EFFECTS OF AQUEOUS EXTRACT OF *Ocimum gratissimum* LEAVES AND VITAMIN C ON LEAD ACETATE-INDUCED CHANGES IN THE THYMUS OF ADULT WISTAR RATS

ABSTRACT

Lead is a heavy metal found in earth crust. It is a widespread and insidious environmental toxin known as a severe and aggressive contaminant to human and animal organisms' health status. This work is aimed at evaluating the effect of aqueous extract of *Ocimum gratissimum* (OG) on lead induced changes in the thymus of adult albino wistar rats. Thirty five male Wistar rats were used in the study and were randomly divided into seven groups with five rats in each group. The rats in Group one (G1) served as the Control and received distilled water. Group 2 received 120 mg/kg body weight (bwt) of lead acetate, G3 received 375mg/kg bwt of OG only. G4 received 120 mg/kg bwt of lead acetate and OG extract at 375 mg/kg bwt., G5 received 120 mg/kg bwt of lead and OG at 750 mg/kg bwt. G6 received 375mg/kg bwt of OG for two weeks followed by120 mg/kg bwt of lead acetate for one week, while G7 received 120 mg/kg bwt of lead acetate and Vitamin C at 11900mg/kg.All the administrations were carried out orally for twenty one (21) days. At the end of the administration, the rats were fasted for 24 hours. They weighted and humanely sacrificed via cervical dislocation. The thymus were harvested in all the groups and prepared for histological studies using routine haematoxylin and eosin (H&E) staining techniques. The result of the present study shows that lead possessed a distortive effect in the histoarchitecture of thymus of wistar rat in G2 when compared with G1. Treatment of these experimental animals with vitamin C prove to posses more ameliorative effect on restoring the histoarchitecture on lead toxicity in the thymus close to normal than OG. Therefore, it is suggested that aqueous extract OG can act via same pathway as vitamin C, in maintaining the normal histological structures of the thymus of adult albino wistar rat exposed to lead toxicity.

Key word: *Ocimum gratissimum,* thymus, vitamin C, lead

Introduction

Various harmful and toxic chemical compounds (lead and mercury) are formed as bye/intermediate products of normal biochemical reactions in the human system; these bye products are eliminated or detoxified under normal physiological conditions. Lead is a heavy metal found in earth crust. Free radicals and reactive oxygen species (ROS) are amongst these chemicals and their accumulation when not eliminated by the endogenous system often lead to oxidative stress [1]. Lead is a chemical element with symbol Pb (from the Latin plumbum) and atomic number 82. It is a heavy metal that is denser than most common materials. Lead is soft and malleable, and has a relatively low melting point. When freshly cut, lead is bluish-white; it tarnishes to a dull gray color when exposed to air. Lead has the highest atomic number of any stable element and concludes three major decay chains of heavier elements. Lead is not naturally present in the human body and, as it currently exists in the environment, has been identified as a health threat. [2]. As suggested by various authors, [3]; [4] lead may contaminate humans from various pathways including: inhalation of airborne lead particulates, consumption of water or food contaminated by lead, and ingestion (due to contamination of hands or other objects) of soil or dust contaminated with lead. Once in the body, lead travels in the blood to soft tissues such as the liver, kidneys, lungs, brain, spleen, muscles, and heart [3].

Cells are equipped with different kinds of mechanisms to fight against radicals and reactive oxygen species and to maintain redox homeostasis of cell. For example, antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) play important roles in scavenging the free radicals and preventing injury [5]. The antioxidant might play a role in the treatment of lead poisoning [6].Vitamins like C is capable to control the critical balance of oxidants and antioxidants in the body. [7] and [8] reported that intake of ascorbic acid decrease the blood lead level and control the lead toxicity.

Ocimum gratissimum belongs to the group of plants known as spices. The plant is an erect small plumb with many barnacles usually not more than 1 m high [9] and [10]. In South East Asia, it is cultivated as a home garden crop but it is grown on a commercial scale in Vietnam. In Nigeria, it is Efinrin in Yoruba, Diadoyal in Hausa and Nchuanwu in Igbo [11] In traditional medicine, the leaves have been used as a general tonic and anti-diarrhea agent and for the treatment of conjunctivitis by instilling directly into the eyes; the leaf oil when mixed with alcohol is applied as a lotion for skin infections, and taken internally for bronchitis [12]. Although, conventional antibiotics have been very useful in orthodox medicine, it has been argued by many that

its concomitant use with herbal extracts is not desirable as one normally antagonizes the activity of the other [13].

The thymus gland is the main organ of the lymphatic system. . Its primary function is to promote the development of specific cells of the immune system called T-lymphocytes. Once mature, these cells leave the thymus and are transported via blood vessels to the lymph nodes and spleen. T-lymphocytes are responsible for cell-mediated immunity, which is an immune response that involves the activation of certain immune cells to fight infection.
In addition to immune function, the thymus also produces hormones that promote growth and maturation. [14]

Material and Method

Collection and identification of plant materials

The fresh leaf of *Occimun gratissimum* were obtained behind the department of Anatomy Federal University, Ndufu-Alike, Ikwo. The specimen was identified by Botanist at Department of Biology Alex-Ekwueme Federal University, Ndufu-Alike, Ikwo. The leaves were washed and shade dried.

Experimental Animals

In order to minimize variations in response, only adult male rats were used for this study. Thirsty-five adult albino wistar rats, weighing between 140-160g were obtained from the Animal House of the Department of Human Anatomy, Alex-Ekwueme Federal University Ndufu-Alike Abakili, Ebonyi State, Nigeria. They were maintained under favorable condition at standard temperature and pressure and fed with top food grower mash (Vital Feed®). Prior to acclimatization which lasted for two weeks, the rats were divided into seven groups, each of five rats. They were fed with feed and water *ad libitum* throughout the experiment.

Plant Extraction Procedure.

It was extracted in the Department of chemistry, University of Nigeria, Nsukka. The dried specimen were grounded into powder of 500g using a Q-link electric blender; Model QBL-18L40, and stored in air-tight containers.[15] Aqueous infusion was done by mixing a calculated volume of distilled water and powdered sample. The mixture was allowed to stand for 30 minutes before filtration. It was then centrifuged at about 3000xg for 5 min and the supernatal collected

Experimental Design

The design consist of thirty-five (35) animals, randomly divided into seven (7) groups each of five rats in each group, and the rats were fed with animal fed and water *ad libitum*. Distilled water were used to dissolve the *Occimum gratissimium* extract, the lead acetate and vitamin C. The treatment was done orally for 21 days. After the administration (24 hours), the rats were sacrificed and the organs harvested. The rats in Group one (G1) served as the Control and received distilled water. Group 2 received 120 mg/kg body weight (bwt) of lead acetate, G3 received 375mg/kg bwt of OG only. G4 received 120 mg/kg bwt of lead acetate and OG extract at 375 mg/kg bwt., G5 received 120 mg/kg bwt of lead and OG at 750 mg/kg bwt. G6 received 375mg/kg bwt of OG for two weeks followed by120 mg/kg bwt of lead acetate for one week, while G7 received 120 mg/kg bwt of lead acetate and Vitamin C at 11900mg/kg.

RESULTS

Animal Sacrifice

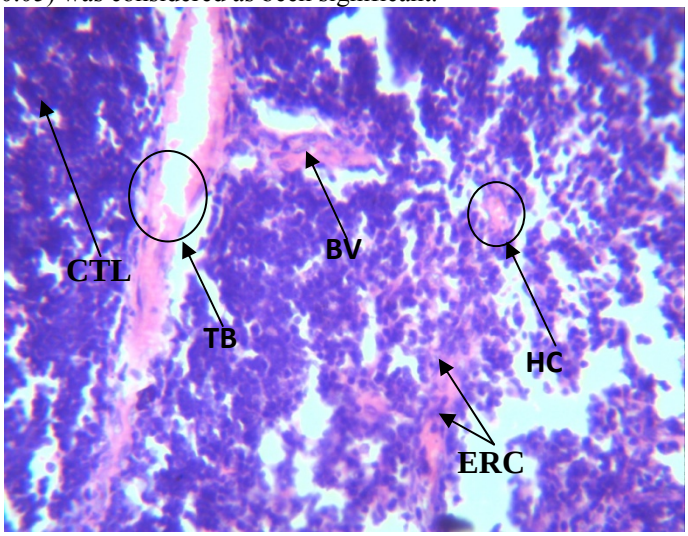
The animals were sacrificed by cervical dislocation followed by midline incision to harvest the organ.
The thymus was procured and cleared from adjoining supporting, connective tissues. The organ was gently blotted with filter paper and weighed. The thymus was immediately immersed in 10% formalin solution to be processed for haematoxylin and eosin staining and light microscopic studies.

Tissue Preparation for Histology

Following sacrifice, the tissue was prefixed in 10% formal saline, after whole body perfusions were transferred to a graded series of ethanol. On Day 1, they were placed in 70% alcohol for 7 hours, then transferred to 90% alcohol and left in the latter overnight. On day 2, the tissues were passed through three changes of absolute alcohol for an hour each and then cleared in xylene. Once cleared, the tissues were infiltrated In molten paraffin wax in the oven at 58^{o}C. Three changes of molten paraffin wax at one-hour interval were made, after which the tissues were embedded in wax and blocked out. Prior to embedding, it was ensured that the mounted sections to be cut by the rotary microtome were orientated perpendicular to the long axes. These sections were designated "vertical sections". Serial sections of 4µm thick were obtained from a solid block of tissue, fixed on clean slides to which Mayer's egg albumin had been coated to cement the sections to the slides properly and were stained. For histological and histochemical study, sections were stained with H & E, after which they were passed through a mixture of equal concentration of xylene and alcohol. Following clearance in xylene the sections were oven-dried between 35°C and 40°C [16{17}].The sections were examined under light microscope at high power magnification(x100 and x400) and photomicrographs taken.

STATISTICAL ANALYSIS

Statistical analysis was done using both electronic calculate and SPSS version 20. All data were expressed as mean ± SD of number of experiments (n = 5). The values will be compared using Student T-test. P value less than 0.05 ($P < 0.05$) was considered as been significant.



It was observed that there was a gradual increase in the mean body weight of the animals in group 1 (control group), and other groups of experimental animals except the animals treated with lead only (Groups 2) and the animals treated with high dose of *Ocimum grattissimu* and lead (group 5) which shows a decrease in the mean body weight.

Thymus exposed to lead show a significant decreased in normal thymic lymphocytes and reticular epithelial cells compare to control. Thymic atrophy was observed in all the groups administered with lead acetate but occurrence was severe in animal treated with lead acetate only (group 2). This atrophy was slight in animals treated with lead acetate and OG, (group 4, 5 and 6) and moderate in animal treated with lead acetate combined with vitamin C (group 7)

BODY WEIGHT DIFFERENCE

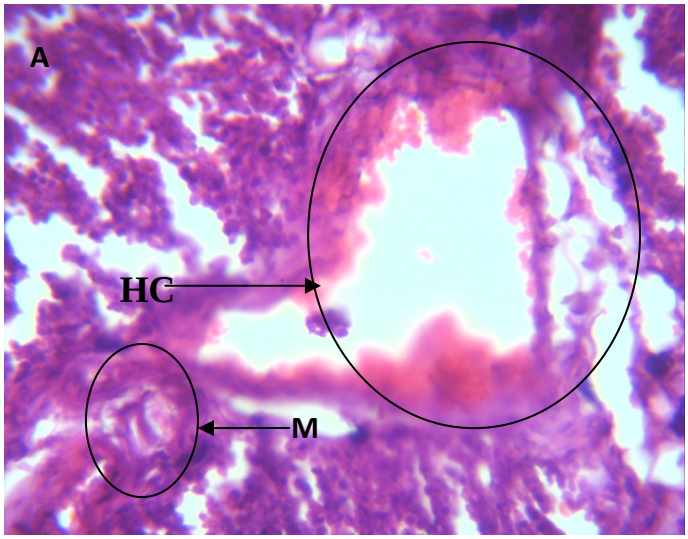
Weight change and weight comparison of Wistar rats at Initial (day 1) and Final (day 21) day of treatment

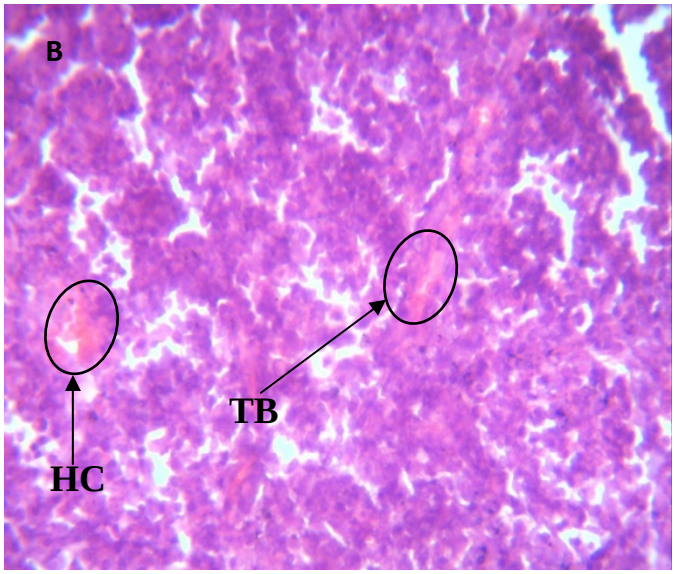
Groups	Initial weight (gram)	Final weight	Change in weight (gram)	Percentage change in weight (%)
1	89.90 ±15.93	114.5±12.99	24.6±2.94*	27.36
2	159.1±25.06	153.9±42.84	-5.2±17.78**	3.27
3	127.4 ±33.84	151.9±49.99	24.5±16.15*	19.23
4	108.7 ±24.00	137.7±19.34	29±4.46*	26.68
5	201.4 ±20.27	189.1±33.11	-12.3±12.84**	6.11
6	110.5 ±6.816	151.2±24.99	40.7±18.17*	36.83
7	118.7 ± 15.23	145.0±11.79	26.3±3.44*	22.17

The values are expressed as Mean ± STD *significant increase **significant decrease

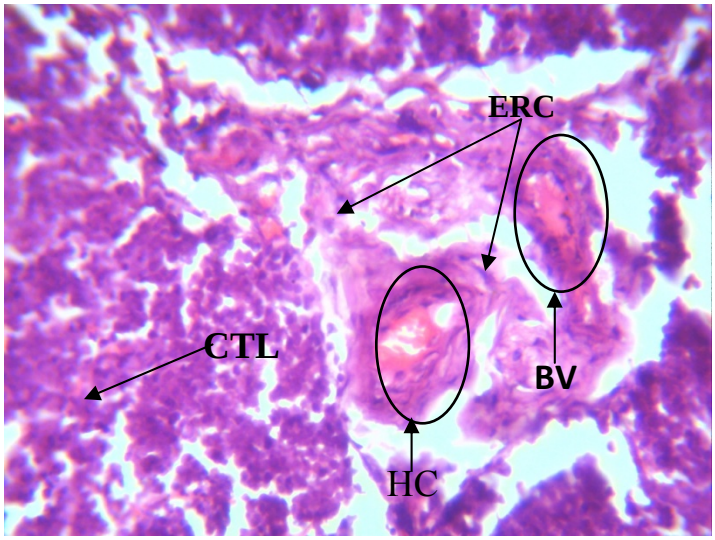
Weight change and weight comparison of Wistar rats at Initial (day 1) and Final (day 21) day of treatment

Photomicrograph of thymus sections from (group 1) , with rat thymus showing; CTL-cortex with thymic lymphocytes, TB- trabecular, BV- blood vessels, ERC- epithelia reticular cells, and HC- hassall's corposules(H&E x 400)

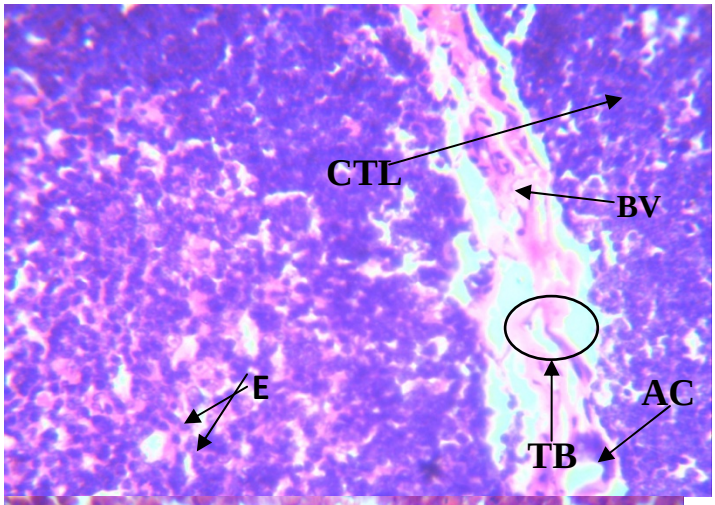


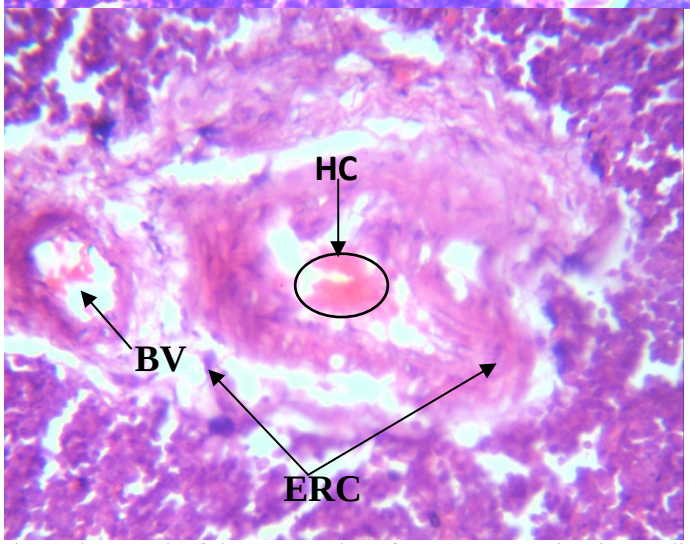


Photomicrographs (A and B) of thymus sections from (group 2) , with rat thymus showing; C-cortex with dense aggregation of thymic lymphocytes, TB- trabecular, M- medullar with distorted epithelia reticular cells, and HC- distorted hassall's corposules (H&E x 400)

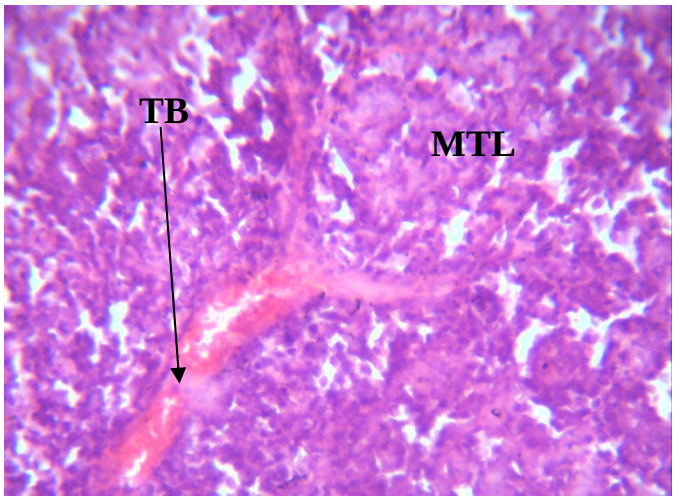


Photomicrograph of thymus sections from (group 3) showing normal and well oriented; HC-hassall's corpuscle, ERC- epithelia reticular cell, BV-blood vessels and CTL- cortex with thymic lymphocytes (H&E x 400)

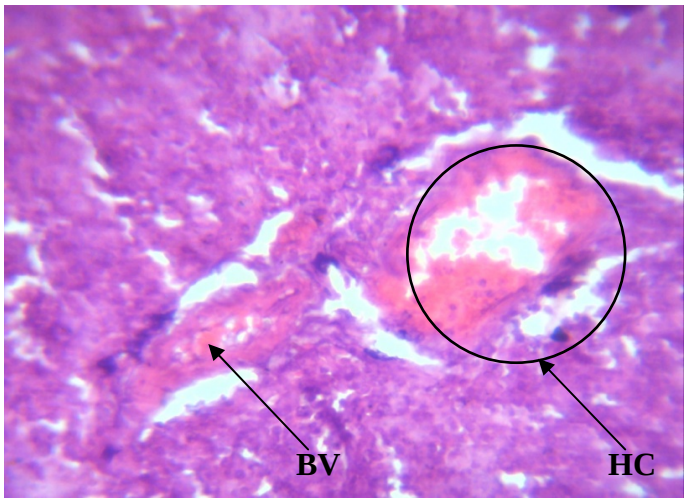




Photomicrograph of thymus sections from (group 4) showing well oriented; BV-blood vessels, TB- trabecular, M-medulla with epithelia reticular cell and CTL- cortex with thymic lymphocytes (H&E x 400)



Photomicrograph of thymus sections from (group 5) showing; shrinked BV- blood vessel, ERC- epithelia reticular cell, distorted or damaged, HC- Hassall's corpuscle, (H&E x400



Photomicrograph of thymus sections from (group 6) showing damaged BV-blood vessels and HC- Hassall's corpuscle (H&E X 400)

Photomicrogragh of thymus section from (group 7) showing damaged TB-trabecular and MTL- medulla with thymic lymphoctyes

DISCUSSION

This study showed that there was reduction in mean body weight of the rats in the lead treated groups only when compared with the rats in the Control group. This observed reduction in the mean bodyweight is in agreement with [18] who stated that that rats that are continuously exposed to heavy metals such as mercury (Hg), Cadmium (Cd), Arsenic (As) and Lead (Pb) usually results in reduction in the body weight. This reduction in the mean body weight with continuous exposure to lead could be explained on the basis of anorexia (loss of appetite) which is caused by lead exposure [19]. The decrease in the mean body weight could be explained more on the basis that the reduction of the mean body weight may be due to the decreased muscle mass and cachexia as a result of the oxidative stress induced by lead. In that, there was evidence that heavy metal toxicity was linked

to oxidative stress [20] which according to many researchers was associated with muscle wasting and cachexia leading to low body weight [21].
Furthermore, this study agrees with [22, 23, 24] who postulated that generation of reactive oxygen species, stimulation of lipid peroxidation and depletion of antioxidants are the major contributors to lead exposure related biochemical and histopathological changes in the animal body organs.

More so, as seen from the animals in G4 and G5, the administration of OG at low dose is an effective therapeutic strategy in restoring the histoarchitecture of the thymus as reported by Patrick, 2006 in one of his studies where he stated that lead exposed animals showed that a therapeutic strategy to increase antioxidant defense system of the body may be of help for long-term effective treatment of lead poisoning. Recently, several chelating agents approaches have been proposed therapeutically, including supplementation with antioxidants and up-regulation of endogenous anti-oxidative defense system for lead induced oxidative stress in various body organs [25, 26, 27]. However, the mechanism of actions of these chelating agents and antioxidants is still indistinct. It is believed that chelating agents reduce the lead toxicity in soft organs through its chelating activity, whereas antioxidants protect the cells from influence of oxidative damage protect the cells from influence of oxidative damage by scavenging the free radical generation and inhibiting of lipid peroxidation [28, 29, 30, 31 and 20]. As indicated by this study and [25], chelating agent, like OG, reduces the toxic effect of heavy metals on the histological sections of the thymus(G4 and G5), although it does not have similar effect in protecting the tissues against toxic effect (G6). Finally this study is in agreement with the believed that vitamins with antioxidant activity protect soft tissues against damaging effect of free radicals which produced as a result of heavy metals toxicity [26].

Conclusion

It could be right to conclude that aqueous extract of *O. gratissimum* in a dose-dependent manner has been proven to be an attractive target very effective in its protective effects against lead acetate induced thymus damages and act via the same route as vitamin C

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