Antioxidative properties of *Ocimum gratissimum* alters Lead acetate induced oxidative damage in lymphoid tissues and hematological parameters of adult Wistar rats

John Chukwuma Oyem [a],*, Lilian Ebite Chris-Ozoko [a], Mamerhi Taniyohwo Enaohwo [a], Francisca Osamahemwem Otabor [a], Vera Anieze Okudayo [a], Onoriode Andrew Udi [b]

[a] *Department of Human Anatomy and Cell Biology, Faculty of Basic Medical Sciences, Delta State University, Abraka, Nigeria*
[b] *Department of Basic Medical Sciences (Anatomy Unit), College of Natural and Applied Sciences, Achievers University Owo, Ondo State, Nigeria*

ABSTRACT

Lead exposure is a well-known environmental hazard. Its accumulation in humans may pose a danger to health. The present study investigated the beneficial effect of *Ocimum gratissimum* extract (OG) in reducing lead acetate (LA) induced oxidative damage in the spleen, thymus, and hematological indices. We employed an *in vivo* model of LA induced Wistar rats and administered 125 mg/kg/bw and 250 mg/kg/bw of OG extracts respectively. Our control groups were divided into 2; the first group received normal saline, feed, and water while the second group was administered OG extracts only. We assessed the levels of malondialdehyde (MDA), glutathione (GSH), superoxide dismutase (SOD), and catalase (CAT) in the thymus and spleen and estimated percentages of blood cells. Our results showed that LA induces oxidative damage by significantly elevating MDA and diminishing GSH levels, SOD, and CAT activities. LA administration led to a significant decline in blood parameters. However, co-administration with OG compensated oxidative stress by significantly reducing MDA, increasing GSH, SOD, and CAT. Oral administration of OG to rats attenuated anemia, thrombocytopenia, leucocytosis, eosinophilia, monocytosis, and neutropenia induced by LA. The present study indicates that LA induced Spleen, thymus, and blood toxicity, which was reversed by oral OG administration.

1. Introduction

Lead is a non-biodegradable toxic element that can cause acute and severe cardiovascular, hematological, reproductive, digestive, immunological, and neurodegenerative diseases [1–3]. To date, different industries apply lead in the productions of cars, paint, pottery, and plastic materials [1] while in modern medicine, it is used as an astringent.

Lead constitutes one of the major environmental pollutants in developing countries [4–6]. According to Alwaleedi [7], lead enters the human body through inhalation, skin, and the alimentary tract. Lead levels in the blood of about 40–60 ug/dL are considered very toxic [8]. A suspected lead poisoning case was reported in Nigeria in Unguwan communities, Niger state, Nigeria [9]. Among the 48 affected individuals were mostly children with blood lead levels of 171.5–224

mg/dL including 14 deaths were reported [9]. Another study conducted in Jos, Nigeria, documented blood lead levels were higher in Muslim subjects, those whose homes are situated close to places where car cells were melted and those using eye cosmetics [10].

Although the mechanism of action of lead is not well elucidated, the prime targets of lead toxicity including heme synthesizing enzymes, thiol-containing antioxidants and oxidative enzymes (superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase, glucose 6-phosphate dehydrogenase and glutathione (GSH)) [11]. Low concentrations of lead in the blood, inhibits the action of oxidative enzymes thus leading to oxidative stress [1,2,11,12]. A prime biomarker of lead oxidation is lipid peroxidation [1,5,13]. The generated free reactive oxygen species traps electrons from intracellular lipids thus leading to cellular damage [1]. Lead induces oxidation of hemoglobin (Hb), which

leads to Red Blood Cells hemolysis (RBC) [1].

Lead directly affects the hematopoietic system by altering the production of hemoglobin through the inhibition of various key enzymes involved in the heme synthesis such as cystolic *δ-aminolevulinic acid dehydratase (ALAD)*, which catalyzes porphobilinogen formation from *δ*-aminolevulinic acid (ALA), *aminolevulinic acid synthetase (ALAS)*, a mitochondrial enzyme that catalyzes aminolevulinic acid (ALA) formation, and, *ferrochelatase* a mitochondrial enzyme that catalyzes iron insertion into protoporphyrin during heme formation [14,15].

Globally, the antioxidative properties of various plants have been applied in the management of diseases especially in developing countries where they have been documented in traditional medicine [13, 16–20,21]. About 80 % of individuals from developing countries are using traditional medicines to meet up their primary health care needs [21]. *Ocimum gratissimum* (OG) commonly called scent leaf is a dietary culinary spice used by different ethnic groups in Nigeria and other countries. It is called "Arurunta" by the Ukwuanis, "Ebe-amwonkho" in Edo, "Tchayo" in Fon, "Efinrin" in Yoruba, "Daidoya" in Hausa, "Nchuanwu" in Igbo, "Ntonng" in Ibibio, "Kunudiri" in Okrika and "Nunum' in Akanb [22,23].

The phytochemical screening of OG revealed that it contains alkaloids, tannins, flavonoids, phytates, and oligosaccharides [24–29]. Matasyoh et al. in 2007, demonstrated the presence of Ocimum oil (essential oil) and non-phlobatannins; Ocimum oil is made up of thymol (48.1 %), as its most constituent, p-cymene (12.5 %) and trace elements. These constituents have been reported to possess several medicinal properties such as antibacterial activities [25,28,30,31] antidiabetic properties [27] and anti-hyperlipidemic effect [22,24]. Also, it was reported to improve blood parameters in experimental animals [23,26, 32].

Although researchers have explored the deleterious effect of lead on the human physiology, prevention of lead exposure on humans is yet to be achieved [1,33]. Suradkar et al. [34], observed degeneration and necrosis of the splenic cells following a 28-day administration of lead acetate while Ekanem et al. [35], reported splenomegaly, a significant reduction in packed cell volume (PCV) and minimal change in the hemoglobin levels in Wistar rats administered lead acetate. A special paper by [36], reported a reduction in RBC count, and Hb White Blood Cell (WBC) count. Similar experimental studies revealed that lead acetate increased malondialdehyde (MDA) and reduced antioxidant enzyme activities in the bone marrow [37,38]. A more recent study by Okechukwu et al. [26], reported that vitamin C and OG restored the distortive histoarchitecture of thymus induced by lead.

Several studies have x-rayed critically the deleterious effects of lead acetate on human physiology [1–3]. Scant literatures have evaluated the effect of lead acetate on the blood, spleen and thymus histology as well as the roles of OG in ameliorating the deleterious effect of LA-induced toxicity [26,34,35]. According to this background, we designed the current study to determine the therapeutic effect of OG against Lead acetate (LA) induced oxidative damage on the thymus, spleen, and hematological parameters.

2. Materials and methods

2.1. Animal care

The Research Ethics Committee on Animal Use of Delta State University Abraka, Nigeria approved every protocol and procedure used with reference number (DELSU/CHS/ANA/12/40), in line with Animal Reforms Guidelines and the National Institute of Health Guide for Care and Use of Laboratory Animals.

2.2. Drugs

LA of 100 % purity manufactured by BDH chemical ltd. England was purchased from a chemical shop in Onitsha, Anambra State, Nigeria. The

dosage of LA (120 mg/kg/bw) administered was adopted from Suradkar et al. [34].

3. Plant material

Fresh OG leaves were obtained from a farmland in Owo, Ondo State, Nigeria. The leaves were identified and authenticated by a plant curator in University Herbarium, Ado-Ekiti, Nigeria with herbarium number (UHAE2019155).

3.1. Plant extracts preparation

Fresh leaves of OG were air-dried in the laboratory at ambient temperature (30 $\pm$ 2°c) for three weeks, and was pulverized using a laboratory mechanical grinder. 247 g of the powdered sample was extracted using 4400 mL of 50 % ethanol (*via* cold maceration) for 48 h. The mixture were decanted and filtered using sterile Whatman paper (3 mm) to obtain a semi-solid residue which was further placed in a sterile glass dish in a desiccator for complete dryness. The extract was reconstituted in water and was administered orally. The dose of OG administered was based on result obtained from our preliminary investigation and that from Mohammed et al. [39], who reported an LD_{50} of LA as 1264.9 mg/kg.

3.2. Experimental design

Thirty (30) adult male Wistar rats (~200 g; Delta State University, Abraka Animal Holdings) were housed under laboratory conditions of humidity, temperature, and unrestricted access to rat chow pelletized meal and water. The animals were left to acclimate. Following a two days acclimation, experimental animals were randomly assigned into 5 groups (control, OG, LA, LA + OG_1, LA + OG_2) and placed in animal house maintained under controlled conditions of temperature (23 $\pm$ 2 °C) and humidity (50 $\pm$ 5 %) and a 12-h light-dark cycle. Animals in the control group were administered normal saline and had *ad libitum* access to drinking water and food only unlike other experimental animals who received food and water freely together with the test compounds. The OG group received 250 mg/kg/bw of aqueous leaf extract of OG only throughout the experiment, the LA group received 120 mg/kg/bw of LA throughout the experiment while group LA + OG_1 and group LA + OG_2 received 120 mg/kg/bw of LA +125 mg/kg/bw of OG and 120 mg/kg/bw of LA +250 mg/kg/bw of OG, respectively for 28 days (each for 14 days respectively) see Table 1 below. All test compounds were administered orally once a day (8:00 am) with the use of an orogastric tube. Specific concentration of OG administration was chosen based on the fact that the LD50 of OG is calculated to be 1264.9 mg/kg body weight [40].

The Percentage extraction yield for *Ocimum gratissimum* extract was calculated using [mass of extract (g)/mass of plant sample (g)] $\times$ 100

Table 1

Experimental design for pre-treatment with ethanolic extract of OG before oral lead acetate administration.

Groups	Dosage
Control (n = 6)	Animals were administered 0.1 mL orally of 0.9 % normal saline orally for 28 days.
OG extract group only (n = 6)	Animals were administered 250 mg/kg/bw of OG extract only orally for 28 days.
LA group only (n = 6)	Animals were administered 120 mg/kg/bw of LA only orally for 28 days.
LA + OG1 (n = 6)	Animals were administered 120 mg/kg/bw of LA orally for 14 days followed by 125 mg/kg/bw of OG extract orally for 14 days
LA + OG2 (n = 6)	Animals were administered 120 mg/kg/bw of LA orally for 14 days followed by 250 mg/kg/bw of OG extract orally for 14 days

Key; OG: *Ocimum gratissimum*; LA: Lead acetate; bw: body weight.

stated by Okoduwa et al. [27]. Mass of extract was 20 g and mass of plant sample was 500 g, which yielded 4 g/100 g.

3.3. Blood collection

After 28 days of administration, blood was collected through the retro-orbital vein into a dipotassium EDTA bottle. It was well mixed with the anticoagulant to prevent coagulation.

3.4. Animal sacrifice

Animals were sacrificed by cervical dislocation. The spleen and thymus tissues were harvested, homogenized, and used for biochemical analysis.

3.5. Biochemical assessment

Biochemical parameters were assayed using homogenates from the spleen and thymus.

3.5.1. Malondialdehyde (MDA) level assessment

This was used to determine lipid peroxidation and was determined quantitatively by measuring MDA content using the Tsika's method [41]. In this test, the reaction mixture contained 1.0 mL tissue homogenate, 1.0 mL of TCA (10 %), and 1.0 mL TBAR (Thiobarbituric acid) (0.67 %). Test tubes were placed in a boiling water bath for 45 min, and were shifted to ice bath. The tubes were then centrifuged at $2500 \times g$ for 10 min. The malondialdehyde (MDA) levels formed in each of the samples were calculated by measuring the optical density of the supernatant at 532 nm. The results were expressed as the nmol MDA formed/gram tissue by using a molar extinction coefficient of 1.56×105 M^{-1} cm^{-1} [41] (Tables 2 and 3).

3.5.2. Reduced glutathione (GSH) levels assessment

Part of the tissues were washed briefly in saline, and then homogenized in an ice-cooled buffer that is composed of 1.15 % KCl, 0.01 M sodium phosphate buffer pH 7.4. Concentrations of GSH were determined using method described by Ellman [42].

3.5.3. Assessment of catalase (CAT) activity

This was determined using the method adopted by Chia with slight modifications [43]. 600 μL of 0.1 M PBS (pH 7.1) was added to 350 μL of 0.059 M hydrogen peroxide (H_2O_2) and 1.0 mL of homogenate. The absorbance was read by measuring the optical density of the supernatant at 340 nm.

3.5.4. Measurement of superoxide dismutase (SOD) activity

SOD was evaluated following the method described by Sirota [44]. This method was based on the ability of SOD to inhibit the autoxidation of epinephrine at pH 10.2.

Table 3
The effect of *Ocimum gratissimum* on lead-induced toxicity of Wistar rats' white blood cell differential counts.

Groups	Eosinophils (%)	Monocytes (%)	Lymphocyte (%)	Neutrophils (%)
CTRL	5.70 ± 0.82	5.60 ± 1.00	29.0 ± 1.00	70.3±0.90
OG	3.60 ± 0.90[a]	6.70 ± 0.38[b]	18.7±0.90	71.0 ± 2.30[a]
LA	6.30 ± 0.90[a]	5.30 ± 0.90[a]	23.3±0.90	68.7±1.86[a]
LA + OG₁	5.70 ± 1.20[ab]	4.00 ± 0.58[ab]	23.3±0.70	70.0 ± 2.3[ab]
LA + OG₂	5.70 ± 0.90[ab]	4.70 ± 0.33[ab]	24.3±0.90	71.0 ± 2.6[ab]

Data were expressed as mean ± SD.
[a] Significant difference when compared to the control group (p < 0.05).
[b] Significant difference when compared to the lead acetate treated group.
[ab] Significant difference when compared to the control, OG extract and LA group (p < 0.05) (n = 5).

3.6. Assessment of hematological parameters

The RBC count, Pack Cell Volume (PCV)), WBC count, monocytes, lymphocytes, neutrophils, eosinophils were adopted from the Hemocytometer method of Thrall et al. [45]. Hemoglobin (Hb) concentration was measured using the Cyanmethemoglobin method of Higgins [46].

3.6.1. Statistical analysis

Data obtained from hematological and biochemical analyses were subjected to Statistical Package for Social Sciences, version 23 (SPSS produced by SPSS Inc. Chicago), and analyzed using one-way analysis of variance (ANOVA). Tukey's post hoc test was used to determine significant differences within each group. The results were expressed as mean ± standard deviation (mean ± SD) and mean differences were considered significant at *p< 0.001, 0.01, 0.05* (Fig. 1).

4. Results

One-Way ANOVA test of (1a: MDA activities in the spleen, 1b: MDA activities in the thymus) shows a significant increase in spleen and thymus MDA actvities of the experimental rats administered only LA when compared with the control *(*p < 0.05, ** p < 0.01);* however, there is a significant decrease in spleen and thymus MDA activities following the administration of varying doses of OG *(*p < 0.05, ** p < 0.01*. CTRL: Control group, OG: *Ocimum gratissimum* group, LA: Lead acetate group, LA + OG₁: Lead acetate + *Ocimum gratissimum* extract (low dose), LA + OG₂: Lead acetate + *Ocimum gratissimum* extract (High dose) (Fig. 2).

A) One-Way ANOVA test shows a significant increase in GSH levels in the spleen of the experimental rats when compared with the control, OG, and LA experimental animals,*(*** p < 0.001).* B) A significant GSH levels were recorded in OG and LA + OG₁ in the thymus. LA led to a significant increase in GSH levels in the thymus which was further increased following the administration of OG. CTRL: Control group, OG:

Table 2
The effect of *Ocimum gratissimum* on lead induced toxicity of Wistar rats' hematological parameters.

Groups	PCV (%)	Hb (g/L)	WBC(x10⁹/L)	RBC (10¹²/L)	Platelet(10⁹/L)
CTRL	44.7±2.73	14.0±0.15	5.3±0.22	7.4±0.33	301±2.40
OG	48.3±1.21[a]	15.1±0.12 [a]	4.1±0.12 [a]	7.7±0.15 [a]	170±9.13 [a]
LA	27.0±1.47 [a]	9.0±0.15 [a]	4.4±0.15 [a]	4.5±0.09 [a]	146±0.91 [a]
LA + OG₁	46.0 ± 1.00[b]	14.5±0.80 [b]	7.0±0.58 [b]	7.5±0.10 [b]	190±1.53 [b]
LA + OG₂	45.0±1.20[b]	14.2±0.10 [b]	5.30 ± 0.70 [b]	7.60 ± 0.23 [b]	209±2.30 [b]

Data were expressed as mean ± SD.
[ab]Significant different when compared to the control, OG and LA group (p < 0.05) (n = 5).
[a] Significant difference when compared to the control group (p < 0.05).
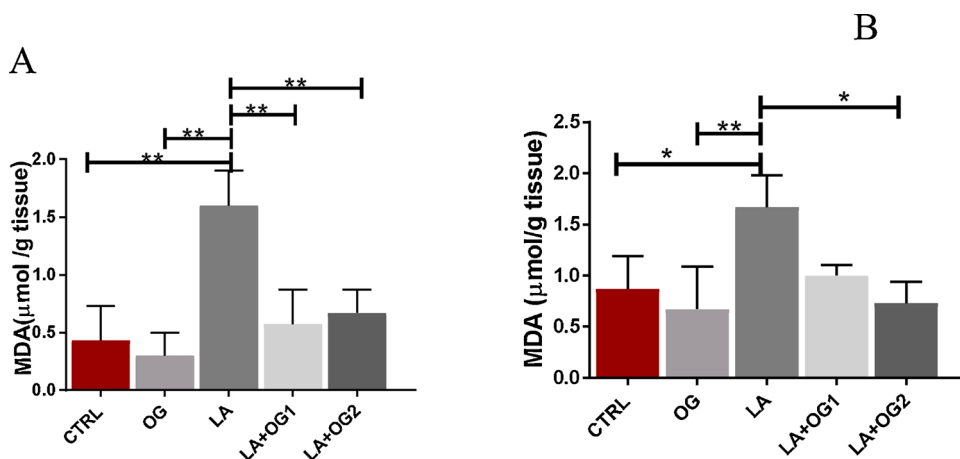[b] Significant difference when compared to the lead acetate treated group.

Fig. 1. The effect of *Ocimum gratissimum* extract on MDA levels of LA induced toxicity on the spleen and Thymus.
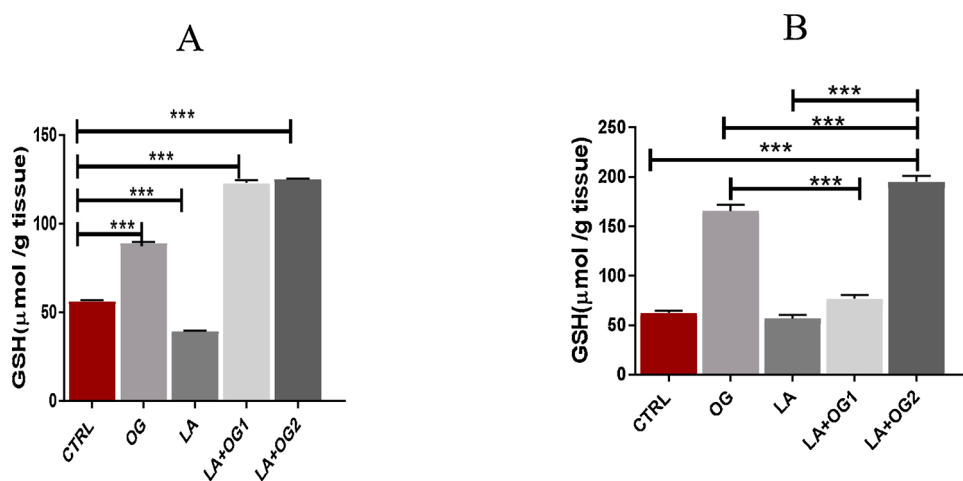

Fig. 2. The effect of *Ocimum gratissimum* on Spleen and Thymus glutathione levels.

Ocimum gratissimum group, LA: Lead acetate group, LA + OG$_1$: Lead acetate + *Ocimum gratissimum* extract (low dose), LA + OG$_2$: Lead acetate + *Ocimum gratissimum* extract (High dose) (Fig. 3).

3a). One-Way ANOVA test shows a significant decrease in the spleen's SOD activities of the experimental rats when compared with LA mice and a significant increase in SOD levels in the spleen when

compared with the OG and CTRL groups, *(*** p < 0.001)*. 3b). It showed a significant decrease *(*** p < 0.001)* in SOD activities in the thymus of experimental animals administered LA only. Further, this SOD activity in the thymus was significantly increased following the administration of OG in groups LA + OG$_1$ and LA + OG$_2$ animals *(*** p < 0.001)*. CTRL: Control group, OG: *Ocimum gratissimum* group, LA: Lead acetate group,
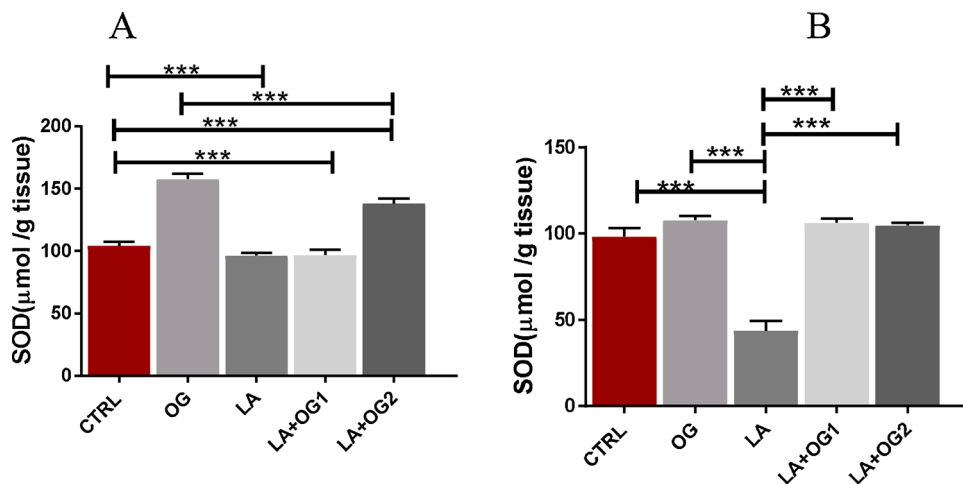

Fig. 3. The effect of LA and *Ocimum gratissimum* administration of SOD activities in the spleen and thymus.

LA + OG$_1$: Lead acetate + *Ocimum gratissimum* (low dose), LA + OG$_2$: Lead acetate + *Ocimum gratissimum* (High dose) (Fig. 4).

One-Way ANOVA test of (4a: CAT activities in the spleen) shows a significant decrease in the spleen's CAT activities of the experimental rats administered LA + OG$_1$ with a significant increase in the CAT activities of animals administered LA + OG$_2$ when compared with the control, OG, and LA groups (*p < 0.05, ** p < 0.01***p < 0.001). However, in the thymus (2b) there is a significant increase in CAT activities in the thymus of experimental animals that received LA + OG$_1$ when compared with control, OG, and LA groups(*p < 0.05, ** p < 0.01***p < 0.001. However, there is no significant difference in the thymus CAT levels in animals in group LA + OG$_2$. CTRL: Control group, OG: *Ocimum gratissimum* group, LA: Lead acetate group, LA + OG$_1$: Lead acetate + *Ocimum gratissimum* (low dose), LA + OG$_2$: Lead acetate + *Ocimum gratissimum* (High dose).

One-Way ANOVA test shows a dose-dependent significant increase in PCV, WBC, RBC, Hb, and platelet counts of the LA + OG$_1$ and LA + OG$_2$ animals when compared with the control and experimental group. CTRL: Control group, OG: *Ocimum gratissimum* group, LA: Lead acetate group, LA + OG$_1$: Lead acetate + *Ocimum gratissimum* (low dose), LA + OG$_2$: Lead acetate + *Ocimum gratissimum* (High dose).

Treatment of experimental animals with lead acetate caused a significant (p < 0.05) decrease in neutrophil and monocytes and a significant increase in eosinophils when compared with the control. These decrease in neutrophil and monocyte were significantly increased and normalized (p < 0.05) in animals that received LA + OG$_1$ and LA + OG$_2$ treatment respectively. There was no recorded significant effect on lymphocyte percentage. CTRL: Control group, OG: *Ocimum gratissimum* group, LA: Lead acetate group, LA + OG$_1$: Lead acetate + *Ocimum gratissimum* (low dose), LA + OG$_2$: Lead acetate + *Ocimum gratissimum* (High dose).

5. Discussion

Even though the ubiquitous nature of lead is beneficial to man, it is still considered an environmental toxin [1]. There is no safe level of lead exposure in the body physiology. Oxidative stress is one of the prime mechanisms of lead-induced toxicity [1,47]. An experimental study presented evidence that stress conditions disrupt the immune function of humans and animals by modifying the pro-oxidant capacity of neutrophils and macrophages [11]. These cause a decline in the immune response to infections [3,11,37]. Findings from the current study demonstrated that oral administration of OG extract attenuated LA induced oxidative damage on the spleen, thymus and hematological parameters by lowering MDA levels, increasing GSH levels, catalase and SOD activities and by improving blood parameters.

Lead-induced oxidative damage involves firstly, the Reactive Oxygen Species (ROS) generation such as singlet oxygen, hydrogen peroxide (H_2O_2), and hydroperoxide, and secondly, the destruction of the body's antioxidant defense system [48]. Due to the reported cases of rapidly increasing levels of lead poisoning in Nigeria, our study was designed to determine the controversial role of lead-induced toxicity concerning its relationship to oxidative damage in spleen, thymus and hematological parameters as well as assessing the roles of OG in interfering with oxidative damage induced by lead.

We hypothesized that OG extract may alter lipid peroxidation by reducing the levels of MDA. In our study, we exposed rats to OG extract, LA, LA + doses of OG extract, and assessed the levels of MDA in the spleen and thymus. Our study revealed that LA caused a significant increase in the MDA levels, which was decreased following co-administration with low and high doses of OG extract. This implied that OG extract attenuates oxidative damage induced by LA in the thymus and spleen by lowering MDA. One of the reasons for increasing levels of MDA in the spleen is that high levels of ROS transverses the cell membrane and destroys neighboring cells which result in an increase in the ROS that facilitates the cellular damage of the spleen [2]. Findings from this study are in line with that of Okechukwu et al. [26], who demonstrated that aqueous extract OG possesses a similar mechanism with vitamin C, in restoring the histoarchitectural details of the thymus of animals exposed to LA.

For the SOD antioxidant system assay, our findings revealed that oral administration of OG extract at low and high doses compensated the activities of SOD in experimental animals administered LA and OG extract. This observation indicated that the chemical constituents of OG extract activated SOD isoenzyme activity, which ameliorated oxidative damage induced by LA in the thymus and spleen. It was clearly shown by the dose significant response in the activity of SOD of animals in groups LA + OG$_1$ and LA + OG$_2$ when compared to LA and control groups. One of the mechanisms for this finding could be that SOD is an inhibitory agent of neutrophil-mediated inflammation and may be a viable therapeutic target for neutrophil induced ROS-dependent tissue damage [49]. Several studies believed that SODs form a very strong antioxidant defense system against oxidative stress and serves as an anti-inflammation agent in the prevention of precancerous progression and hemoglobinopathies [49–54].

Redox biomarkers such as GSH and CAT have been implicated in understanding the mechanisms related to the action of mixtures of xenobiotics on animal oxidative profile based on the current toxicological approach termed "the real-life exposure scenario" [55]. In the present study, we investigated the role of GSH and CAT, a powerful antioxidant for disrupting ROS in lead-induced toxicity in the spleen and thymus. LA administration led to a significant decrease in GSH levels in both organs.
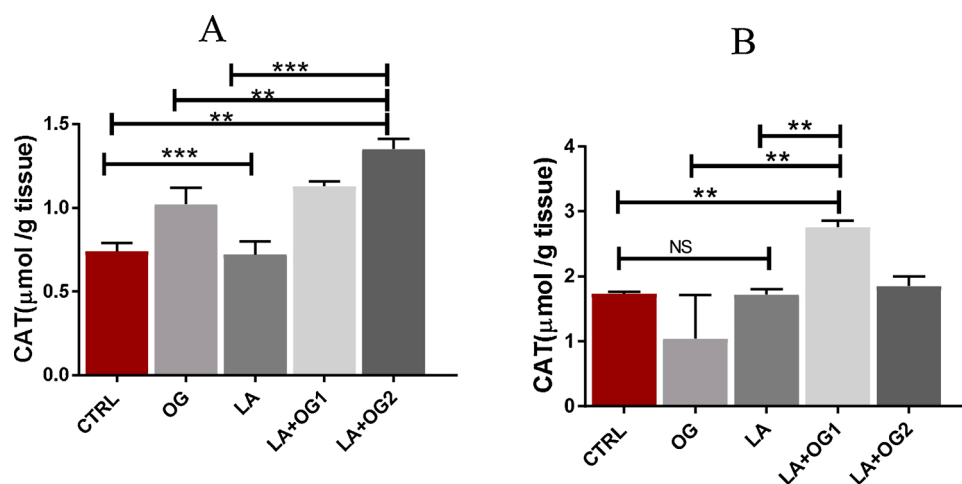

Fig. 4. The effect of *Ocimum gratissimum* on CAT activities of LA induced toxicity on the spleen and Thymus.

However, following oral administration of low and high doses of OG extract to LA induced animals led to a dose dependent significant increase in levels of GSH. This implied that exogenous administration of OG extract increases GSH antioxidant levels, which was depleted by LA. This is really a significant observation since the elevated increase of GSH in OG groups attenuated LA-induced impairment in the intrinsic antioxidant defense mechanisms. Similar finding was reported by Offor et al. [56], who noted that activated charcoal administration led to a significant increase in GSH levels of LA acetate induced rats. According to Hultberg [57], GSH in cells is rendered inactive by lead which leads to GSH synthesis from cysteine *via* the γ-glutamyl cycle, and it becomes ineffective in restoring the supply of GSH. Moreover, lead inactivates the activities of δ-aminolevulinic acid dehydratase (ALAD), glutathione reductase (GR), glutathione peroxidase (GPX) and glutathione-S-transferase, which further depresses the of levels glutathione [58].

Catalase, functions by modulating hydrogen peroxide at the cellular level which implies that the catabolism of CATs is protective [59]. In this study, we quantified and compared the activities of catalase in control, OG, LA, and LA + OGs experimental animals. We discovered a dose dependent significant increase in CAT activity in experimental animals co-administered with OG compared with the control and LA groups. This result pattern thus implies that the oral administration of OG extract at low and high doses increased the activities of CAT already compromised by LA induction. Reduction in the activity of CAT causes the generation of Hydrogen peroxide (H_2O_2) in cells [59]. Further, H_2O_2 associates with hemoglobin-containing iron to stimulate the Fenton and Haber-Weiss reactions as well as elevating other ROS activities [60,61],

One of the major functions of the thymus is to promote the development of T-lymphocytes whose major function is cell-mediated immunity [26]. Upon maturation, this T-lymphocytes exits the thymus and is channeled to the lymph nodes and spleen *via* blood vessels [26]. The spleen is a primary lymphoid organ that destroys red blood cells and recycles heme iron [2]. Lead exposure is known to induce anemia, leukocytosis, monocytopenia, polychromatophilia, glycosuria, increased serum urobilinogen, and hematuria while in chronic cases of lead exposure it leads to neutrophilia, leukocytosis, eosinopenia, and monocytopenia. In the current study, we hypothesized that the antioxidative properties of OG extract will ameliorate LA induced blood aberration by administering OG extract orally to LA induced experimental animals for an additional 14 days. Interestingly, our result revealed that oral administration of OG extract following LA exposure led to a significant increase in PCV, RBC, Hb, WBC, and platelet count. This implied that the antioxidative properties of OG extract are potent in averting LA induced anemia, leucocytosis, and thrombocytopenia.

Furthermore, we investigated the effect of LA following OG extract administration on circulating WBC in the spleen and thymus. The spleen is the largest peripheral lymphatic organ which contains about one-fourth of all the lymphocytes in the body [2]. The results regarding the white blood differential count are somewhat surprising giving that oral administration of OG led to a significant elevation in neutrophil count and a significant decline in the percentages of monocytes, and eosinophils with no observable significant changes in lymphocyte count of the experimental animal. Our results indicated that oral administration of OG extract reversed eosinophilia, monocytosis, and neutropenia induced by LA. These findings may also be mediated by the roles of neutrophils in inflammation [62]. Activated neutrophils attach to the endothelial layers of blood vessels and migrate to the extravascular space where ROS, proteases enzymes, and chemokines, which damage normal tissues and extracellular matrix proteins, are released. In addition, O_2 activates cells of the endothelium which enhances neutrophil infiltration [63].

Lead-induced toxicity on the circulating blood may be mediated by the activities of lead on key enzymes of heme synthesis [1,47]. Lead has also been demonstrated to reduce the life span of RBC in circulation by increasing the vulnerability of their cellular membranes [1]. The

consequence of these mechanisms leads to a decrease in RBC (anemia) [2,9,38]. According to Vij [64], lead-induced anemia is divided into; frank anemia which is caused by a prolonged elevation of blood lead level and hemolytic anemia which has been linked to chronic lead exposure.

Although the exact immunomodulatory mechanisms of co-administration of OG extract on LA induced lymphoid toxicity has not been elucidated, our findings propose that OG extract protects the spleen and thymus from lipid peroxidation by increasing and reducing the levels of GSH and MDA respectively, and increasing the activities of antioxidants such as CAT and SOD. This may be attributed to the antioxidative properties of OG extract which has been demonstrated to possess protective properties [23,26,28,30,65–68]. These plant may exert similar protective mechanism of action with flaxseed isolate incorporated with lemon juice which was reported to exert protective properties on lead induced kidney and liver toxicity [69]. Antioxidants prevent lead-induced toxicity by; inactivating the activities of generated ROS at the gene level, lead ion chelating, and prevention of ROS formation in its maintenance in a redox state thus contributing to its weakness in reducing molecular oxygen [65,70,71]. Also, assessing the hazard index (HI) and hazard quotient (HQ) of Lead will shed more light in understanding the level of risk and toxicity of lead in the experiment animals so as to proffer an analysis for the mechanism of any outcomes with exposure to lead and OG [72,73].

6. Conclusion

Lead acetate exposure is deleterious in experimental animals including hematological alterations, lipid peroxidation, and disruption of the body's antioxidant defense system. However, oral administration of OG protected the blood, spleen, and thymus from lead acetate induced oxidative stress. This indicates that the antioxidative properties of OG extract may be a viable therapeutic target in LA-induced blood, spleen and thymus toxicity in Wistar rats. Future studies should explore the exact mechanisms of actions of OG extract in attenuating lead acetate toxicity by carrying out histological, immunohistochemical, and molecular studies. Furthermore, phytochemical screening should be conducted on this agent to ascertain the major chemical compound of OG, which possesses the antioxidant properties.

CRediT authorship contribution statement

John Chukwuma Oyem: Formal analysis, Writing - original draft, Writing - review & editing. **Lilian Ebite Chris-Ozoko:** Conceptualization, Supervision. **Mamerhi Taniyohwo Enaohwo:** Data curation, Validation. **Francisca Osamahemwem Otabor:** Project administration, Methodology. **Vera Anieze Okudayo:** Project administration, Methodology. **Onoriode Andrew Udi:** Investigation, Resources.

Declaration of Competing Interest

The authors declare no conflict of interest.

Acknowledgement

References

[1] G. Flora, D. Gupta, A. Tiwari, Toxicity of lead : a review with recent updates, Interdiscp Toxicol. 5 (2) (2012) 47–58, https://doi.org/10.2478/v10102-012-0009-2.

[2] C. Rocha, D. Santos, A. Luiza, M. Cavalcante, R.A. Hauser-davis, R. Matos, R. De Cássia, O. Da, C. Mattos, Ecotoxicology and environmental safety effects of sub-

lethal and chronic lead concentrations on blood and liver ALA-D activity and hematological parameters in Nile tilapia, Ecotoxicol. Environ. Saf. 129 (2016) 250–256, https://doi.org/10.1016/j.ecoenv.2016.03.028.

[3] S. Sinha, A.M. Thaker, Study on the impact of lead acetate pollutant on immunotoxicity produced by thiamethoxam pesticide, Ind J Pharmacol. 46 (6) (2014) 596–601, https://doi.org/10.4103/0253-7613.144910.

[4] M. Bleasdale, H.R. Watch, A Heavy Price : Lead Poisoning and Gold Mining in Nigeria's Zamfara State. Photographs by Marcus Bleasdale / VII for Human Rights Watch, 2011. https://nairametrics.com/wp-content/uploads/2012/02/Heavy-Price-Nigeria-Lead-Brochure_lowres.pdf.

[5] S. Kianoush, M. Balali-, Clinical, toxicological, biochemical, and hematologic parameters in lead exposed workers of a Car battery industry, Iran. J. Med. Sci. 38 (1) (2013) 30–37.

[6] A.S. Nizami, M. Rehan, M. Waqas, M. Naqvi, O.K.M. Ouda, K. Shahzad, R. Miandad, M.Z. Khan, M. Syamsiro, I.M.I. Ismail, Waste biorefineries: enabling circular economies in developing countries, Bioresour. Technol. 241 (2017) 1101–1117.

[7] S.A. Alwaleedi, Hematobiochemical changes induced by lead intoxication in male and female albino mice, J. Physiol. Pharm. Pharmacol. 6 (1) (2016) 46–51, https://doi.org/10.5455/njppp.2015.5.0910201578.

[8] M. Lotfi-ghahramanloo, H. Baghshani, Ameliorative effects of caffeic acid on lead accumulation and oxidative stress in lead-exposed mice, Zahedan J. Res. Med. Sci. 18 (5) (2016) 4–8, https://doi.org/10.17795/zjrms-6674.Research.

[9] L.A. Alli, Blood level of cadmium and lead in occupationally exposed persons in Gwagwalada, Abuja, Nigeria, Interdiscip. Toxicol. 8 (3) (2015) 146–150, https://doi.org/10.1515/intox-2015-0022.

[10] N.J. Wright, T.D. Thacher, M.A. Pfitzner, P.R. Fischer, J.M. Pettifor, Causes of Lead Toxicity in a Nigerian City, 90, 2005, pp. s262–266, https://doi.org/10.1136/adc.2003.043562.

[11] E. İnce, F. Curabeyoğlu, S. Akyol, Oxidative stress in lymphoid tissues and complement activation in alcoholic mother rats and their newborns, Gen. Physiol. Biophys. 38 (1) (2019) 91–100, https://doi.org/10.4149/gpb.

[12] K. Nemsadze, T. Sanikidze, L. Ratiani, L. Gabunia, T. Sharashenidze, Mechanisms of lead-induced poisoning, Georgian Med. News (2009) 92–96.

[13] M.E. El-Boshy, B. Refaat, A.H. Qasem, A. Khan, M. Ghaith, H. Almasmoum, A. Mahbub, R.A. Almaimani, The remedial effect of Thymus vulgaris extract against lead toxicity-induced oxidative stress, hepatorenal damage, immunosuppression, and hematological disorders in rats, Environ. Sci. Pollut. Res. 26 (22) (2019) 22736–22746.

[14] N.N. Dongre, A.N. Suryakar, A.J. Patil, J.G. Ambekar, D.B. Rathi, Biochemical effects of lead exposure on systolic & diastolic blood pressure, heme biosynthesis and hematological parameters in automobile workers of north karnataka (India), Indian J. Clin. Biochem. 26 (4) (2011) 400–406, https://doi.org/10.1007/s12291-011-0159-6.

[15] C. Sachdeva, K. Thakur, A. Sharma, K.K. Sharma, Lead: tiny but mighty poison, Indian J. Clin. Biochem. 33 (2018) 132–146.

[16] K.K. Anachuna, C.J. Oyem, B.C. Nwogueze, J.N. Asiwe, Glucose lowering effects and histomorphological changes ofVernonia amygdalina on pancreatic compromised Wistar rats using alloxan monohydrate, Trop. J. Heal. Sci. 25 (2) (2018) 27–31.

[17] B.O. Anyanwu, C.N. Orish, A.N. Ezejiofor, I.L. Nwaogazie, O.E. Orisakwe, Neuroprotective effect of Costus afer on low dose heavy metal mixture (lead, cadmium and mercury) induced neurotoxicity via antioxidant, anti-inflammatory activities, Toxicol. Rep. 15 (7) (2020) 1032–1038, https://doi.org/10.1016/j.toxrep.2020.08.008.

[18] S. Asagba, P. Ichipi-Ifukor, R. Ichipi-Ifukor, J. Oyem, Palm oil fractions alter acute cadmium mediated haematotoxicity, Galician Med. J. 26 (3) (2019) E201933, https://doi.org/10.21802/gmj.2019.3.3.

[19] V. Atuadu, B.-A. Benneth, J. Oyem, E. Esom, C. Mba, K. Nebo, G. Ezemeka, C. Anibeze, Adansonia digitata L. leaf extract attenuates lead-induced cortical histoarchitectural changes and oxidative stress in the prefrontal cortex of adult male Wistar rats, Drug Metab. Pers. Ther. (2020), https://doi.org/10.1515/dmdi-2020-0116.

[20] L.E. Chris-ozoko, O. Okpe, W.J. Iju, J.C. Oyem, Histomorphological effects of cottonseed oil on testes in adult male Wistar rats, Gal Med. J. 25 (4) (2018), https://doi.org/10.21802/gmj.2018.4.2.

[21] V. Joshi, R.P. Joshi, Some plants used in ayurvedic and homoeopathic medicine, IC J. J. Pharmacogn. Phytochem. 2 (1) (2013), 2668735–5.

[22] M.T. Ayinla, S.O. Dada, S.T. Shittu, L.A. Olayaki, A.O. Akiode, S.L. Ojulari, Anti-hyperlipidemic effect of aqueous leaf extract of Ocimum gratissimum in alloxan induced diabetic rats, Int. J. Med. Med. Sci. 3 (12) (2011) 360–363, https://doi.org/10.5897/IJMMS.9000022.

[23] S.-T. Shittu, W. Oyeyemi, T. Lasisi, S.-S. Shittu, T. Lawal, S. Olujobi, Aqueous leaf extract of Ocimum gratissimum improves hematological parameters in alloxan-induced diabetic rats via its antioxidant properties, Int. J. Appl. Basic Med. Res. 6 (2) (2016) 96, https://doi.org/10.4103/2229-516x.179016.

[24] P.-Y. Chao, J.A. Lin, W.-J. Ting, W.-H. Lee, K. Hsieh, Y.-W. Chiu, T.-J. Lai, J.-M. Hwang, J. Liu, C.-Y. Huang, Ocimum gratissimum aqueous extract reduces plasma lipid in hypercholesterol-fed hamsters, Int. J. Med. Sci. 13 (11) (2016) 819–824, https://doi.org/10.7150/ijms.16474.

[25] R.S. Melo, Á.M. Albuquerque Azevedo, A.M. Gomes Pereira, R.R. Rocha, R. M. Bastos Cavalcante, M.N. Carneiro Matos, P.H. Ribeiro Lopes, G.A. Gomes, T. H. Soares Rodrigues, H.S.Dos Santos, I.L. Ponte, R.A. Costa, G.S. Brito, F.E. A. Catunda Júnior, V.A. Carneiro, Chemical composition and antimicrobial effectiveness of Ocimum gratissimum L. Essential oil against multidrug-resistant

isolates of Staphylococcus aureus and Escherichia coli, Molecules 24 (21) (2019) 3864, https://doi.org/10.3390/molecules24213864.

[26] N. Okechukwu, F. Elizabeth, U. Godwin, Effects of aqueous extract of Ocimum gratissimum leaves and vitamin C on lead acetate-induced changes in the Thymus of adult Wistar rats effects of aqueous extract of Ocimum gratissimum leaves and vitamin C on lead acetate-induced changes in the Thymus of adult Wistar rats, IJBCCR 26 (1) (2019) 1–9, https://doi.org/10.9734/ijbcrr/2019/v26i130087.

[27] S.I.R. Okoduwa, I.A. Umar, D.B. James, H.M. Inuwa, Anti-diabetic potential of Ocimum gratissimum leaf fractions in fortified diet-fed streptozotocin treated rat model of Type-2 diabetes, Med. (Basel, Switzerland) 4 (4) (2017) 73, https://doi.org/10.3390/medicines4040073.

[28] O.D. Omodiaro, M.A. Jimoh, Antioxidant and antibacterial activities of Ocimum gratissimum, Am. J. Phytomedicine Clin. Ther. 12 (3) (2015) 10–19.

[29] J.Y. Talabi, S.A. Makanjuola, Proximate, phytochemical, and in vitro antimicrobial properties of dried leaves from Ocimum gratissimum, Prev. Nutr. Food Sci. 22 (3) (2017) 191–194, https://doi.org/10.3746/pnf.2017.22.3.191.

[30] L.G. Matasyoh, J.C. Matasyoh, F.N. Wachira, M.G. Kinyua, A.W. Thairu Muigai, T. K. Mukiama, Chemical composition and antimicrobial activity of the essential oil of Ocimum gratissimum L. growing in Eastern Kenya, African J. Biotechnol. 6 (2007) 760–765, https://doi.org/10.5897/AJB2007.000-2085.

[31] S.Lda S. Pereira, J.W.G. de Oliveira, K.K.S.V. Ângelo, A.M.A. da Costa, F. Costa, Clinical effect of a mouth rinse containing Ocimum gratissimum on plaque and gingivitis control, J. Contemp. Dent. Pract. 12 (5) (2011) 350–355, https://doi.org/10.5005/jp-journals-10024-1058.

[32] O.E. Ofem, E.J. Ani, A.E. Eno, Effect of aqueous leaves extract of Ocimum gratissimum on hematological parameters in rats, Int. J. Appl. Basic Med. Res. 2 (1) (2012) 38–42, https://doi.org/10.4103/2229-516X.96807.

[33] K. Kalia, S.J.S. Flora, Strategies for safe and effective therapeutic measures for chronic arsenic and lead poisoning, J. Occup. Health 47 (2005) 1–21, https://doi.org/10.1539/joh.47.1.

[34] S.G. Suradkar, P.D. Vihol, J.H. Patel, D.J. Ghodasara, B.P. Joshi, K.S. Prajapati, Patho-morphological changes in tissues of Wistar rats by exposure of Lead acetate, Vet. World 3 (2) (2010) 82–84.

[35] A.U. Ekanem, H.D. Kwari, S.H. Garba, H.A. Salami, Effect of lead acetate on spleen and blood parameters in albino rats, IOSR J. Dent. Med. Sci. Ver. I (14) (2015) 2279–2861, https://doi.org/10.9790/0853-14314349.

[36] N.M. Ibrahim, E.A. Eweis, H.S. El-Beltagi, Y.E. Abdel-Mobdy, Effect of lead acetate toxicity on experimental male albino rat, Asian Pac. J. Trop. Biomed. 2 (1) (2012) 41–46, https://doi.org/10.1016/S2221-1691(11)60187-1.

[37] B. Debnath, W.S. Singh, K. Manna, Sources and toxicological effects of lead on human health, Ind. J. Med. Spec. 10 (2) (2019) 66–71, https://doi.org/10.4103/INJMS.INJMS.

[38] N. Haleagrahara, S. Chakravarthi, A.B. Kulur, A. Radhakrishnan, Effects of chronic lead acetate exposure on bone marrow lipid peroxidation and antioxidant enzyme activities in rats, African J. Pharm. Pharmacol. 5 (7) (2011) 923–929.

[39] S.A. Mohammed, M.A. Razzaque, A.E. Omar, W.M. Al-gallaf, Biochemical and hematological profile of different breeds of goat maintained under intensive production system, Afr. J. Biotechnol. 15 (24) (2016) 1253–1257, https://doi.org/10.5897/AJB2016.15362.

[40] K.S. Prabhu, R. Lobo, A.A. Shirwaikar, A. Shirwaikar, Ocimum gratissimum : a review of its chemical, Pharmacol. Ethnomed. Prop. 1 (2009) 1–15.

[41] D. Tsikas, Assessment of lipid peroxidation by measuring malondialdehyde (MDA) and relatives in biological samples: analytical and biological challenges, Anal. Biochem. 1 (524) (2017) 13–30, https://doi.org/10.1016/j.ab.2016.10.021.

[42] G.L. Ellman, Tissue sulfhydryl groups, Arch. Biochem. Biophys. 82 (1959) 70–77.

[43] Y.Y. Chia, M.S. Kanthimathi, K.S. Khoo, J. Rajarajeswaran, H.M. Cheng, W.S. Yap, Antioxidant and cytotoxic activities of three species of tropical seaweeds, BMC Complement. Altern. Med. 15 (2015) 339, https://doi.org/10.1186/s12906-015-0867-1.

[44] T.V. Sirota, A novel approach to study the reaction of adrenaline autoxidation: a possibility for polarographic determination of superoxide dismutase activity and antioxidant properties of various preparations, Biochem. Suppl. Ser. B Biomed. Chem. 5 (2011) 253, https://doi.org/10.1134/S1990750811030139.

[45] M.A. Thrall, G. Weiser, R. Allison, T. Campbell, Veterinary Hematology and Clinical Chemistry, John Wiley & Sons, 2012.

[46] C. Higgins, Hemoglobin and Its Measurement, Acutecaretesting.Org, 2005, pp. 1–10.

[47] T.O. Obafemi, A. Onasanya, A. Adeoye, J.A. Falode, D.J. Daniel, E.F. Irefo, A. O. Ojo, A. Fadaka, O.B. Afolabi, J.O. Awe, B.O. Omiyale, Protective effect of methanolic and flavonoid-rich leaf extracts of Synsepalum dulcificum (Danielli) on lead-acetate-induced toxicity in Wistar albino rats, J. Appl. Pharm. Sci. 9 (5) (2019) 65–72, https://doi.org/10.7324/JAPS.2019.90508.

[48] A. Barbouti, P.V.S. Vasileiou, K. Evangelou, K.G. Vlasis, A. Papoudou-bai, V. G. Gorgoulis, Review Article Implications of Oxidative Stress and Cellular Senescence in Age-Related Thymus Involution, 7986071, 2020, https://doi.org/10.1155/2020/7986071.

[49] H. Younus, Therapeutic potentials of superoxide dismutase, Int. J. Health Sci. (Qassim). 12 (3) (2018) 88–93.

[50] B. Hu, G.W. Wu, J. Yan, Z.M. Mao, Achievement in SOD mimics with antioxidant and anti-inflammation functions, Prog. Chem. 21 (5) (2009) 873.

[51] G.N. Landis, J. Tower, Superoxide dismutase evolution and life span regulation, Mech. Ageing Dev. 126 (3) (2005) 365–379, https://doi.org/10.1016/j.mad.2004.08.012.

[52] C. Li, H.M. Zhou, The role of manganese superoxide dismutase in inflammation defense, Enzyme Res. (2011) 387176, https://doi.org/10.4061/2011/387176.

[53] N. Yahfoufi, N. Alsadi, M. Jambi, C. Matar, The immunomodulatory and anti-inflammatory role of polyphenols, Nutrients 10 (11) (2018) 1618, https://doi.org/10.3390/nu10111618.

[54] K. Yasui, A. Baba, Therapeutic potential of superoxide dismutase (SOD) for resolution of inflammation, Inflamm. Res. 55 (9) (2006) 359–363.

[55] P. Fountoucidou, A.S. Veskoukis, E. Kerasioti, A. Oana, A mixture of routinely encountered xenobiotics induces both redox adaptations and perturbations in blood and tissues of rats after a long-term low-dose exposure regimen: the time and dose issue, Toxicol. Lett. 317 (2019) 24–44, https://doi.org/10.1016/j.toxlet.2019.09.015.

[56] S.J. Offor, H.O.C. Mbagwu, O.E. Orisakwe, E. Robinet, Lead induced hepato-renal damage in male albino rats and effects of activated charcoal, Front. Pharmacol. 8 (2017) 1–10, https://doi.org/10.3389/fphar.2017.00107.

[57] M. Hultberg, Cysteine turnover in human cell lines is influenced by glyphosate, Environ. Toxicol. Pharmacol. 24 (1) (2007) 19–22, https://doi.org/10.1016/j.etap.2007.01.002.

[58] A. Carocci, A. Catalano, G. Lauria, M.S. Sinicropi, G. Genchi, Lead toxicity, antioxidant defense and environment. Reviews of Environmental Contamination and Toxicology, Springer, 2016, pp. 45–67.

[59] A. Nandi, L.J. Yan, C.K. Jana, N. Das, Role of catalase in oxidative stress- and age-associated degenerative diseases, Oxid. Med. Cell. Longev. 2019 (2019), 9613090, https://doi.org/10.1155/2019/9613090.

[60] B. Halliwell, J.M.C. Gutteridge, Free Radicals in Biology and Medicine, Free Radicals in Biology and Medicine, Oxford University Press, USA, 2015, https://doi.org/10.1093/acprof:oso/9780198717478.001.0001.

[61] M. Lawson, K. Jomova, P. Poprac, K. Kuca, K. Musílek, M. Valko, Free radicals and antioxidants in human disease, Nutr. Antioxid. Ther. Treat. Perspect. 39 (2018) 283–305, https://doi.org/10.1007/978-3-319-67625-8_12.

[62] U. Kulkarni, D.R. Goldstein, Divergent roles for neutrophils: promoting and resolving inflammation? Transplantation 102 (4) (2018) 542–543, https://doi.org/10.1097/TP.0000000000002062.

[63] X. Jiang, T.T. Nguyen, W. Tian, Y.K. Sung, K. Yuan, J. Qian, J. Rajadas, J.-M. Sallenave, N.P. Nickel, V. de Jesus Perez, M. Rabinovitch, M.R. Nicolls, Cyclosporine does not prevent microvascular loss in transplantation but can synergize with a neutrophil elastase inhibitor, Elafin, to maintain graft perfusion during acute rejection, Am. J. Transplant. Off. J. Am. Soc. Transplant. Am. Soc. Transpl. Surg. 15 (7) (2015) 1768–1781, https://doi.org/10.1111/ajt.13189.

[64] A.G. Vij, Hemopoietic, hemostatic and mutagenic effects of lead and possible prevention by zinc and vitamin C, Al Am. J. Med. Sci. 2 (2) (2009) 27–36.

[65] M.T. Antonio-García, E.L. Massó-Gonzalez, Toxic effects of perinatal lead exposure on the brain of rats: involvement of oxidative stress and the beneficial role of antioxidants, Food Chem. Toxicol. 46 (6) (2008) 2089–2095, https://doi.org/10.1016/j.fct.2008.01.053.

[66] E.O. Igbinosa, E.O. Uzunuigbe, I.H. Igbinosa, E.E. Odjadjare, N.O. Igiehon, O.A. Emuedo, In vitro assessment of antioxidant, phytochemical and nutritional properties of extracts from the leaves of Ocimum gratissimum (Linn), African J. Tradit. Complement. Altern. Med. AJTCAM 10 (5) (2013) 292–298.

[67] C. Onyebuchi, D. Kavaz, Chitosan and N, N, N-Trimethyl chitosan nanoparticle encapsulation of Ocimum gratissimum essential oil: optimised synthesis, in vitro release and bioactivity, Int. J. Nanomed. 14 (2019) 7707–7727, https://doi.org/10.2147/IJN.S220202.

[68] M. Rezzoug, B. Bakchiche, A. Gherib, A. Roberta, FlaminiGuido, Ö. Kilinçarslan, R. Mammadov, S.K. Bardaweel, Chemical composition and bioactivity of essential oils and Ethanolic extracts of Ocimum basilicum L. and Thymus algeriensis Boiss. & Reut. from the Algerian Saharan Atlas, BMC Complement. Altern. Med. 19 (2019) 146, https://doi.org/10.1186/s12906-019-2556-y.

[69] R.S. Mohamed, K. Fouda, E.M. Akl, Hepatorenal protective effect of flaxseed protein isolate incorporated in lemon juice against lead toxicity in rats, Toxicol. Rep. 3 (7) (2020) 30–35, https://doi.org/10.1016/j.toxrep.2019.12.001.

[70] A. Alhusaini, L. Fadda, I.H. Hasan, E. Zakaria, A.M. Alenazi, A.M. Mahmoud, Curcumin ameliorates lead-induced hepatotoxicity by suppressing oxidative stress and inflammation, and modulating Akt/GSK-3β signaling pathway, Biomolecules 5 (9(11)) (2019) 703, https://doi.org/10.3390/biom9110703.

[71] S.-Z. Cai, L.-N. Zhao, J. Liu, Y.-T. Ji, X.-Y. Shi, Z.-R. Ma, X.-H. Lv, K. Chen, Y. Chen, Allicin alleviates lead-induced hematopoietic stem cell aging by up-regulating PKM2, Biosci. Rep. 39 (7) (2019), BSR20190243, https://doi.org/10.1042/BSR20190243.

[72] M. Goumenou, A. Tsatsakis, Proposing new approaches for the risk characterisation of single chemicals and chemical mixtures: the source related Hazard Quotient (HQS) and Hazard Index (HIS) and the adversity specific Hazard Index (HIA), Toxicol. Rep. 6 (2019) 632–636, https://doi.org/10.1016/j.toxrep.2019.06.010.

[73] A.M. Tsatsakis, D. Kouretas, M.N. Tzatzarakis, P. Stivaktakis, K. Tsarouhas, K.S. Golokhvast, V.N. Rakitskii, V.A. Tutelyan, A.F. Hernandez, R. Rezaee, G. Chung, Simulating real-life exposures to uncover possible risks to human health: a proposed consensus for a novel methodological approach, Toxicol. Lett. 317 (2017) 24–44, https://doi.org/10.1177/0960327116681652.