

Full Length Research paper

Hepatoprotective and some haematological effects of *Allium sativum* and vitamin C in lead-exposed wistar rats

G. O. Ajayi^{1,2}, T. T. Adeniyi^{1*} and D. O. Babayemi¹

¹Department of Biochemistry, College of Natural Sciences, University of Agriculture, P. M. B. 2240, Abeokuta, Ogun State, Nigeria.

²Department of Medical Biochemistry, Faculty of Basic Medical Sciences, Lagos State University College of Medicine, P. M. B. 21266, Ikeja, Lagos, Nigeria.

Accepted 12 September, 2019

The hepatoprotective and some haematological effects of *Allium sativum* (Garlic) and vitamin C were studied on experimental rats that were exposed to lead (Pb) for one week. Twenty – four female Wistar rats were divided into four groups of six rats per group. Rats in group A were kept as the control. Rats in groups B, C and D were exposed to 100 µmol/kg body weight of lead acetate intraperitoneally for seven days. Garlic diet (200 g minced garlic/kg diet) and vitamin C (500 mg/kg body weight) were given to rats in groups C and D for additional seven days respectively. Garlic and vitamin C produced significant reduction at $p < 0.05$ in the levels of ALT, ALP and PCV while the level of AST increases significantly at $p < 0.05$. The level of Hb increases significantly at $p < 0.05$ in rats treated with garlic and reduces significantly at $p < 0.05$ in rats treated with Vitamin C. This study, therefore suggests that garlic and vitamin C have some hepatoprotective and haematological effects.

Key words: Hepatoprotective, haematological, *Allium sativum*, vitamin C, Lead (Pb).

INTRODUCTION

Lead (Pb), a heavy metal, has continued to pose health hazards in animal and man in Nigeria and many other parts of the world. Many people who are exposed to gasoline, paints, exhaust fumes from automobile through inhalation, oral or dermal route have suffered from lots of health problems (Ademuyiwa et al., 1994). Pb is a cumulative poison and it affects every organ and system in the body. It is accumulated in the bone, brain, muscle, liver, kidney, haematopoietic system, central nervous system and gastrointestinal system (Goyer, 1990; Senapati et al., 2001; Adeniyi et al., 2008). Lead accumulation and intoxication in adults and children has also resulted in various behavioural and physiological disorders (Adeniyi et al., 2008). Several chelating agents have been used to reduce the burden of the toxic effect of lead, but these have also produced a toxic potential themselves. This has necessitated researches into the therapeutic potential of

various medicinal plants and herbs (Senapati et al., 2001).

Allium sativum which is commonly called garlic belongs to the family *Liliaceae* and genus *Allium* (Krishnaraju et al., 2006). Garlic is like onion made up of bulbs called cloves. It is cultivated in some parts of Nigeria and used as meat tenderizer and spice in many delicacies (Mora-kinyo et al., 2008). The traditional medical practitioners have considered garlic as an excellent medicinal plant that has a lot of therapeutic potentials. Garlic is used as an anti-hypertension (Krishnaraju et al., 2006), anti-rheumatic and stimulant, and in the treatment of various ailments like asthma, diabetes, cold, paralysis, forgetfulness, tremor, colicky pain and chronic fever (Morakinyo et al., 2008; Nadkarni, 1994). It has been found that garlic lowered blood pressure and cholesterol level (Garouri et al., 1989; Siligaly and Neil, 1994) and possesses strong anti-microbial activity (Sood, 2003).

Vitamin C (Ascorbic acid), a water soluble vitamin is derived from dietary sources such as citrus fruits, grapefruits, berries, cabbage, tomatoes, pepper and leafy vegetables. The therapeutic potential of vitamin C is as a

*Corresponding author. E-mail: ttadeniyi@yahoo.com. Tel.: +234-803-389-2442.

Table 1. Effect of *A. sativum* and vitamin C on the serum levels of AST, ALT and ALP in post-lead treated rats.

Group	Treatment	Serum ALT (U/L)	Serum AST (U/L)	Serum ALP (U/L)
A	Control	158.33±24.01	466.67±55.74	44.77±25.74
B	Lead only	223.33±38.82 ^a	241.67±86.81 ^a	75.75±46.33 ^a
C	Lead + <i>A. sativum</i>	166.67±24.22 ^b	375.01±101.34 ^b	32.70±27.19 ^{a,b}
D	Lead + Vitamin C	161.67±34.30 ^b	333.30±98.12 ^{a,b}	43.92±27.53 ^b

^a and ^b represent significant values at $p < 0.05$ when compared to control (group 1) and lead only (group 2) values respectively.

result of its antioxidant effect on free-radicals (Adeniyi et al., 2008). It improves neutrophil migration in chronic granulomatous disease *in vitro* decreases the frequency of bacterial infection used clinically on this condition. Excess vitamin C may predispose premature infants to haemolytic anaemia due to fragility of the red blood cells (Cook and Monsen, 2000).

The recent published report by us, Adeniyi et al. (2008), that ascorbic acid and *A. sativum* have ameliorative potential on tissue lead level in female *Rattus navigicus* prompted the design of this study.

MATERIALS AND METHODS

Plant materials and diet preparation

Methods of collection of materials and preparation of diet were as reported by Adeniyi et al. (2008).

Experimental animal

Twenty – four healthy female rats (*R. navigicus*) weighing between 125 and 200 g were obtained from the Animal House of the College of Veterinary Medicine (COLVET), University of Agriculture, Abeokuta (UNAAB), Ogun State, Nigeria. These rats were treated in accordance with the internationally accepted principles for laboratory animal use and care. They were housed in standard cages, under clean environmental conditions ($23 \pm 1^\circ\text{C}$, with $55 \pm 5\%$ humidity and a 12 h / 12 h light / dark cycles) and allowed to acclimatize for 14 days.

Experimental design and animal treatment

Animals were divided into four groups consisting six rats per group. Group A was the control, fed with animal feed and water *ad libitum* only for fourteen days. Group B was injected intraperitoneally for seven days with 100 $\mu\text{mol/kg}$ body weight lead acetate- $[(\text{CH}_3\text{COO})_2\text{Pb} \cdot 3\text{H}_2\text{O}]$ supplied by J. T. Baker Chemical Company, Dagentiam, England in addition to the feed and water *ad libitum*. Group C was also injected with lead acetate for seven days and then fed with garlic supplement diet (200 g minced garlic/kg diet) for seven days. Group D was injected with lead acetate for seven days and was later given vitamin C (500 mg/kg body weight) supplied by Sigma Chemical (Germany) for another seven days.

Finally, group B rats were sacrificed after one week of lead acetate injection while groups A, C and D rats were sacrificed at the end of second week of garlic and vitamin C treatment. The sacrifice was done twenty – four hours after the last injection or treatment

under ether anaesthesia. Blood samples were collected by heart puncture into clean heparinized bottles for plasma preparation for AST, ALT and ALP activities assay and whole blood collected for the haematological analysis.

Enzymatic assays

Alanine and aspartate aminotransferases determination

Plasma assays for tests on the function of liver vis- a-viz serum aspartate amino transferase (AST) and alanine amino transferase (ALT) activities were estimated with the Randox reagent kit using 2,4-dinitrophenylhydrazine as substrate according to the method described by Reitman and Frankel (1957).

Alkaline phosphatase determination

Alkaline phosphatase (ALP) activity was determined with the Randox reagent kit using the p-nitrophenylphosphate as substrate according to the method described by Bassey et al. (1946).

Haematological analysis

The blood samples collected for haematological parameters were analyzed for packed cell volume (PCV) and haemoglobin (Hb) level.

Statistical analysis

Results are reported as mean \pm standard deviation (SD). Statistical analysis was carried out using two-way analysis of variance (ANOVA). Statistical significance was considered at $p < 0.05$.

RESULTS

Effect of *A. sativum* and vitamin C on the levels of plasma ALT, AST and ALP enzymes in post-lead treated rats

Administration of lead significantly increased ($p < 0.05$) plasma ALT and ALP activities in the rats (Table 1). Specifically, the activities of ALT and ALP were increased by 29.1 and 69.2% respectively. Plasma AST activity was significantly decreased ($p < 0.05$) by 48.3% compared to the control. However, treatment with *A. sativum* produced significant decrease ($p < 0.05$) on the activities of plasma ALT and ALP by 25.4 and 56.8%, respectively while it produced significant increase ($p < 0.05$) on plasma AST

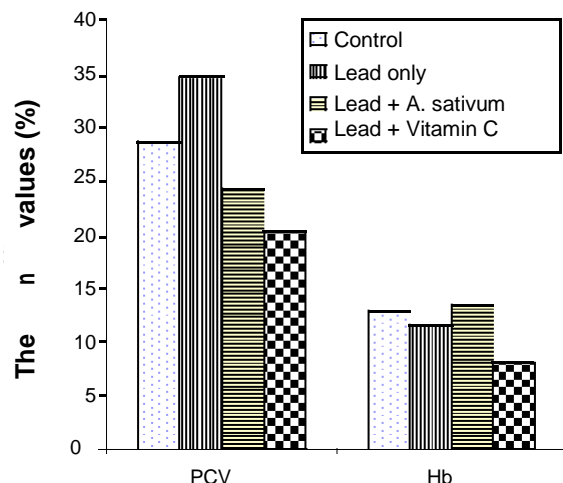


Figure 1. Effects of *Allium sativum* and vitamin C on percentage (%) PCV and Hb in post-lead treated rats.

activity by 55.2% in post-lead treated rats compared to the lead treated rats. Furthermore, treatment with vitamin C also followed the same trend as treatment with *A. sativum*. Vitamin C produced significant decrease ($p < 0.05$) on the levels of plasma ALT and ALP by 27.6 and 42.0% respectively while it produced significant increase ($p < 0.05$) on the level of serum AST by 37.9% in the post-lead treated rats compared to the lead treated rats.

Effect of *A. sativum* and vitamin C on the percentage (%) PCV and Hb in post-lead treated rats

Administration of lead significantly increased ($p < 0.05$) the percentage (%) PCV by 21.1% and non-significantly decreased ($p < 0.05$) the percentage (%) Hb by 10.4% compared to the control (Figure 1). However, treatment with *A. sativum* produced a significant decrease ($p < 0.05$) PCV by 30.0% and non-significant increase ($p < 0.05$) Hb by 16.3% in the post-lead treated rats compared to the lead treated rats (Figure 1). The treatment with vitamin C produced significant decrease ($p < 0.05$) PCV and Hb by 41.4 and 30.0% respectively compared to the lead treated rats. It was found that the values of PCV and Hb were further significantly lowered ($p < 0.05$) by 29.2 and 37.3% respectively in vitamin C treated rats and PCV value non-significantly lowered ($p < 0.05$) by 15.2% in *A. sativum* treated rats compared to the control.

DISCUSSION

Lead has been known to be environmental pollutant and its toxicity has also been associated with some health hazards. Liver enzymes such as ALT, AST and ALP are marker enzymes for liver function and integrity (Jens and Hanne, 2002; Adaramoye et al., 2008). These enzymes are usually raised in acute hepatotoxicity or mild hepato-

cellular injury, but tend to decrease with prolonged intoxication due to damage to the liver (Cornelius, 1979; Jens and Hanne, 2002).

In this study, administration of lead showed significant increase in plasma ALT and ALP activities, and conversely decrease plasma AST activity level. The present available data suggest that lead exerts possible hepatotoxic effect as the increase in ALT and ALP suggest liver damage. Lead is known to bind to the sulfhydryl groups of enzymes containing cysteine, and found to form complexes with amino acids and protein. Since ALT is liver enzyme, lead will alter the level of ALT activity in the tissues by disrupting their membrane. Consequently, there will be a discharge of the cell content into the blood stream and ALT activity is known to increase only in heavy metal poisoning, toxic hepatosis, and muscular dystrophy (Nduka, 1999). Post-lead treatment with *A. sativum* and vitamin C significantly reduced the activities of ALT and ALP, and increased the activity of AST when compared to the rats treated with lead alone. The reduced serum ALT and ALP activities may generally be attributed to decreased production of these enzymes from these sources (Olagunju et al., 2006) hence denotes the reversing effect of lead toxicity in rats.

The haematological effect of lead led to significant increase of PCV and non-significant increase of Hb. However, post-lead treatment of rats with *A. sativum* and vitamin C results into significant decrease of PCV and Hb compared to rats treated with lead only and control. This effect may be attributed to the combined effects of the inhibition of Hb synthesis and shortened life-span of circulating erythrocytes, and these may result in anaemia. This is contrary to the various reports by Oluwole (2001) and Bartimaeus et al. (2002) that administration of garlic and vitamin C in rats not exposed to lead has the ability to increase PCV and Hb; because of their ability to bind lead and chelate the effect of lead toxicity in rats. The effect noticed in this research work may be as a result of short duration of post-lead treatment of only one week.

Conclusion

Overall, our results confirm that administration of lead in rats caused some level of liver or hepatic damage in the animals and post-lead treatment with *A. sativum* (garlic) and vitamin C also have some hepatoprotective and haematological effects on these rats. Moreover, efforts are currently underway to look at the effect of increasing the post-treatment period in rats.

REFERENCES

- Adaramoye OA, Osaimoje DO, Akinsanya MA, Nneji CM, Fafunso MA, Ademowo OG (2008). Changes in antioxidant status and biochemical indices after acute administration of artemether, artemether-lumefantrine and halofantrine in rats. Authors J. Compilation: Basic Clin. Pharmacol. Toxicol. 102: 412- 418.

- Ademuyiwa O, Adesanya O, Ajuwon OR (1994). Vitamin C in CCl₄ hepatotoxicity - A preliminary report. *Hum. Expt. Toxicol.* 13: 107-109.
- Adeniyi TT, Ajayi GO, Akinloye OA (2008). Effect of Ascorbic acid and *Allium sativum* on Tissue lead level in female *Rattus navigicus*. *Niger. J. Health Biomed. Sci.* 7(2): 38-41.
- Bartimaues ES (2002). Toxicological effects of garlic (*Allium sativum*) on some haematological and biochemical parameters in rats. *Global J. Environ. Sci.* 2(1): 11-16.
- Bassey OA, Lowry OH, Brock MJ (1946). A method for the rapid determination of alkaline phosphatase with five cubic millimetres of serum. *J. Biol. Chem.* 164: 321-325.
- Cook A, Monsen P (2000). Biochemistry and effect of vitamins on iron and zinc absorption. *Gastroenterol.* 9: 1408 -1413.
- Cornelius CE (1979). Biochemical evaluation of hepatic function in dogs. *J. Am. Anim. Hosp. Assoc.* 15: 25- 29.
- Garouri YH, Moreau MK, Jain GH, Deltass RV (1989). The chemistry of garlic health benefits. *Biochem. Biophys. Acta.* 1006: 137-139.
- Goyer RA (1990). Lead toxicity: from overt to sub-clinical to subtle health effects. *Environ. Health Perspectives.* 86: 177-181.
- Jens JJ, Hanne H (2002). A Review on Liver Function Test. The Danish Hepatitis C: website
http://home3.inet.tele.dk/omni/hemochromatosis_iron.htm
- Krishnaraju AV, Rao TVN, Sundararaju D, Tsay MH-S, Subbaraju GV (2006). Biological screening of medicinal plants collected from Eastern Ghats of India using *Artemia salina* (Brine shrimp test). *Inter. J. Appl. Sci. Eng.* 4(2): 115-125.
- Morakinyo AO, Oloyo AK, Raji Y, Adegoke OA (2008). Effects of Aqueous extract of garlic (*Allium sativum*) on testicular functions in the rats. *Niger. J. Health Biomed. Sci.* 7(2): 26-30.
- Nadkarni AK (1994). *Indian Materia Medica I and II*. Popular Prakashan, Bombay. pp. 66-71.
- Nduka N (1999). *Clinical Biochemistry for Students of Pathology*. Longman Nigerian Plc: pp.1-236.
- Olajunju JA, Fagbohunka BS, Oyedapo OO, Abdul-Azeez IA (2006). Effects of an ethanolic root extract of *Plumbago zeylanica* Linn on some serum parameters of the rat. *RPPM – Drug Dev. Mole.* 11: 267-276.
- Oluwole FS (2001). Effect of garlic on some haematological and biochemical parameters. *Afr. J. Biomed. Res.* 4: 139-141.
- Reitman S, Frankel S (1957). A colorimetric method for the determination of glutamic oxaloacetic and glutamic pyruvic transaminases. *Am. J. Clin. Pathol.* 28: 56-66.
- Senapati SK, Dey S, Dwivedi SK, Swarup D (2001). Effect of garlic (*Allium Sativum* L.) extract on tissue lead level in rats. *J. Ethnopharmacol.* 76: 229-232.
- Siligaly CS, Neil HAW (1994). Garlic as lipid lowering agent: a metal analysis. *J. Royal College Phys.* 28(1): 3-45.
- Sood DR, Vinod Chhokar and Shilpa (2003). Effect of Garlic (*Allium sativum* L.) extract on degree of hydration, fructose, sulphur and phosphorus contents of rat eyelens and intestinal absorption of nutrients. *Indian J. Clin. Biochem.* 18(2):190-196.