control (distilled water), n = 6

Groups	Day - 1	Day - 2	Day - 3	Day - 4
Control (Distilled water)	5.00 $\pm$ 0.41	19.50 $\pm$ 0.50	22.00 $\pm$ 0.41	25.00 $\pm$ 1.08
200 mg/kg Garlic Extract	10.00 $\pm$ 0.40	20.00 $\pm$ 1.41	30.00 $\pm$ 1.08 ^a	26.00 $\pm$ 1.08
400 mg/kg Garlic Extract	15.00 $\pm$ 1.22 ^a	15.00 $\pm$ 1.47	33.00 $\pm$ 2.48 ^a	27.00 $\pm$ 0.82
200 mg/kg Garlic Extract + Enriched En.	22.00 $\pm$ 2.16 ^a	30.00 $\pm$ 0.41 ^a	31.00 $\pm$ 0.82 ^a	32.00 $\pm$ 0.82 ^a
400 mg/kg Garlic Extract + Enriched En.	30.00 $\pm$ 0.41 ^a	26.25 $\pm$ 2.87 ^a	35.00 $\pm$ 1.08 ^a	34.00 $\pm$ 0.41 ^a
Enriched En Only	15.00 $\pm$ 0.40 ^a	20.00 $\pm$ 0.41	25.00 $\pm$ 0.82 ^a	35.00 $\pm$ 1.09 ^a
Imipramine	15.00 $\pm$ 1.47 ^a	25.00 $\pm$ 1.08 ^a	30.00 $\pm$ 1.22	30.00 $\pm$ 0.41

Fig. 3. Effect of chronic administration of aqueous methanol extract of garlic (*Allium sativum*) on malondialdehyde (MDA) concentration in mice data presented as mean $\pm$ SEM; $p=0.05$ (compared with control) En = environment; a = statistical significance when compared to control (distilled water) group; b = statistical significance when compared to imipramine (standard drug); n = 6

Fig. 4. Effect of chronic administration of aqueous methanol extract of garlic (*Allium sativum*) on superoxide dismutase (SOD) concentration in mice data presented as mean $\pm$ SEM; $p=0.05$ (compared with control) En = environment; a = statistical significance when compared to control (distilled water) group; b = statistical significance when compared to imipramine (standard drug); n = 6

Fig. 5. Effect of chronic administration of aqueous methanol extract of garlic (*Allium sativum*) on glutathione peroxidase (GPx) concentration in mice. data presented as mean $\pm$ SEM; $p=0.05$ (compared with control) En = environment; a = statistical significance when compared to control (distilled water) group; b = statistical significance when compared to imipramine (standard drug); n = 6

Fig. 6. Effect of chronic administration of aqueous methanol extract of garlic (*Allium sativum*) on brain-derived neurotrophic factor (BDNF) concentration in mice. data presented as mean $\pm$ SEM; $p=0.05$ (compared with control) En = environment; a = statistical significance when compared to control (distilled water) group; b = statistical significance when compared to imipramine (standard drug); n = 6

Fig. 7. Effect of chronic administration of aqueous methanol extract of garlic (*Allium sativum*) on tumour necrosis factor alpha (TNF α) concentration in mice. data presented as mean $\pm$ SEM; $p=0.05$ (compared with control) En = environment; a = statistical significance when compared to control (distilled water) group; b = statistical significance when compared to imipramine (standard drug); n = 6

The effect on BDNF showed that there was significant increase in BDNF level in the EE only group as well as the 400 mg/kg with EE and the imipramine group (Fig. 6). And TNF- α reduced significantly in the groups that received the extract (200 mg/kg & 400 mg/kg) and housed in EE. These results showed that EE, extract of *allium sativum* alongside EE boost the level of BDNF and this might have played a role in mitigating depression in the mice [48] in their finding stated that garlic oil significantly increase hippocampal BDNF and reversed the sucrose preference index. Just as [55] found that animal with low immobility showed significant reductions of BDNF expression. It correlates with the finding of [56] that that EE may be useful for mitigating the detrimental effects of chronic stress in patients with depression. It exerts neuromodulatory effects across species, reducing vulnerability to stress and its biochemical impact [57].

The reduction in the TNF- α suggests that EE and the extract might have given neuroprotection to the mice since increase in the TNF- α could be due to chronic unpredictable mild stress and depression-like behavior may be mediated by TNF- α and subsequent damage to neurons, suggesting that TNF- α might be an important biomarker [58,59].

4. CONCLUSION

Mild chronic stress induces depressive behaviour in mice leading to the production of reactive oxygen species but administration of aqueous methanolic extract of *Allium sativum* as well as EE this ameliorates these effects. And combination of EE with aqueous methanolic extract of *Allium sativum* give a better amelioration as comparable with a standard drug. This suggests that garlic could be effective in the management of depression, so also enriching the environment and their combination give a better management.

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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