CIBTech Journal of Microbiology ISSN: 2319-3867 (Online)
An Online International Journal Available at http://www.cibtech.org/cjm.htm
2016 Vol. 5 (3) July-September, pp.6-15/Okekeani et al.
Research Article

PROTECTIVE EFFECTS OF *OCIMUM GRATISSIMUM* ON MERCURY INDUCED HEPATOTOXICITY IN ADULT WISTAR RATS

Okekeani M.C[1], *Ezejindu DN[1], Anokwulu I.O[1], Chukwujekwu I.E[1], Nzeako H.C[2], Sobanke A.O[3], Ekezie Jervas[4], Ezejindu C.N[5] and Nwachukwu N.O[5]

[1]*Department of Anatomy, Faculty of Basic Medical Sciences, Nnamdi Azikiwe University, Nnewi Campus, Anambra State, Nigeria*

[2]*Department of Surgery, Faculty of Medicine, Nnamdi Azikiwe University, Awka, Anambra State*

[3]*Department of Physiology, Faculty of Basic Medical Sciences, Nnamdi Azikiwe University, Nnewi Campus, Anambra State, Nigeria*

[4]*Department of Anatomy, School of Basic Medical Sciences, Federal University of Technology, Owerri, Imo State, Nigeria*

[5]*Department of Microbiology, Faculty of Biological and Physical Sciences, Abia State University, Uturu, Abia State*

**Author for Correspondence*

ABSTRACT

Ocimum gratissimum is one of the essential medicinal plants whose leafs are used in folk medicine for treatment of different diseases. The present study is amid at studying the hepatoprotective effects of *Ocimum-gratissimum* on mercury induced wistar rats. Twenty adult wistar rats weighing 200-3200g were used for the study and were allocated into four (4) groups of five animals each. Group A served as the control and received 0.5ml of distilled water orally; the experimental groups B, C, D received different doses of drugs; group B received 0.3ml of mercury, group C received 0.5ml of *Ocimum-gratissimum* while group D received 0.3ml of mercury in the first two weeks and 0.5ml of aqueous leaf extract of *Ocimum-gratissimum* for the last two weeks. The administration was done orally for twenty eight days (28). Twenty four hours after the last administration, animals were weighed, sacrificed under the influence of chloroform vapour and dissected. Liver tissues were harvested, weighed and trimmed down to a size of 3mm×3mm thick and fixed in 10% formalin for histological studies. The final body weight result showed significantly decrease in group B when compared with the experimental control group A while groups C and D increased significantly relative to the control group A. The relative organ weight result showed that group B animals had elevated weight when compared with the control group. The present study therefore suggests that consumption of aqueous leaf extract of *Ocimum-gratissimum* could be protective to the liver cells against mercury induced toxicity.

Keywords: *Ocimum-Gratissimum, Hepatoprotective, Mercury, Wistar Rats*

INTRODUCTION

The use of plant and herb extract in the treatment of human ailment is a very ancient art, a practice that has been passed on for generations and scientists in Africa and other developing countries are conducting research into local plant abundant in the continent for their possible use in traditional medicine (Nneamaka, 1991).

Research into traditional plant and herb received further boost due to the increasing resistance to many orthodox medicine and thus a search for new organic molecules of plant with antimicrobial properties (Sofowora, 1993).

Ocimum-gratissimum (Scent leaf) is one of the plants of interest in the quest for treatment of human ailment.

The benefits of *Ocimum gratissimum* have been implored in both human and animal science. Other benefit includes antioxidant properties (Bhanl *et al.,* 2011), anitleishmanial activity (Tania *et al*., 2006) and possession of Novel cancer-fighting compounds that needs to be isolated, purified and characterized (Stephen *et al.,* 2010).

Ocimum gratissmum has been used extensively in the traditional system of medicine in many countries. In the Northeast of Brazil, it is used for medicinal, condiment and culinary purposes. The flowers and leaves of this plant are rich in essential oil and so it is used in preparation of teas and infusion (Rabelo *et al.*, 2003).

In the coastal areas of Nigeria, the plant is used in the treatment of epilepsy, high fever and diarrhea (Ephraim *et al*., 2003). In the Savannah areas decoction of the leaves are used to treat mental illness (Akinmoladun *et al.*, 2007).

Mercury is a highly hazardous pollutant with an estimated global natural mercury emission of 1,800-5,800 tons per annum and global anthropogenic mercury emission to the atmosphere was estimated to be 2,190 tons in 2000. Since mercury is ubiquitous in the environment, it is nearly impossible for most human and animal to avoid exposure to some form of mercury, be it elementary, organic or inorganic mercury through biotransformation and bioaccumulation has found its way through the food chain to human.

Anthropogenic activities and industrialization are also sources of mercury pollution that had resulted in several catastrophe of mercury poisoning in Japan, the Amazon basin and Iraq. Moreover, mercury pollution and poisoning have imposed a huge economic cost on environment remediation and public health.

All forms of mercury causes toxic effects in the number of tissues and organs depending on the chemical form of mercury as well as the level, duration and the route of exposure. Exposure to mercury compounds typically occurs by inhalation or ingestion. Ingested mercury is absorbed in the gastrointestinal tract (GIT) and it is distributed to all tissues in about 30h while inhaled through mercury vapour accumulates in red blood cells and is carried to all tissues in the body in less than 24h. Mercury undergoes extensive biliary-hepatic cycling. It is secreted into bile and partially reabsorbed into the portal circulation and thereby returned to the liver. The high mobility of mercury in the body is attributed to the formation of water-soluble mercury complexes that are mainly, if not exclusively, attached to the sulfur atom thiol group such as glutathione.

Mercury as a bio hazardous metal which is found naturally in the environment in different chemical species shows in sample analysis that average 70kg man has mercury deposited of about 13mg in the skin, nail, hair, kidney and liver as well. In growing children mercury tend to have a prorogued neurotoxic effect in the central nervous system (CNS) and peripheral nervous system (PNS). To some group of genetically prone individual exposure to the metal lead to the development of immune-dysfunction. Toxicity associated with mercury arises through avid bounding with sulfhydryl (-SH) and to a lesser degree hydroxyl, carboxyl and phosphryl group. The linking's modifying signal transduction events in the body.

The liver is the largest of the abdominal viscera, occupying a substantial portion of upper abdominal cavity. It prefers a wide range of metabolic activities necessary for homeostasis, Nutrition and immune defense. It is composed largely of epithelia cell (Hepatocytes), which are bathed in blood derived from hepatic portal vein and hepatic arteries. The liver is the key organs regulating homeostasis in the body, the liver is involved with almost all the biochemical pathway related to growth, fight against diseases, nutrient supply, energy production and reproduction. Because of its unique metabolism and relationship to the gastrointestinal tract, is important target for toxicity produced by drugs, xenobiotics and oxidative stress (Anusha *et al.*, 2011, Jaescke *et al.*, 2002)

Therefore, this research is aimed at determining the effects of aqueous extract of *Ocimum gratissimum* on the liver of the adult wistar rats.

MATREIALS AND METHODS

Location and Duration of the Study

This experiment was carried out at the Animal House of Human Anatomy Department, Nnamdi Azikiwe University, Nnewi Campus, Anambra State, Nigeria. The rats were made to acclimatize for two weeks after which the test substance were administered for six weeks

Research Article

Procurement of Plant

The leaves of *Ocimum gratissimum* was procured from Nnewi in Anambra North (Anambra state, Nigeria) and authenticated at the herbarium unit Department of Botany, Nnamdi Azikiwe University, Awka, Nigeria.

Preparation of Extract

Fresh leaves of *Ocimum gratissimum* were air-dried and grinded into fine powder with hand grinder. The powder was macerated into absolute alcohol at room temperature. The filtrate was concentrated under reduced pressure and later evaporated in a water bath using evaporating dish at 45oC. A greenish paste of *Ocimum gratissimum* extract measuring 0.5litre was obtained.

Experimental Procedure

Twenty (20) adult wistar rats weighing 150 to 80g were obtained for the study. The animals were fed with standard diet and water and were adapted to the laboratory environment in the Department of Human Anatomy for two weeks in order to acclimatize.

The animal care and handling was conducted in compliance with the National Regulations for Animal Research. University Ethical committee reviewed the protocols, which were consistent with International Animal Welfare Guidelines.

The drugs were administered once in a day between the hours of 12 to 30pm for a period of twenty eight days. Wistar rats weighing between 320g and 200g were grouped into four (4) groups of A, B, C and D of five animals each. Group A served as control and received 0.5ml of distilled water. Group B, C and D received different doses of mercury and *Ocimum gratissimum* extract as follows: Group B received 0.3ml of mercury, Group C received 0.5ml of extract while Group D received 0.3ml of mercury in the first two wees and 0.5ml of extract of *Ocimum gratissimum* in the last two weeks. Oral route of administration was used and the administration lasted for twenty eight days. After the last administration, the animals were weighed and their weight recorded.

Twenty four hours after the last administration, the animals were anaestathized under chloroform vapour and were dissected. Blood samples were collected by cardiac puncture using sterile syringes with needles. Blood for serum preparation was collected into sterile plain tubes without an anti-coagulant. Serum samples were separated from the clot by centrifugation at 3,000g for 5minutes using bench top centrifuge (MSE, Minor, England).

Serum samples were separated into sterile plain tubes and were stored in the refrigerator for analysis. All analysis on blood serum samples completed within 24hours of sample collection. Liver tissues were harvested from the animals and weighed. They were trimed down to a size of 3mm x 3mm thick and fixed in zenkers fluid for four (4) hours for histological studies.

Tissue Preparation

Tissue sections of the organ were produced through normal histological procedures; fixation, dehydration, clearing, embedding, sectioning, mounting and stained with hematoxylin and eosin.

RESULTS AND DISCURSSION

Results

Analysis of Changes in Body Weight of the Rats

Table 1: Initial and Final Body Weight of the Rats

Group	Initial Weight	Final Weight	t-Stat	P-Value
A	196.00±16.73	254.00±16.73	-8.744	0.001
B	260.00±43.21	220.00±46.90	5.657	0.011
C	222.50±5.00	245.00±5.78	7.000	0.006
D	212.50±9.57	219.50±22.17	5.196	0.014

Paired sample t-test showed that while there was a significant increase in final body weight in group A ($p<0.05$), groups B, C and D indicated a significant decrease in body weight between initial and final body weights ($p<0.05$).

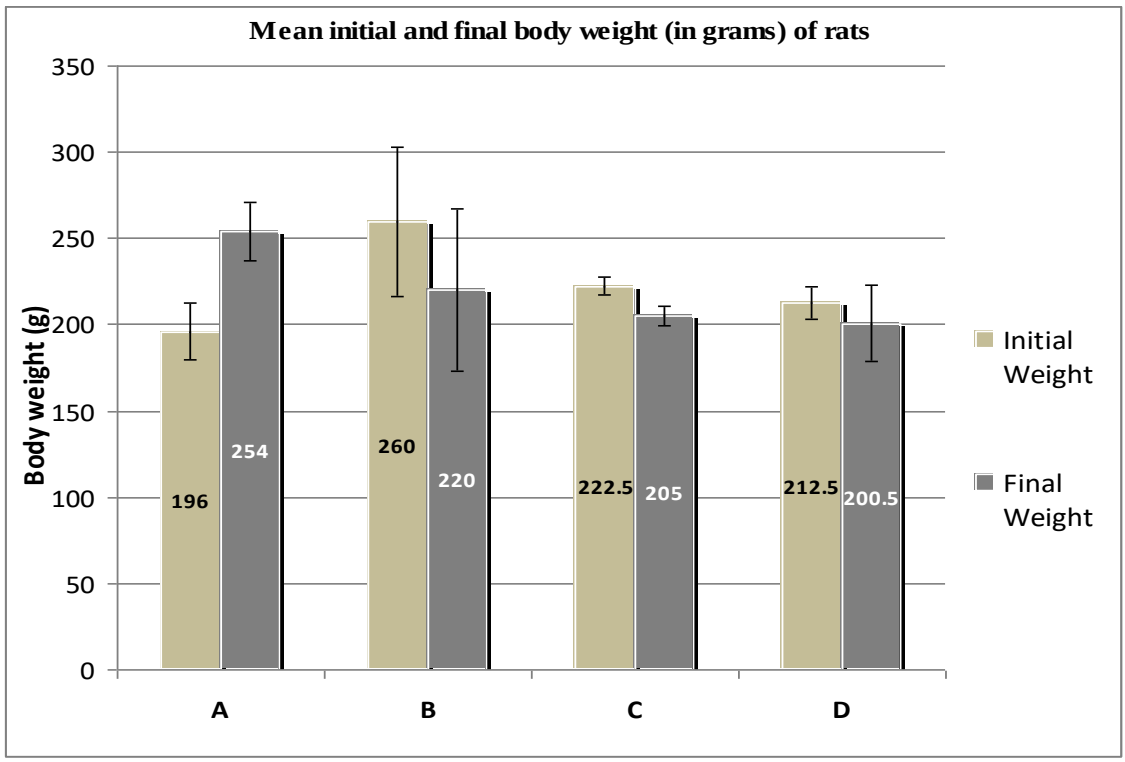

Figure 1: The Chart above Depicts the Mean Initial and Final Body Weights of the Rats in Groups A, B, C and D
[* Indicates Significant Increase in Body Weight; # Indicates Significant Decrease in Body Weight]

Analysis of Organ Weight

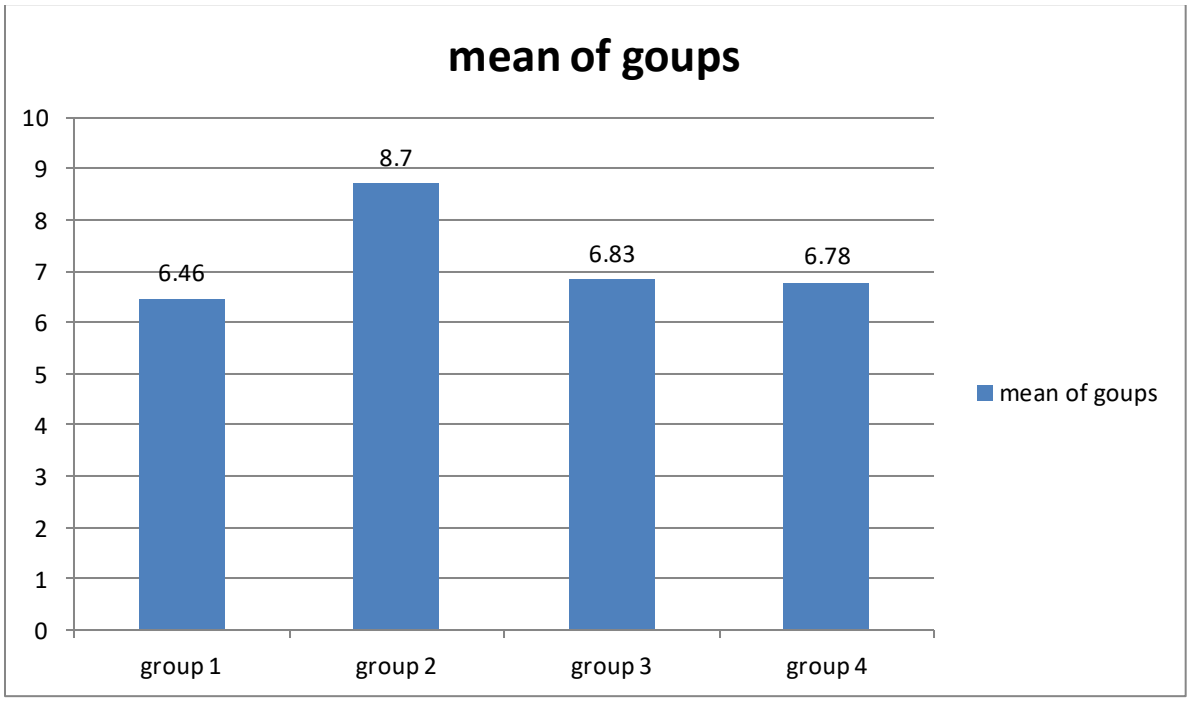

Figure 2: The Chart above Depicts the Mean Liver Weights of the Rats in Groups A, B, C and D

Table 2: Liver Weight of the Rats

Group	Liver Weight (g)	F-Ratio	P Value
A	6.46±0.75	9.169	0.002
B	8.70±1.04#		
C	6.83±0.74*		
D	6.78±0.51*		

[# significant increase compared to control; * no significant difference compared to control]

Activities of Serum Levels of Aspartate Aminotransferase (AST) Alkaline Aminotransferase (ALT) and Alkaline Phosphatase (ALP)

Table 3: Comparison of Activities of Serum Level of AST, ALT & ALP in all the Groups (Mean ± SEM Given for each Groups)

Groups	Group A	Group B	Group C	Group D	F-Ration	Prob. of Sig.
AST	70.50±2.30	85.60±2.70	76.30±4.60	73.10±3.40	27.60	<0.001
ALT	60.20±2.60	78.20±2.40	70.30±3.40	61.40±2.80	33.40	<0.001
ALP	175.30±3.30	192.30±5.10	182.20±4.50	177.60±2.80	11.50	<0.001

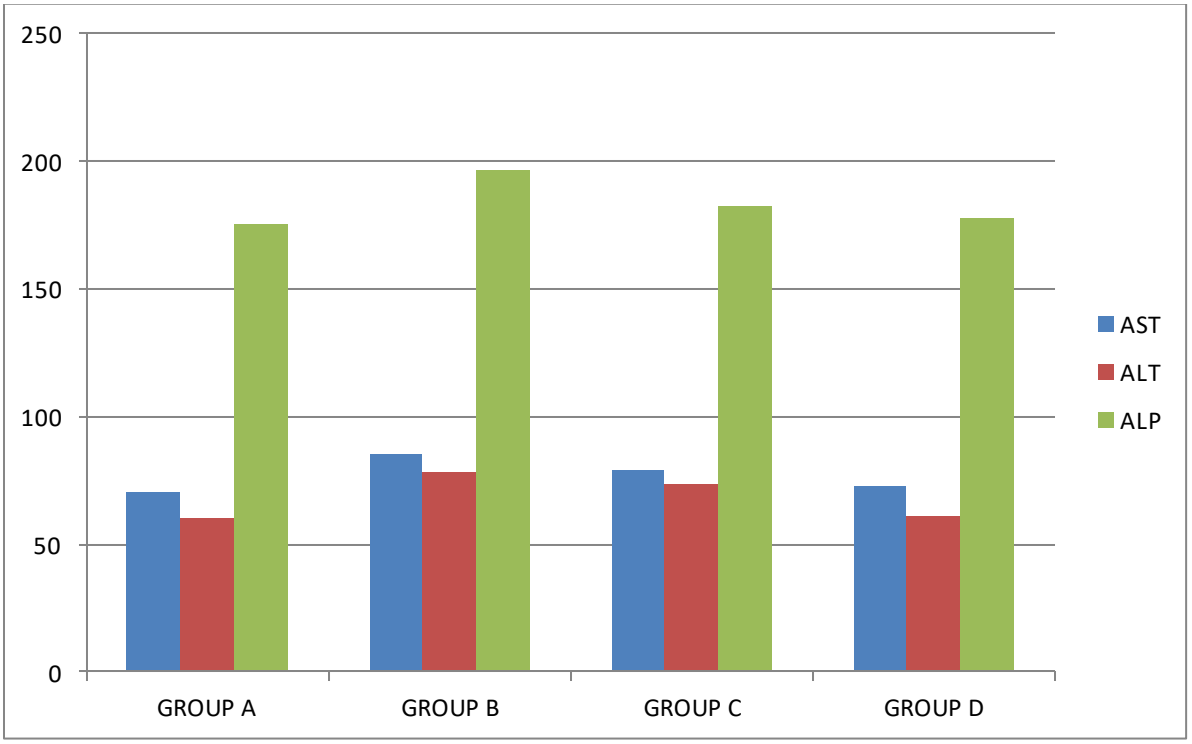

Figure 3: Bar Chart Showing the Comparison of Activities of Serum Level of AST, ALT & ALP in all the Groups

RESULTS AND DISCUSSION

Histopathological Findings

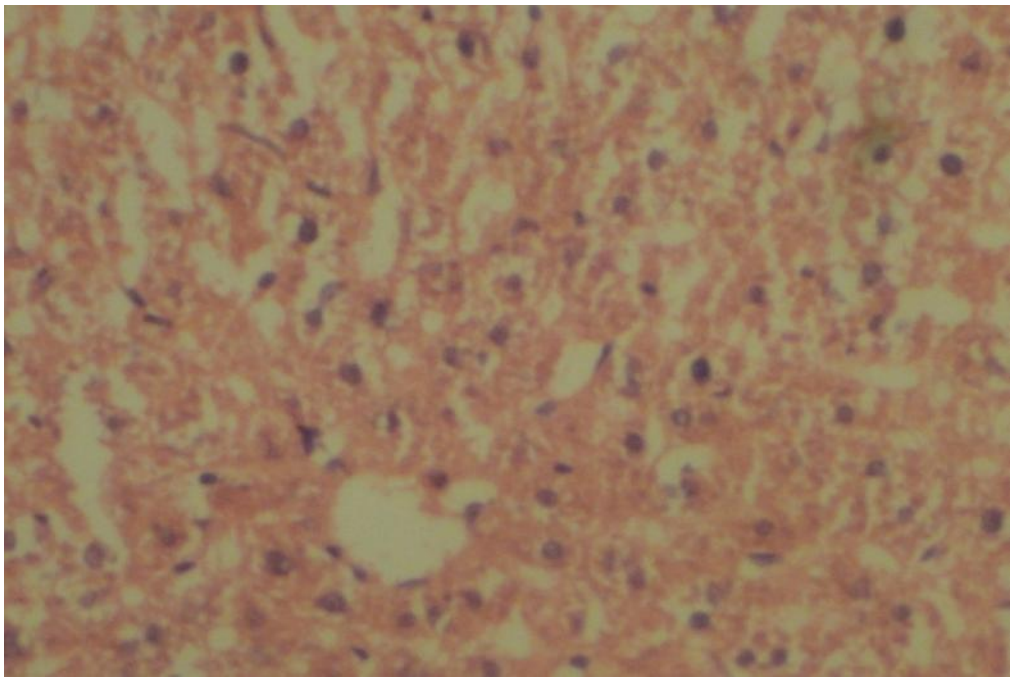

Plate 1: Control Group (A): Showing H & E Section of Liver with Normal Histology (x100)

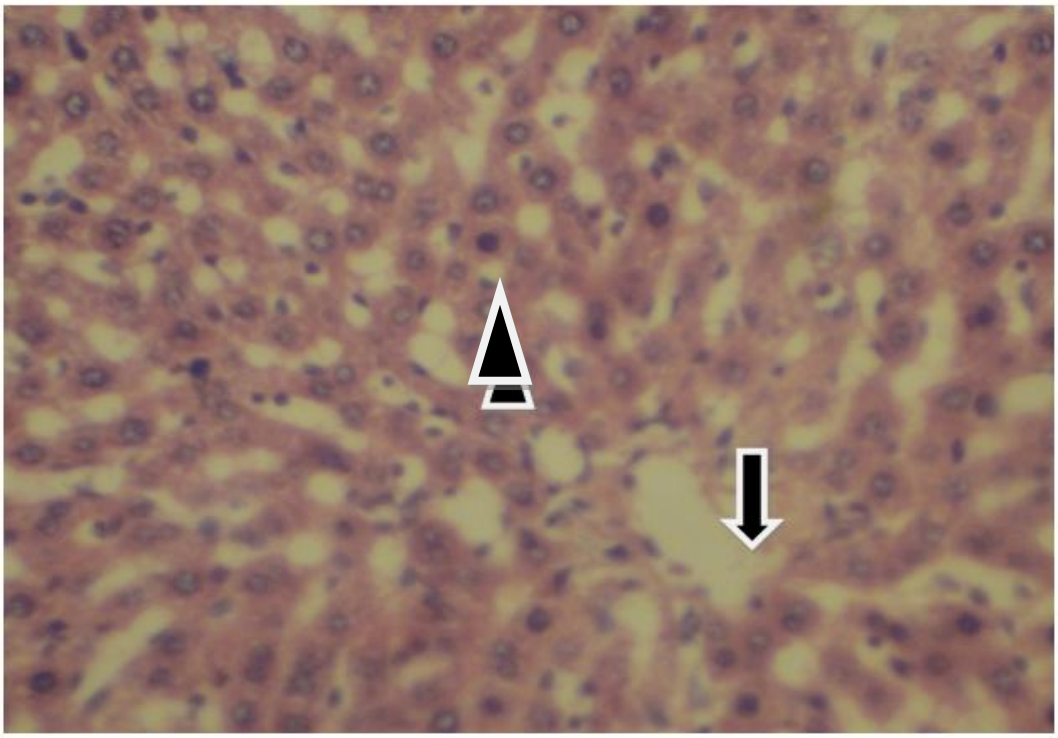

Plate 2: Group B: Showing H & E Section of Liver with Vacoulated Hepatocytes and Severe Distorted Stroma (x100)

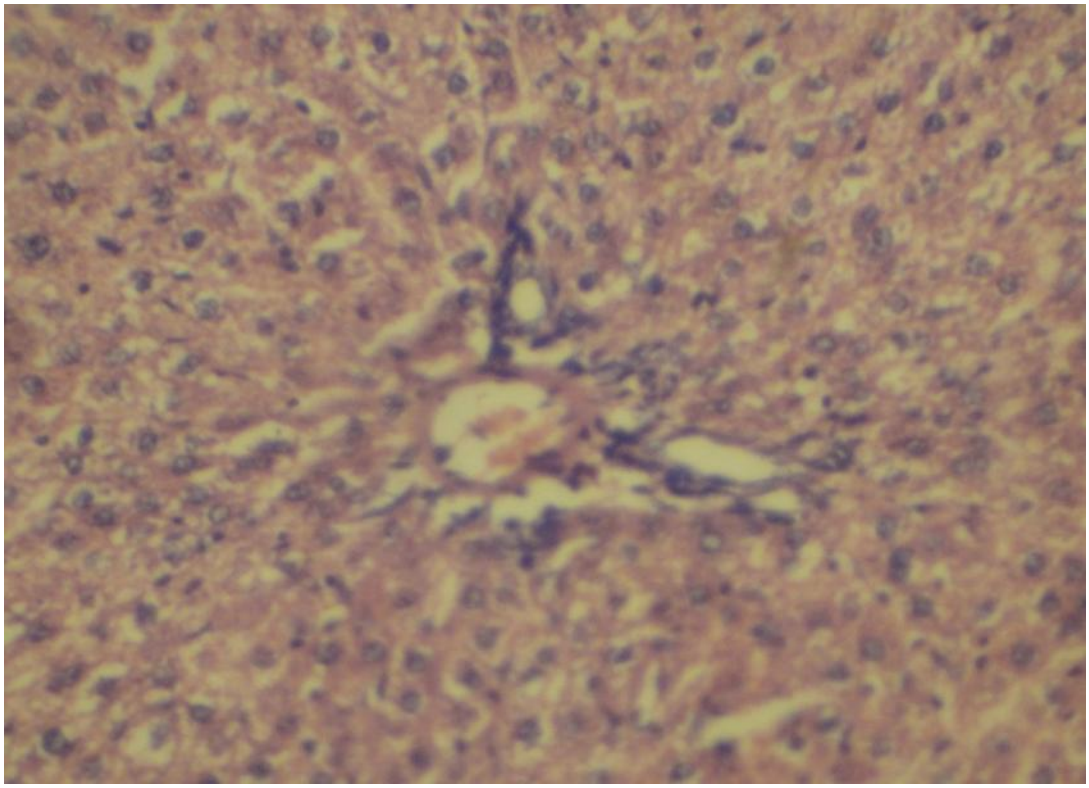

Plate 3: Group C: Showing H & E Section of Normal Histology of Liver with Centrally Placed Portal Artery (x100)

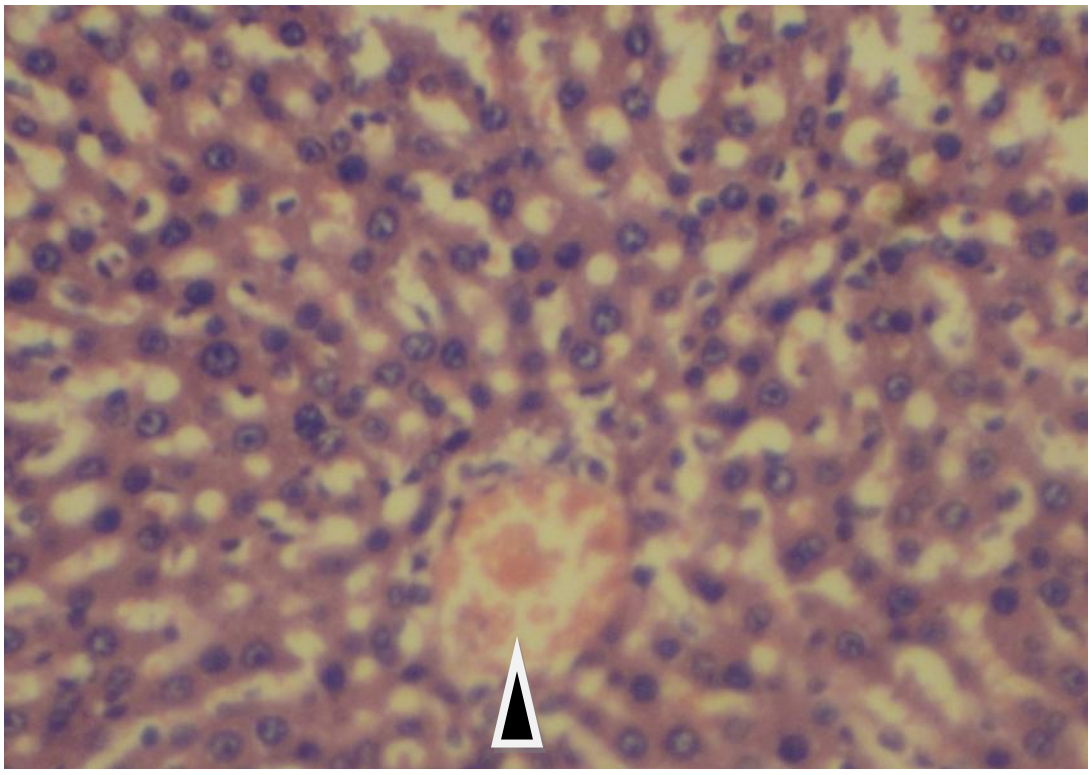

Plate 4: Group D: Showing H & E Section of Liver with Congested Central Vein (x100)

Discussion

Mercury is a toxic substance which can as well be regarded as an important factor in hepatotoxicity. The toxicity of mercury depends on its chemical form, while in some cases various mercury compounds have different toxicities depending on physical and chemical properties that affect absorption, distribution, tissue affinities and stability within the biosystem. For instance, elemental mercury in the liquid state has unique toxic effects that differ from those of mercury vapors; likewise organic mercury and molecules with toxicological different from inorganic forms (NTP, 1993). Mercury as a common environmental and occupational toxic heavy metal is also known to have direct and indirect effects on biological system and cell (Hesse, 2007; Bjomberg *et al*., 2011) one of the major ways that mercury exert its toxic effects is through oxidative stress that may be an important contributor to the negative pathogenesis observed after mercuric chloride exposure (Valera *et al*., 2008; ATSDR, 2011).

In this study, mercury was used to induce the liver damage. The result of this study agrees with previous researchers that mercury is a high toxic metal that causes toxic effect in a number of tissues and organs depending on the chemical form, level, duration and route of exposure (ATSDR, 1999; Clarkson, 2002). Liver plays a very important role in the metabolism of foreign compound entering the body. The exposure to the foreign compound could equally be through contaminated food or through synthetic drugs consumed for various pathological conditions. These foreign compounds have many toxic manifestations in human liver (Rajesh and Latha, 2004). Liver diseases remain one of the serious health problems and medicinal plants and herbs have been in use for treating these. The present modern age demands proof on a scientific basis to justify the various medicinal uses of herbs (Pohocha and Grampurohit, 2001).

Result from this study showed infiltration by inflammatory cells, vacuolated hepatocytes and severe distorted stoma, which tends to agree with (Agarwal *et al*., 2010; Ibegbu *et al*., 2013) that under the conditions of liver toxicity, some cells become infiltrated close to the damaged hepatocytes and play a major role in the development of fibrosis, including the fibrosis secondary to alcoholic liver disease. And this fibrosis may become irreversible, leading to liver cirrhosis (Junqueira and Caneiro, 2005; Hesse, 2007; Quirino *et al*., 2012).

It was observed that the group in which the rats were treated with the aqueous extract of *Ocimum gratissmum* and mercury tolerated mercury to an extent and there was much difference in the liver compared with the group B which received only the mercury. Thus, these findings implied the potential of *Ocimum gratissimum* aqueous extract on increasing antioxidant activity and reducing inflammatory associated proteins in liver of rats induced with mercury. This is similar to the reports by Ighodaro and Ebuehi, (2009), which indicated that administered orally aqueous extract of *Ocimum gratissimum* leaf could reduce oxidative and toxicant activity and then enhances specific activities of hepatic antioxidant enzymes in rats.

Observation of the body weight differences in the groups revealed gradual increase in final body weight of animals in control group A. This could have been physiological as the only substance they were exposed to was water and food. Then, comparing the result of weight differences also revealed severe loss of final body weight of the mercury exposed group. This is probably as a result of loss of appetite by the animals in the group. Group C that received only the extract of *Ocimum gratissimum* and group D that received both the extract and mercury had a mild decrease in final body weight when compared with the mercury group. These could be as a result of inhalation and ingestion of mercury during administration, which agrees with (Clarkson, 2002), that exposure to mercury could occur by inhalation or ingestion. Ingested mercury is absorbed in the gastrointestinal tract and it is distributed to all tissues in about 30hours, while inhaled through mercury vapour accumulates in red blood cells and is carried to all tissues in the body in less than 24hours. These means that group B, C and D indicated a significant decrease in body weight between the initial and final body weight ($P < 0.05$).

The relative organ weight also showed significant differences in the groups. As a result of these differences, there was relative increase in the liver weight for the mercury exposed animals compared to the control group and group D animals exposed to both mercury and the extract. This organ weight increase was irrespective of the fact that there was total body weight loss. This could have been

pathological and one may deduce that the increase in liver weight was not growth but as a result of inflammation.

Antioxidant properties of *Ocimum gratissimum* could have been responsible for the control group or prevention of inflammation in group C and group D respectively. This is in line with (Okonkwo and Njoku, 2011) that carried out the antioxidant properties of *Ocimum gratissimum*. Therefore, the current result indicated significant increase in the liver weight of group B and significant increase in group C and D when compared to the control group.

Conclusion

Ocimum gratissimum has proofed its ameliorative and hepatoprotective effect in this study. It was observed that aqueous extract of *Ocimum gratissimum* has an appreciable ability to prevent damage to the liver following exposure to mercury.

REFERENCES

Agarwal R, Raisuddin S, Tewari S, Goel S, Raizada RB and Behari JR (2010). Evaluation of comparative effect of pre and post treatment of selenium on mercury induced oxidative stress, histological alterations and metallothionein mRNA expression in rats. *Journal of Biochemistry and Molecular Toxicology* **24**(2) 123-135.

Agency for Toxic Substances and Disease Registration (ATSDR) (1999). *Toxicology Profile for Mercury*, (Atlanta, GA: US Department of Health and Human Services) 345-347.

Akinmoladum AC, Ibukun EO, Emmanuel A, Obuafor EM and Fawmbi EO (2007). Photochemical constituent and antioxidant activity of extract from the leaves of *Ocimum gratissimum*. *Science Research Essay* **2** 163-166.

Bhanu P, Ravindra S, Pwinyanka S, Prashant M, Nawai KD and Ravindra NK (2011). Efficiency of chemically characterized *Ocimum gratissimum* L. Essential oil as an antioxidant plant based antimicrobial against fungal and aflatoxin BI contamination of species. *Food Research International* **44**(1) 385-390.

Bjornberg A, Vahter M, Bergland B, Nilasson B, Blennow M and Sandborgh G (2011). Transport of methylmercury and inorganic mercury to the fetus and breast-fed infant. *Environmental Health Perspectives* **113**(10) 114-119.

Clarkson TW (2002). The three modern faces of mercury. *Environmental Health Perspectives* **110** 11-13.

Ephraim KD, Jack TW and Sodipo OA (2003). Histopathological studies on toxicity of *Ocimum gratissimum* leaf extract on some organs of rabbit. *African Journal of Biomedical Research* **6** 21-25.

Hesse RW (2007). *Jewelry Making through History*, (Greenwood Publishing Group Connecticut, USA) 120.

Ibegbu AO, Alatise AT, Babatunde AO, Dassah JS, Umana UE, Humman WO, Musa SA and Apulu PS (2013). Effects of vitamin E on phostoxin induced changes in the liver and biochemical parameters of adult wistar rats. *Journal of Biological and Environmental Science* **7**(21) 121-129.

Ighodoro MO and Ebuechi OA (2009). Aqueous leaf extract of *Ocimum gratissimum* potentiates activities of plasma and hepatic antioxidant enzymes in rats. *Nigerian Quarterly Journal of Hospital Medicine* **19**(2) 106-109.

Janine AL, Xisto SP, Orionaida FL, Jose RP, Pedro HF, Lucia HS, Aline AL and Maria RS (2005). Antifungal activity from *Ocimum gratissimum* L. towards Cryptococcus neoformans. Mem. *Institute of Oswaldo Cruz* **100** 55-58.

Nneamaka AI (1991). Screening of some Nigerian medical plants for bacterial activity. M.Sc. Thesis A. B. U Zaria.

Okonkwo CJ and Njou OU (2011). Antioxidant effect of *Ocimum gratissimum* Linn leaf extract against carbon tetrachloride induced oxidative stress in wistar rats. *International Journal of Current Research* **2**(1) 001-007.

Pohocha N and Grampurohit ND (2001). Antispasmodic activity of the fruits of helicteres isora linn. *Phytotherapy Research* **1** 49-52.

Quirino CJ, Marcilia AF and Renerio FJ (2012). Depression, insomnia, and memory loss in a patient with chronic intoxication by inorganic mercury. *The Journal of Neuropsychiatry and Clinical Neurosciences* **1** 191-205.

Rabelo M, Souza IP, Soames PM, Miranda AV, Matos FS and Cuddle DN (2003). Antinociceptive properties of the essential oil of *Ocimum gratissimum* L. in mice. *Brazilian Journal of Medical Biology* **36** 521-524.

Rajesh MG and Latha MS (2004). Protective activity of glycyrrhiza glabra in carbon tetrachloride induced peroxidative damage. *Indian Journal of Pharmacology* **36** 284-287.

Sofowara IA (1982). *Medicinal Plants and Traditional Medicine in Africa*, (John Wiley, Chichester, UK) 256.

Stephen IN, Melvanique ST, Xuan L, Hengshan W, Yong C, Xiaopu Z and Gregond BB (2010). Potential cancer-fighting *Ocimum gratissimum* leaf extract; increased anti-proliferation activity of partially purified fractions and their spectral fingerprints. *Ethnicity & Disease* **20** 12-16.

Tania UN, Ricardo RM, Jose AM, Paloma KM, Benedito PD, Diogenes GC, Daniela SA and Celso VN (2006). Antileishmanial activity of eugenol-rich essential oil from *Ocimum gratissimum*. *Parasitology International* **55**(21) 99-105.

Valera B, Dewailly E and Poirier P (2008). Cardiac autonomic activity and blood pressure among Nunavik Inuit adults exposed to environmental mercury: a cross-sectional study. *Environmental Health* **28** 924-926.