Research Article

Genomic Modification by *Ocimum canum* Against Lead-Induced Chromosome Aberration and It's Effect on Antioxidant Enzymes

Tugbobo Oladimeji Samuel[1], Abegunde Segun Michael[2], Idowu Kayode Solomon[3]

Abstract

Introduction: Anticlastogenic potential of *Ocimum canum* (Black leaf) extract was studied in bone marrow cells of mice using micronucleus assay.

Experimental: 200mg/kg of *Ocimum canum* aqueous extract was administered as dietary supplement for 30-days. The mice were divided into three groups A, B and C. Animals in group A were fed with distilled water, B were treated with 2.5mg/kg lead acetate while group C were fed with 200mg/kg *Ocimum canum* aqueous extract and 2.5mg/kg lead acetate simultaneously. After 30-days, mice were sacrificed and chromosome preparations were made from bone marrow according to colchicines hypotonic-fixation air drying Giemsa schedule. The cytogenic end-point observed was chromosomal aberration which increased significantly ($P<0.05$) in group B animals treated with lead acetate only. However, the chromosomal aberration was significantly ($P<0.05$) reduced by the extract fed to animals in group C. In addition, the effect of the extract on the defensive antioxidant enzymes of the test animals was also assessed.

Result: The results indicate synergistic effect of the extract on the antioxidant enzymes in the liver tissues.

Conclusion: Hence, the results of this study suggest viable anticlastogenic and antioxidant potentials of *Ocimum canum* extract which could protect against lead-induced chromosomal aberration and as well enhance activities of antioxidant enzymes.

Keywords: Chromosomal-aberration, *Ocimum canum*, Lead acetate, Anticlastogen, Antioxidant

Introduction

Heavy metals are mostly clastogens in the environment causing oxidative burst in the exposed individuals leading to tissue damage. Damage to DNA and other body tissues by these metals is likely to be a major cause of cancer and genetic birth defects and may as well contribute to aging and cardiovascular diseases (1). The metals are mostly chemicals present in the diets as complex mixture, or as contaminants as well as e-waste. Large amount of these chemicals were tested on their ability to cause damage with newly developed short and long-term tests that accounts for mutagenicity, clatogenicity and carcinogenicity among which lead, cadmium, arsenite, mercury are notable (2). Studies conducted about a decade ago by Environmental Protection Agency and other associated International Regulatory agencies showed that low level exposure to lead is associated with societal problems such as brain dysfunction, neurobehavioural changes as well as kidney and liver diseases (3). Medicinal plants from time immemorial have been used in virtually all

[1,2,3]Department of Science Technology, Federal Polytechnic, Ado-Ekiti, Nigeria.

Correspondence: Dr. Tugbobo Oladimeji Samuel, Department of Science Technology, Federal Polytechnic, Ado-Ekiti, Nigeria.

E-mail Id: tugbobooladimeji@gmail.com

Orcid Id: https://orcid.org/0000-0002-0490-8775

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cultures for healing purposes (4). They are considered to be the backbone of traditional medicine and are widely used to treat acute and chronic diseases. The World Health Organization estimated that about 80% of the world population relies mainly on traditional medicines. *Ocimum canum* is grown for its medicinal and culinary value and it is highly useful in treating various types of diseases and in lowering blood glucose, especially in type 2 diabetes levels (5). The plant is reported to be rich in volatile essential oils of therapeutic importance (6). Its anticlastogenic and antioxidant potentials against lead-induced chromosome aberration would be investigated in this study.

Materials and Methods

Experimental design

The in vivo experiment was conducted using twelve male albino mice weighing between (80-100)g housed in stainless cage with temperature maintained at 25±2^{0}C and 12h alternating day/night cycle and mice were fed standard pellets and water *ad libitum*. The animals were divided into three groups with four in each group. Mice in group A served as control and were treated with distilled water only; B received 2.5mg/kg lead acetate; group C mice were fed simultaneously with 2.5mg/kg lead acetate and 200mg/kg *Ocimum canum* aqueous extract (1:1). The concentration of lead salt was made equivalent to $^1/_{10}$ of LD_{50} (7). The dose of *Ocimum canum* used was equivalent to exact concentration used for beneficial effect against specific disease conditions (8). Each dose was administered via oral gavage into the mice on daily basis consecutively for 4-weeks. The handling and use of the animals were in strict compliance with national institute of animal husbandry (NIAH) guide for the care and management of laboratory animals.

Chromosome aberrations

Chromosomes were examined from bone marrow cells following the usual colchicines-hypotonic fixation technique (9). Animals were sacrificed and their femurs quickly removed where the bone marrow was flushed out into the centrifuge tube with freshly prepared and pre-warmed (36^{0}C), 0.56% KCl solution into 8ml centrifuge tubes. The fat lumps were removed with fine tipped pipette and was allowed to stand for 1hr to allow cells swell in the hypotonic solution which were later centrifuged (100rpm) for 5min where the supernatant was separated using Pasteur pipette (10). Freshly prepared glacial acetic acid methanol [1:3] was added to the cells and vigorous agitated (11).The slides were coded and scored blind and were stained with Giemsa for 15min, rinsed, dried at room temperature and were carefully placed on the microscope (40x) for better view of chromosomes. The identified chromosome spreads were later viewed under (100x) oil immersion objective. The end-points scored were chromosomal aberrations and damage cells.

Reduced glutathione (GSH)

0.2ml of liver homogenate was added to 1.8ml distilled water and 3ml of sulphosalicylic acid was mixed with 2.5ml of *Ocimum canum* extract. This was centrifuged at 3000g for 5min and 0.5ml supernatant was added to 4.5ml of Ellman's reagent. A blank was prepared with 0.5ml phosphate buffer with dilute precipitating reagent and 0.5ml Ellman's reagent. The absorbance of the reaction mixture was taken within 30min of color development at 412nm and GSH concentration was extrapolated from its standard curve (12).

Superoxide dismutase (SOD)

The activity of superoxide dismutase in the homogenates was determined according to the method described by (13). 0.2ml aqueous extract was added to 2.5ml of 0.05M carbonate buffer of pH 10.2 to equilibrate in the spectrophotometer. The reaction was initiated by the addition of 0.3ml of freshly prepared 0.3mM adrenaline to the mixture which was quickly mixed by inversion. The reference cuvette contained 2.5ml buffer, 0.3ml of adrenaline and 0.2ml distilled water. The increase in absorbance at 480nm was monitored every 30secs for 150secs.

Catalase (CAT)

The activity of catalase in the liver homogenate was determined using method described by (14). 70µl aqueous extract was mixed with 920µl sodium phosphate buffer at pH 7.0 containing 0.1mM EDTA. The reaction started by adding 10µl of hydrogen peroxide and the decrease in H_2O_2 concentration was taken by reading the absorbance at 240nm at 10secs intervals for 180sec.

Results

Table 1.Treatment with 2.5mg/kg lead acetate and 200mg/kg aqueous extract of O. canum

Group	Treatment (mg/kg)	% G	B	RR	Mean ± SD
A	distilled water	2	0	0	1.40 ± 1.95
B	2.5 lead acetate	32	37	25	19.80 ± 16.36
C	200 O.canum+ 2.5 lead acetate	7	12	2	7.20 ± 6.91

Mean of 4 mice ± standard error of the mean; G – chromosome gap, B = chromosome break, RR= chromosome rearrangement.

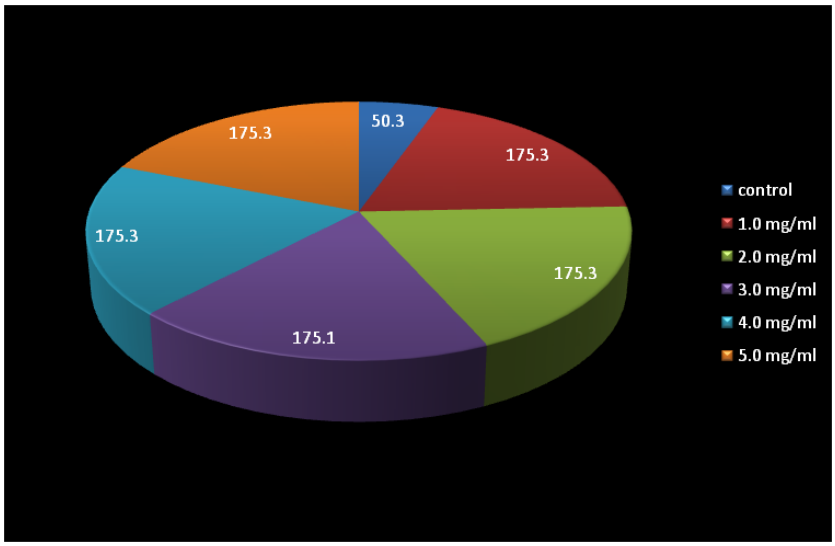

Figure 1.Effect of *Ocimum canum* aqueous extract on concentration level of reduced glutathione

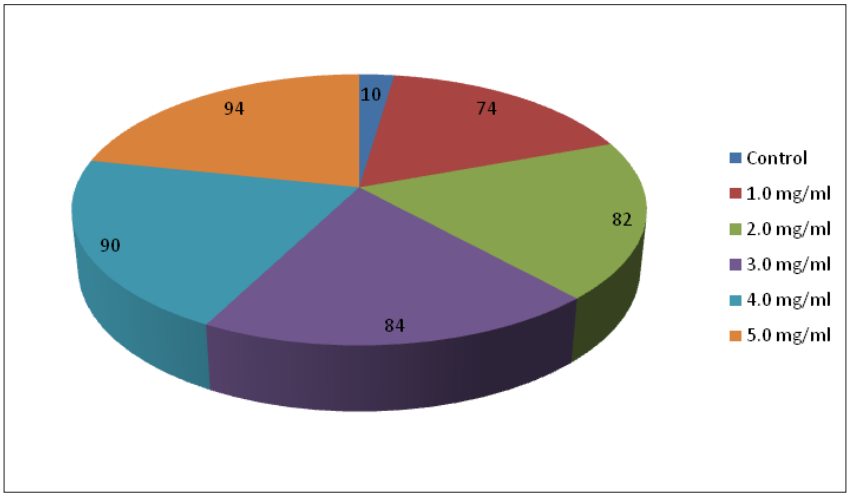

Figure 2.Effect of *Ocimum canum* aqueous extract on superoxide dismutase activity

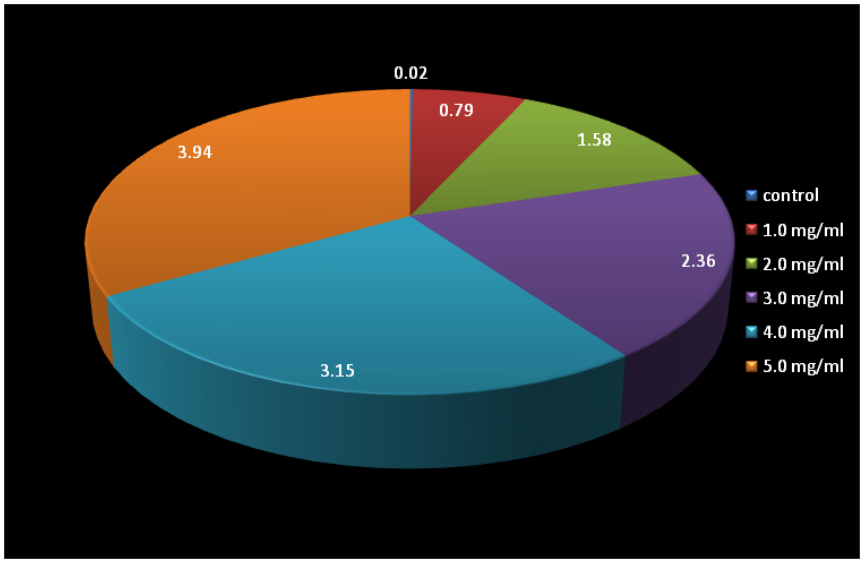

Figure 3.Effect of *Ocimum canum* aqueous extract on catalase activity

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Discussion

The result of chromosomal aberration shown in Table 1 above reveals the clastogenic effect of lead acetate in the test animals. The total chromosomal aberration recorded shows highest percentage of chromosomal aberration at 25% in animals in group B fed with lead acetate only while lowest percentage (2%) was recorded for animals in group C fed with the antioxidant extract (*Ocimum canum*) with simultaneous administration of lead acetate, and there was no significant development in animals fed with distilled water alone in group A that serves as 'control'. The result shows that the frequency of chromosome aberration of damaged cells was significantly ($P<0.05$) reduced in animals fed with aqueous extract *Ocimum canum* in group C. The toxicity of the divalent lead in the animals is caused by its binding to thiol groups, thus inhibiting some enzymatic reactions (15). Although, many plant products or extracts are known to reduce the toxic effects of clastogens (16), the mode of action of *Ocimum canum* extract used for this study could be attributed to its different phytochemical components. The toxicity of the lead is predisposed by its divalent ions (Pb^{2+}) and its affinity to bind onto the thiol groups thus, inhibiting some vital enzymatic reactions (17), hence, the significant reduction in the percentage chromosomal aberrations by the administration of the extract may be attributed to the protective activity of its inherent bioactive components. The effect of aqueous extract of *Ocimum canum* leaf on reduced glutathione (GSH) in rat liver presented in Figure 1, caused a significant ($P<0.05$) increase in concentration (175.3µg/ml) of the antioxidant enzyme compared with the control (50.3µg/ml). The result from this study indicates synergistic effect of the extract on these antioxidant enzymes with concomitant increase in their activities corresponding to increase in extract concentration as indicated in Figures 2 and 3 respectively. This effect could be attributed to the presence of selenium and zinc in the extract which generally activate the defensive antioxidant enzymes, thus, preventing rise in concentration of superoxide anions and hydrogen peroxide that generate cellular assault.

Conflict of Interest: None

References

1. Alan, G. and Housecroft, D. (2008). Pathophysiologic disorders of lead toxicity. Toxicol. Appl. Pharmacol. 132: 156-163.
2. Poddar, S. (2000). Reduction of cytotoxic effect of chronic exposure to arsenic. Mutation Res. 22 (10): 113-114.
3. Brautbar, M.D. (2005). Neurobehavioural changes, hypertension and kidney disease by lead exposure. JAMA, 253: 530-534.
4. Yakubu, M.T., Bukoye, B, Oladiti, A.T., Akanji, M.A. (2009). Toxicological implications of aqueous extract of Bambusa vulgaris leaves in pregnant Dutch rabbits. Human and exp. Toxicol. 28 (9): 591-598.
5. Nyarko, A.K, Asare-anane, H., Ofosuhene, M., Andy, M.E. (2002). Extract of Ocimum canum lowers blood glucose and facilitates isulin release by isolated pancreatic β-islet cells. Phytomedicine, 9: 346-351.
6. Preston, R.J., Dean, B.S., Galloway, S., Shelby, M. (1987). Mammalian in vivo cytogenetic assay; micronucleus assay in bone marrow cells. Mutat. Res. 189: 157-165
7. Choudhury, A.R., Das, T., Sharma, A., Talukder, G. (1997). Inhibition of clastogenic effect of arsenic through continued oral administration of garlic extract in mice in vivo. Mutat. Res. 392: 237-242.
8. Marsell, P. and Reckless, JPD. (1991). Garlic effect on serum lipid, blood pressure, platelet segregation and vasodilation. Brit. Med. Journal 303: 379- 388.
9. Sharma, A. and Sharma, A.K. (1994). Chromosome techniques manuals. Sixth Ed. Pp. 368, Haedwood Publishers, Switzerland.
10. Sharma, A. and Talukder, G. (1987). Effects of metals on chromosomes of higher organisms. Environ Mutagen, 9: 191-226.
11. Al-Shehri, A.H. and Al-Saleh, A.A. (2005). Karyotype of amphibians in Saudi Arabia (Rana ridibunda), Journal of Biol. Sci., 5: 535-538.
12. Butler, J. and Gyamfi, M.A. (1986). Estimation of glutathione in the liver and brain supernatants. General Pharmacology, 32: 661-667.
13. Misra, H.P. and Fridovich, I. (1972). The role of superoxide anion in auto-oxidation of epinephrine and simple assay for superoxide dismutase. J. Biol. Chem. 247 (12): 3170-3175.
14. Aebi, I.C. (1994). Determination of catalase activity. Plant Physiol. 33: 1071-1074.
15. Leonard, A. (1991). Metals and their compounds. Berlin: VCH, pp.751-774.
16. Afolabi, O.B., Oloyede, O.I., Tugbobo, O.S. (2014). Effect of Talinum triangulae in lead-induced chromosomal aberration. J. Phytopharmacol., 3 (4): 238-241.
17. Rodamilans, M., Osaba, M.J., Figneras, J., Magner, J.M. (1998). Lead toxicity on endocrine, testicular functions in occupationally exposed population. Toxicol. 7 (2): 125-128.

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