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Ocimum basillicum protective effect against Hg^{2+}- induced hematologic disorders and genetic birth defects in Wistar rats

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Abstract

Human indiscriminate exposure to heavy metal contaminants such as mercury through long-term ingestion of contaminated drinking water with notable attendant health burdens has been widely reported. In this study, the protective potential of *Ocimum basillicum* aqueous leaf extract was examined on mercury-induced hematologic disorders and chromosome aberrations in wistar rats. Mercury chloride (1.5mg/kg bd. wt.) and *Ocimum basillicum* leaf extract (100 mg/kg bd. wt.) were administered alone (group B) and simultaneously (group C) to the rats by gavage consecutively for 14-days. The control (group A) was fed with distilled water only while animals in group D were administered the extract alone. The hematologic assay was conducted using hematology-analyzer while the chromosomal aberrations were examined with the use of micronucleus assay. The results from micronucleus assay show that mercury is a pro-oxidant capable of inducing clastogenic effect in rats where mercury chloride caused significant ($P<0.05$) increase in frequency of micronucleated polychromatic erythrocytes (MPCEs) in group B (10.75 ± 1.25^{d}) animals compared with the control (0.50 ± 0.29^{a}) while appreciable reduction in frequency of MPCEs in group C (4.01 ± 0.90^{bc}) animals was observed. However, significant ($P<0.05$) decrease in hematologic parameters was observed in group B animals fed with mercury chloride when compared with others in group C and D. This study indicates that mercury is a potential clastogen which could possibly induce chromosome aberration and hematologic disorders in rats as observed in group B animals while *Ocimum basillicum* is suggested to have demonstrated metal chelating ability against the mercury chloride where it offered protective effect to animals in group C.

Keywords: Hematology; *Ocimum basillicum*; Mercury chloride; Micronucleus; Clastogen

1. Introduction

Mercury (Hg) as heavy metal is also a notable and well recognized environmental toxicant (Elais, 2005). Exposure to mercury especially through ingestion or consumption of contaminated water and food has been associated with various health burdens varying from skin lesions, certain forms of cancer especially leukemia, non-cancer diseases such as neurological disorders and impaired cognitive development in children (Parvez et al., 2016). There have been some research findings on heavy metals deleterious effect leading to genetic disorders in man via inhalation of heavy metals such as lead, arsenic as well as mercury due to indiscriminate environmental exposure (Valverde *et al.*, 2022). Besides, occupational and environmental co-exposure to mercury and other heavy metals have also been documented (Nehez *et al.*, 2010). Such exposure emanated from industries where electrical tools, cables, batteries are galvanized and recycled (Elias et al., 2005; Frisbie *et al*., 2002). The toxicity of mercury is largely due to its capacity and high propensity to mimic calcium in the body and substitute it in many of the functional cellular processes that largely depend on calcium (Kim and Leonard, 2016). Plant based drugs usually refers to as medicinal plants are still very relevant and remain important source of therapeutic agent for myriad of infections and diseases due to availability, accessibility as well as relatively

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non-toxic nature compared to unorthodox medicine (Aghor and Ngogang, 2005). Medicinal plants are considered to be the bedrock of folk medicines and are widely used for the treatment of acute and chronic diseases (WHO, 2013). *Ocimun basillicum* is grown for its culinary value and it is highly useful in treating some kind of diseases in lowering blood glucose level especially in type 2 diabetes mellitus (Nyako et al., 2002). Hence, its acclaimed purgative and anticlastogenic potentials against mercury-induced hematologic disorders and genetic birth defects would be investigated in this study.

2. Materials and methods

2.1. Preparation of Extract

120g of the powdered *Ocimum basillicum* were extracted with distilled water (1000ml) via maceration for 48hrs using method described by (Aguawa and Mittal, 2008). The mixture was decanted and filtered using sterile Whatman filter paper No 1. The filtrate measured up to 425ml and was evaporated to dryness using freeze dryer to obtain 9.56% yield. The crude extract was subjected to bioassay analysis where stock solution were concentrated as (1.0, 2.0, 3.0, 4.0 and 5.0) mg/ml via serial dilution.

2.2. Experimental animals

Twenty (20) male wistar rats average weight of approximately 110 ± 5g were purchase from Agricultural Technology Department, Federal Polytechnic, Ado-Ekiti, Nigeria. The animals were housed in the Experimental Animal House, Department of Science Laboratory Technology, Federal Polytechnic, Ado-Ekiti, Nigeria. They were fed with rat pellets containing 25% protein, 3% fat, 10% fibre, 2.5% calcium, 0.5% phosphorous, vitamin; mineral per mix; with water ad libitum. The room temperature was 29 ± 2^0C with 12hrs dark/light cycle and relative humidity of 60 ± 5%.

2.3. Experimental design

The wistar rats were distributed randomly into four groups of five animals each. The rats in group A serves as negative control and were administered distilled water only throughout the four weeks exposure. Group B animals were fed 1.5mg/kg bd. wt. of mercury chloride only via gavage daily for 14-days while those in group C were administered 1.5mg/kg mercury chloride simultaneously with 100mg/kg crude *Ocimum basillicum* leaf extract. The rats in group D were administered with 100mg/kg crude *Ocimum basillicum* leaf extract only. The concentration of mercury salt was made equivalent to $1/10^{th}$ of the LD50 (Preston *et al*., 2007) while the extract dose was equivalent to the exact concentration used for beneficial effect against specific disease condition (Das *et al.*, 2003). Each dose was administered to the animals on daily basis for four weeks.

2.4. Hematologic assay

The rats were sacrificed and blood samples were quickly collected via cardiac puncture and were immediately transferred into tubes containing EDTA (BD Diagnostics, Pre-analytical systems, Midrand, USA) for analysis of hematological parameters using Hematology Analyzer Sysmex XS8000i.

2.5. Micronucleus assay

Chromosome were examined from bone marrow cells following the usual colchicine-hypotonic fixation technique (Sharma and Sharma, 2004). Animals were sacrificed and their femurs quickly removed where the bone marrow was flushed out in 75 mM KCl-hypotonic solution, incubated for 20min at 37^0C and fixed in methanol glacial acetic acid (3:1). Chromosome preparation was made following the standard procedure of air-drying and then stained in 7% Giemsa solution and the slides were scored blind (Sharma and Sharma, 2004).

2.6. Statistics analysis

The data from the groups were pooled and statistically analyzed using one-way analysis of variance ANOVA (Sokal and Rohlf, 1987). This was followed by Duncan's multiple range test in order to compare the significant differences among different experimental rats.

3. Results

Table 1 Hematological parameters of wistar rats on administration of *Ocimum basillicum* crude extract and mercury chloride

Treatment Groups	A	B	C	D
White blood cell count (x10/L)	5.76 ±1.02^{a}	2.80 ± 0.49^{ab}	9.25 ± 1.77^{bc}	10.60 ±1.85^{c}
Hemoglobin (g/dl)	12.53± 4.38^{a}	6.78 ±6.74^{a}	13.73 ± 1.30^{a}	15.70 ± 8.24^{a}
Red blood cell (x10^{12}/L)	4.63± 0.59^{a}	1.38± 2.02^{a}	4.70 ± 1.70^{a}	6.08 ±2.27^{a}
Packed cell volume	32.67 ± 2.09^{a}	18.50 ±5.02^{a}	30.00 ± 4.41^{a}	33.50 ± 4.95^{a}
Neutrophils (%)	32.0 ±3.06^{a}	12.00 ± 2.83^{a}	30.18±2.57^{a}	36.40 ± 4.73^{a}
Lymphocytes (%)	44.00 ± 0.23^{a}	25.00 ± 9.89^{a}	56.50 ± 4.83^{ab}	54.00 ±3.46^{b}
Monocytes (%)	10.00 ± 2.01^{a}	5.01 ± 0.80^{b}	11.03±1.41^{ab}	13.67± 1.15^{b}
Basophils (%)	2.30 ± 0.03^{a}	0.37 ± 0.02^{a}	4.29 ± 0.05^{a}	5.26 ± 0.13^{a}
Eosinophils (%)	5.23± 0.58^{a}	0.02 ± 0.05^{b}	6.18±0.64^{b}	9.39± 0.55^{b}

Table 2 Induction of micronucleated polychromatic erythrocytes (mPCEs) in wistar rats exposed to mercury chloride ($HgCl_2$) and Ocimum basillicum in vivo

Group	Treatment	Number of mPCEs/1000PCEs
A	Distilled water	0.20 ± 0.12^{a}
B	$HgCl_2$ only	10.75 ± 1.25^{bc}
C	$HgCl_2$ + Ocimum basillicum	4.20 ± 0.64^{d}
D	Ocimum basillicum only	.35 ± 0.91^{a}

Values with same superscripts are not significant at 5% level while values with different superscripts are significant (P<0.05)

4. Discussion

The hematological parameters evaluated on Table 1 were significantly (P<0.05) reduced with lowest values obtained mainly in group B animals administered mercury chloride only. There was appreciable increase in values obtained in group C animals fed simultaneously with mercury chloride and the crude extract while in group D animals fed with the crude extract only, the values of hematological parameters obtained were significantly (P<0.05) high. Results from Table 1 is a clear indication that mercury is a pro-oxidant capable of inducing microcytosis in animal hematologic system with deleterious effects. However, the significant increase in hematological values observed in D animals harps more on the inherent antioxidant potential of crude *Ocimum basillicum* leaf extract. The toxicity of mercury is largely due to its capacity and high propensity to mimic calcium in the body and substitute it in many of the functional cellular processes that largely depend on calcium (Kim and Leonard, 2016). Besides, the results obtained from Table 2 clearly indicate that mercury chloride when administered alone significantly (P<0.05) induced the formation of micronuclei in the polychromatic erythrocytes (PCEs) of the rat bone marrow. This observation is consistent and aligns with earlier findings on the genotoxic potential of lead acetate in bone marrow and other tissues of albino rats (Moore et al, 1993; Tugbobo *et al.*, 2017).

The mercury chloride administered alone in group B animals ($1/10^{th}$ of the LD_{50}, 1.5mg/kg bd.) compared to approximate environmental daily exposure in man ; 14mg/kg) as compared with the micronucleated polychromatic erythrocytes (mPCEs) induced in bone marrow of animals in group A given distilled water alone (Table 2). The mercury chloride administered in group B results in enhanced genotoxic effect in rat bone marrow with marked induction of micronucleated polychromatic erythrocytes (mPCEs) which further confirms the potent clastogenic effect of most heavy metals in mammalian system (Das et al., 1993). The simultaneous administration of *Ocimum basillicum* extract with mercury chloride in group C animals indicates appreciable reduction (4.20) in the frequency of micronucleated

polychromatic erythrocytes (mPCEs) compared with (10.75) obtained in group B animals fed with the clastogen alone. The extract reduces the clastogenic effect in the bone marrow of animals in group C and this could be attributed to inherent antioxidant and metal chelating potential of *Ocimum basillicum* leaf extract. However, group D animals fed with the extract alone indicates significantly ($P<0.05$) low frequency of micronucleated polychromatic erythrocytes (0.35) when compared with animals in group B. The results from this study suggest that mercury chloride is a potential pro-oxidant and a potent clastogen while *Ocimum basillicum* leaf extract is a promising dietary supplement which could protect against mercury-induced toxicity.

5. Conclusion

This study reveals the deleterious clastogenic potential of mercury as a heavy metal and as well harps on the potentials of *Ocimum basillicum* leaves as dietary supplement against clastogenic toxicity of heavy metals such as mercury and also show case its protective activity on mammalian hematologic system due to its inherent antioxidant bioactive components.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors hereby declare no conflict of interest in this research work.

Statement of ethical approval

This research work was approved under the authority of Ethical Research Management of the Centre for Research Innovation and Development (CRID), Federal Polytechnic, Ado-Ekiti, Nigeria.

Statement of informed consent

Informed consent was obtained from all individual participants included in this study.

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