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AMELIORATIVE EFFECTS OF AQUEOUS GARLIC EXTRACTS ON LEAD- INDUCED NEUROBEHAVIOURAL CHANGES IN WISTAR RATS

Hamza G. Adamu[1] **and Rabiu A. Magaji**[2]

[1]Department of Human Anatomy, Faculty of Basic Medical Sciences, Yusuf Maitama Sule University, Kano, Nigeria.
[2]Department of Human Physiology, Faculty of Basic Medical Sciences, Ahmadu Bello University, Zaria, Nigeria.

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ABSTRACT

Body exposure to heavy metals including lead has been found to cause adverse effects especially on the nervous system which is the primary target. The study was intended to evaluate the ameliorative effect of aqueous garlic extracts on lead-induced neurobehavioural changes in Wistar rats. Twenty five Wistar rats were randomly divided into five groups. Control group (C) received distilled water. Lead only (L) group received lead acetate (120 mg/kg). Lead low dose garlic (L+LG) and Lead high dose garlic (L+HG) groups received aqueous garlic extract at 300 and 500 mg/kg, respectively after pretreatment with 120 mg/kg lead acetate. Lead DMSA (L+ DMSA) group received succimer (30 mg/kg) after pretreatment with the 120 mg/kg lead acetate. Morris water maze and beam walk test were employed to assess the spatial learning and memory and motor coordination, respectively. Lead acetate caused significant ($p \leq 0.05$) changes on the spatial learning and memory and motor coordination compared with the control. In the Morris water maze test, there was a significant reduction of latency in the lead and aqueous garlic extract or succimer groups while rats that were exposed to lead acetate only had an increased latency. Beam walking test results also showed significant increases in the latency and foot slips of the treated groups compared with the control. These results clearly showed that the aqueous garlic extract could ameliorate the effects of lead acetate on spatial learning, and memory and motor coordination.

Key words: *Lead, Memory and learning, Succimer, Morris water maze, Beam Walking, Garlic*

INTRODUCTION

Lead poisoning (also known as plumbism, colica pictorum, saturnism, devon colic, or painter's colic) is a medical condition in humans and other vertebrates caused by increased levels of the heavy metal lead in the body (Karri et al. 2008). Lead can be found in air, drinking water, soil and many industrial by products such as pipes, storage batteries, pigments and paints, glazes, automobiles, vinyl products, ceramic, ammunition, cable covers and radiation shielding as well as many agricultural products (Salisu et al. 2015). It enter body mainly through eating, drinking or inhalation where its absorption occur primarily in gastrointestinal tract and respiratory tract. Once injected, it is carried in blood and transported to various tissues such as liver, kidney, bone and brain (Saleh et al. 2018). Body's organs and systems exposure to heavy metals including lead has been found to cause a number of disturbances which include neurological, haematological, gastrointestinal, reproductive, circulatory, and immunological disorders Owolabi et al. 2012).

Heavy metals have shown demonstrable neurological effects (Reddy et al. 2003).and behavioural disorders which include; deficits in learning, spatial memory and motor skills (Prasanthi et al. 2005). However, previous data suggested that the neurotoxic effects of lead are mediated through interference with

Correspondence: Hamza G. Adamu, MSc, Department of Human Anatomy, Faculty of Basic Medical Sciences, Yusuf Maitama Sule University, PMB 3220, Kano. Nigeria. hgadamu@gmail.com; +2348036944764

cholinergic and aminergic system (Xu et al. 2009). Oxidative stress has also been reported as the most important mechanism in lead toxicity (Sherif et al. 2014). Lead catalyzed oxidative reactions and generate reactive oxygen species. These reactive oxygen species (ROS) inhibit the production of sulphurous antioxidants, inhibit enzyme reactions impairing heme production, cause inflammation in vascular endothelial cells, damage nucleic acids and inhibit deoxyribonucleic acid repair, and initiate lipid peroxidation in cellular membranes (Patrick 2006). The major treatments approaches are; the removal of the source of lead and chelation therapy (administration of agents that bind lead so it can be excreted) (Garcia and Gonzalez 2008; Flora et al. 2003). The effects of lead on different site of brain were reported by several studies but the hippocampus has been the focus of much research on lead effects (Petit et al. 1983; Soodi et al. 2008). Hippocampus is an area, known to undergo morphological changes in rats exposed to lead. It is also reported that effect of lead on NMDA (a glutamate receptor) on hippocampal cells is greater than on cortical cells (Guilarte et al. 1994).

Recently, the utilization of plants herbal has received significant attention where there has been a rising awareness in their therapeutic usage; among these is the Garlic. Garlic is one of the most researched plant with long history of medicinal use (Omotoso et al. 2009). Garlic contains sulphur, phosphorus, potassium and zinc ions, moderate amounts of selenium, vitamin A, vitamin C and smaller amounts of calcium, magnesium, sodium, iron, B complex vitamins and allicin, a compound to trap free radicals (El Demerdash et al. 2005). As such in this study we evaluated the ameliorative effects of aqueous garlic extract on the performance of Wistar rat in Morris water maze and beam walking test after exposure to lead, aqueous garlic extract or both.

MATERIALS AND METHODS

Ethical Approval/Clearance

Ethical approval/clearance was obtained from research and ethic committee of Ahmadu Bello University, Zaria, Nigeria.

Management and Treatment

Twenty five Wistar rats (both sexes) were used in this study and were purchased from the animal house, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria, Nigeria. The animals were maintained at the animal house, Department of Human Anatomy, Faculty of Medicine, Ahmadu Bello University, Zaria, Nigeria, under a controlled condition.

Experimental Groups

The Wistar rats were randomly divided into five Groups (n = 5) as follow:

Control (C): served as a control and received distilled water.

Lead only (L): each rat received 20% of LD_{50} of lead acetate (120 mg/kg body weight only) dissolved in distilled water once a day.

Lead + low dose garlic (L+LG): each rat received aqueous garlic extract (300 mg/kg body weight) and 20% LD_{50} of lead acetate (120 mg/kg body weight) dissolved in distilled water once a day by oral gavages.

Lead + high dose garlic (L+HG): each rat received aqueous garlic extract (500 mg/kg body weight) and 20% LD_{50} of lead acetate (120 mg/kg body weight) dissolved in distilled water once a day by oral gavages.

Lead + Meso 2, 3 dimercaptosuccinic acid (L+DMSA): each rat received Meso 2, 3 dimercaptosuccinic acid (30 mg/kg body weight) and 20% LD_{50} of lead acetate (120 mg/kg body weight) dissolved in distilled water once a day. All the treatments were carried out orally once a day for twenty one (21) days.

Lead Acetate and Its Preparation

Lead solution was prepared by dissolving 20% (120 mg/kg body weight) of the LD_{50} (600 mg/kg body weight) of the lead acetate in 1ml of distilled water for each of the adult Wistar rat according to their body weight (Magaji et al. 2014).

Source of Garlic and Preparation of the Extract

Fresh garlic bulbs was obtained from Abubakar Rimi Market, Kano and authenticated at the Department of Biological Science (Voucher No: 423) by Sani Muhammed, Ahmadu Bello University Zaria, Nigeria. The garlic bulbs were separated, peeled and washed with distilled water. After drying, the clean garlic bulbs were crushed with an electric grinder and then dissolved in distilled water. The extract are then decanted carefully using muslin cloth (Senapati et al. 2000).

Source of Succimer (Meso 2, 3 dimercapto-succinic acid)

Succimer (DMSA) was purchased from ABCAM Plc in United Kingdom with quote order reference number (#1887607) and product key (ab142294) respectively.

Neurobehavioural Study

Spatial memory and learning test (Morris Water Maze)

Morris water maze test was used to study spatial memory and learning according to the method of Morris (1981). The Morris water maze used in this study was a black circular pool (130 cm in diameter, 60 cm high) filled with water (30 cm depth) at 24 ± 2ºC. The pool was divided into 4 quadrants of equal

size. An invisible escape platform (10 cm diameter) was placed in the middle of one of the quadrants (2 cm below the water surface) equidistant from the side wall and middle of the pool.

The basic procedure for the Morris water maze test was that the rat was placed in a pool of water which contains an escape platform 2 cm away and below the water surface. The rat was released and allowed to swim around the pool in search of an exit and subsequent trials was performed to know if the rat was able to locate the platform in a shorter time. As the rat was learning to locate the hidden platform, any improvement in the time to find the platform was recorded. If the rat did not find the escape within 60 sec, it was manually guided to the escape platform by the experimenter. After the learning, the rats were administered with lead acetate and aqueous garlic extract once a day over a period of three (3) weeks and the above procedures were repeated during and after the administration. The memory of the rats were evaluated by the time the rats spent in locating the platform before and after administration.

Beam Walking Test

Fine motor coordination and balance was assessed by beam walking test. The apparatus used; consisted of a metre beam with flat surface resting 50 cm above the table top on two poles. A black box was placed at the end of the beam as the finishing point. Performance on the beam was quantified by measuring the time it takes for the rat to traverse the beam and the number of paw slips that occur in the process. The test was done in four days; three days of training and one day of testing respectively.

Statistics

All the results were analyzed using statistical package for social sciences (SPSS version 20) and the results were expressed as mean ± standard error of mean (SEM). The statistical significance between the means were analyzed using one way analysis of variance and p- value ≤ 0.05 was considered statistically significant..

RESULTS

Effect of aqueous Garlic extract on lead induced changes on Body Weight of the Rats

The results on the effect of lead administration on body weight changes are shown in Table 1. The results showed a significant difference in body weight between the control and the treated Groups ($p < 0.05$). There was a gradual increase in the mean body weight of the rats in the control while there was a gradual decrease in body weight of the rats in the lead only (L) group as compared with the rats in the control.

Table 1 Effect of Aqueous Garlic Extract on Lead Induced Changes on Body Weight of Wistar Rats

	0 Week	1st Week	2nd Week	3rd Week
Group	Mean ± SEM (g)	Mean ± SEM (g)	Mean ± SEM (g)	Mean ± SEM (g)
Control	096.00±0.71	103.00±2.52	119.00±5.57	121.33±1.86
Lead (L)	144.60±4.42	37.60±3.80**	137.60±3.80**	134.60±3.80**
L+LG	119.00±6.54	125.00±3.00*	125.00±3.00**	125.00±2.65
L+HG	120.00±6.18	137.40±3.42*	137.40±3.42*	141.80±3.48
L+DMSA	099.25±6.10	119.40±1.81	119.40±1.81	125.20±1.66

Significantly different at *p= < 0.05 or **p = < 0.01. g = grams

However, the rats in the lead and aqueous garlic extract or Succimer treated groups, showed gradual increases in the mean body weight which when compared with the rats in the control group were not significant after third week of administration but was statistically significant after the first and second weeks of administration ($p < 0.05$) as shown in Table 1.

Effect of Aqueous Garlic Extract on Lead Induced Changes on Spatial Learning and Memory Using Morris Water Maze Test

The results of the effect of lead acetate exposure on spatial learning and memory using Morris water maze test are shown in Table 2. The result showed that after four days of Morris water maze training for all groups (control and treated groups), the rats learnt how to locate the hidden platform indicated by the decreasing mean time taken by the rats to complete Morris water maze which showed no significant difference between groups ($p < 0.05$). However, after administration, the result showed that there were significant reduction in the meantime taken by the rats in control group to complete Morris water maze task, while the rats that were exposed to lead acetate only (L) had increases in the meantime taken to complete Morris water maze task which when compared with the rats in control group was found to be statistically significant ($p< 0.01$). However, the rats in the lead and aqueous garlic extract or succimer treated groups had a marked reduction in the

meantime taken to complete Morris water maze task which when compared with the rats in control group the difference was not significant ($p < 0.05$).

Table 2: Effect of Aqueous Garlic Extract on Lead Induced Changes on Morris Water Maze Test

	0 Week	1st Week	2nd Week	3rd Week
Group	Mean ± SEM (s)	Mean ± SEM (s)	Mean ± SEM (s)	Mean ± SEM (s)
Control	05.20±0.84	04.35±0.28	01.89±0.23	02.17±0.28
Lead (L)	06.11±0.88	11.16±0.88**	11.79±0.68**	17.23±1.02**
L+LG	03.63±0.71	02.49±0.27	03.70±0.45	16.89±0.82**
L+HG	07.06±3.25	04.81±0.38	03.59±0.22	03.75±0.42
L+DMSA	12.49±4.82	03.92±0.54	03.43±0.23	03.36±0.42

** significantly different at $p < 0.01$

Effect of aqueous Garlic extract on lead induced Changes on Motor Coordination Using Beam Walking Test

The results of beam walking test are shown in Table 3. The results showed a significant difference across all the treated groups ($p < 0.05$). In the control, the results showed that there was non-significant increase in the meantime taken by the rats to traverse the beam while significant increase were observed in the meantime taken by the rats in lead treated groups to traverse the beam which when compared with the control group was found to be statistically significant ($p < 0.05$) during the first and second weeks of administration. However, The result also showed a significant decrease in the time (s) taken by the rats in L+HG and L+DMSA groups to traverse the beam when compared to the rats in lead only and L+LG groups ($p < 0.05$).

Table 3: Effect of Aqueous Garlic Extract on Lead Induced Changes on Motor Coordination Using Beam Walking Assay of Wistar Rat

	0 Week	1ST Week	2nd Week	3rd Week
Group	Mean ± SEM (s)	Mean ± SEM (s)	Mean ± SEM (s)	Mean ± SEM (s)
Control	41.95±1.31	41.66±0.85	42.16±1.26	42.94±3.61
Lead (L)	52.63±1.06**	83.32±1.34**	54.88±1.29**	75.66±3.13**
L+LG	53.40±0.87**	79.91±4.34**	55.50±1.15**	78.27±5.69*
L+HG	55.46±1.42**	59.77±3.96**	52.22±2.42*	63.39±4.44
L+DMSA	54.33±0.70**	54.68±1.59*	50.37±3.70	56.24±1.15

Significantly different at *p = < 0.05 or **p= < 0.01.

DISCUSSION

The present study showed that there were reduction in mean body weight of the rats in the lead treated group only when compared with the rats in the control group. This reduction in the mean body weight with continuous exposure to lead could be explained on the basis of anorexia which is induced by lead exposure (Klaassen 2001). Another possible explanation for the reduction of the mean body weight could be as result of the decreased muscle mass and cachexia due to the oxidative stress induced by lead. However, many study have showed that heavy metal toxicity was associated with oxidative stress (Yeh et al. 2009) which according to many researchers was associated with muscle wasting and cachexia leading to low body weight (Buck et al. 1998).The result of the effect of lead on body weight of the Wistar rats agreed with many other studies of the effect of lead on body weight of Wistar rat, one of such studies was the work of Amjad et al. (2013) in which lead was given, to study its effect on the body and kidney weights of Wistar albino rats which was ameliorated by *Ginkgo biloba* extract. The result of the present study also showed that rats treated with lead acetate and aqueous garlic extract or succimer (DMSA) showed slight increase in the body weight which when compared with the rats in the control group was not significant. The slight increase in the mean body weight in treated groups, which were given both lead acetate as well as aqueous garlic extract, could be attributed to the antioxidant property of garlic.

On the day 4 of training no significant differences in meantime taken by the rats to complete Morris water maze task were observed which indicated that all groups learned how to find the hidden platform. During administration, the result of the behavioural

studies in the present study showed that there was decrease in the meantime taken by the rats in control group to completing the Morris water maze task while there were significant increase in mean time taken by the rats to complete the Morris water maze task in the lead treated group only and this could be due to lead's neurotoxicity on the membrane ionic channels and signalling molecules. Lead interferes with the most common neurotransmitter in the brain, glutamate, which is critical for learning in the brain (Cory-Slechta 1995). The N-methyl-D-aspartate receptor (NMDA-r) is an ionotropic receptor for glutamate. The activation of the NMDA-r results opens an ion channel t hat is nonselective to cations, thereby allowing small amounts of Ca^{2+} into the cell. This calcium flux is thought to play a critical role in the development of synaptic plasticity, a cellular mechanism for learning and memory. Lead (Pb^{2+}) acts as a non-competitive, voltage-independent antagonist of the NMDA-r channel, disrupting long-term potentiation, a process believed to be responsible for the acquisition of information, thereby compromising the retention of newly learned information (Zhu et al. 2005). The result obtained from the present study agreed with many other studies on the effect of lead on neurobehavioural changes. One of such work was by Bazrgar et al. (2015), on the effect of postnatal lead exposure on the spatial learning and memory in male rats. The main finding of the present study was that the administration of aqueous garlic extract or succimer (DMSA) caused better performance in Morris water maze task and beam walking compared with the lead treated group only. The rats treated with lead and aqueous garlic extract or succimer had decrease mean time taken to complete Morris water maze test. This could be attributed to garlic's antioxidant property due to the presence of sulphur-containing amino acids and compounds having free carboxyl (C=0) and amino (NH_2) groups in their structures. These biologically active compounds might have chelated lead and enhanced its excretion from the body resulting in reduced lead accumulation in brain and blood (Senapati et al. 2000).

On the other hand, the result of the present study on motor coordination showed a significant increase in the meantime taken by the rats in lead only treated group to traverse the beam which when compared with the control group was found to be significant While the results in lead and aqueous garlic extract or succimer (DMSA) treated groups showed that there were slightly decrease in the meantime taken by the rats to traverse the beam which indicated the effectiveness of aqueous garlic extract on induced changes on the cerebellum.

Conclusion

In the present study, we demonstrated that lead exposure resulted in significant learning and memory deficits as well as motor coordination in Wistar rats, as indicated by longer escape latency compared to control group. Moreover, rats that are exposed to the same concentration of lead and aqueous garlic extract or succimer did not showed spatial learning and memory deficits or motor coordination deficits. From this data, we can conclude that aqueous garlic extract could have a beneficial effect against lead neurotoxicity.

Conflict of Interest

None declared.

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