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Research Article

AMELIORATING EFFECTS OF *OCIMUM GRATISSIMUM* ON LIVER ENZYMES OF MERCURY INDUCED WISTAR RATS

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ABSTRACT

Ocimum gratissimum has been used extensively in traditional system of medicine in many countries. The present study is amid at studying the ameliorating effects of *Ocimum-gratissimum* on liver enzymes of 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. Blood for serum preparation was collected through cardiac puncture for histochemical studies. Liver tissues were harvested, weighed and trimmed down to a size of 3mm×3mm thick and fixed in 10% formalin for histological studies. The activities of serum levels of aspartate aminotransferase (AST), alanin aminotransferase (ALT) and alkaline phosphotase (ALP) were determined using randox kit method. 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 A. There were significant increase in serum levels of aspartate aminotransferase (AST), alanin aminotransferase (ALT) and alkaline phosphotase (ALP) in group B animals when compared with the control group A. The present study therefore, suggests that consumption of aqueous leaf extract of *Ocimum-gratissimum* could ameliorate the toxic effects of mercury on liver enzymes.

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

INTRODUCTION

Ocimum gratissimum "scent leaf" (linn), family labiaceae is a shrub commonly found around village huts and gardens (Iwu, 1993). The plant is popular among various ethnic groups in Nigeria. It is known as *Effirin* in Yoruba, *Daidoya* in Hausa, *Nchuawu* in Igbo and *Ntoung* in Ibibio. Mostly a weed of the road side and wasteland, but is also important in pastures. It prefers moist and fertile soils during growth, but will tolerate drought after flowering (Swabirk, 1997). The plant occurs in deciduous forest and savannah and is usually cultivated for its medicinal uses and as food flavor. *Ocimum gratissimum* is propagated by seed or cuttings (Rabelo *et al.*, 2003). It is used by Ibos of south eastern Nigeria in the management of the baby's cord, to keep the wound surface sterile. It is also used in the treatment of fungal infections, fever, cold and catarrh (Ijeh *et al.*, 2005). The oil is known to exhibit antimicrobial, insect repellant and

antihelminthic activities (Sofowora, 1982). The extract of *Ocimum gratissimum* exhibited antibacterial activity (Ofokansi *et al.,* 2003).

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 (ATSDR, 1999).

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 biohazardous 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 (Valera *et al*., 2008; Clarkson, 2002).

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 work is aimed at evaluating the effects of aqueous leaf extract of *Ocimum gratissimum* on liver enzymes of mercury induced 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

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 200g and 220g were grouped into four (4) groups of A, B, C and D of five aknimals 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.

Enzyme Analysis

The activities of serum level of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphotase (ALP) were determined using randox kit method.

RESULTS AND DISCUSSION

Results

Physical and Behavioral Changes

The animals were weighted before and after administration and the initial and final body weight was recorded. Before the administration, the animals looked so healthy and strong but on the process of the administration the animals.

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	205.00±5.78	7.000	0.006
D	212.50±9.57	200.50±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 ($p<0.05$)

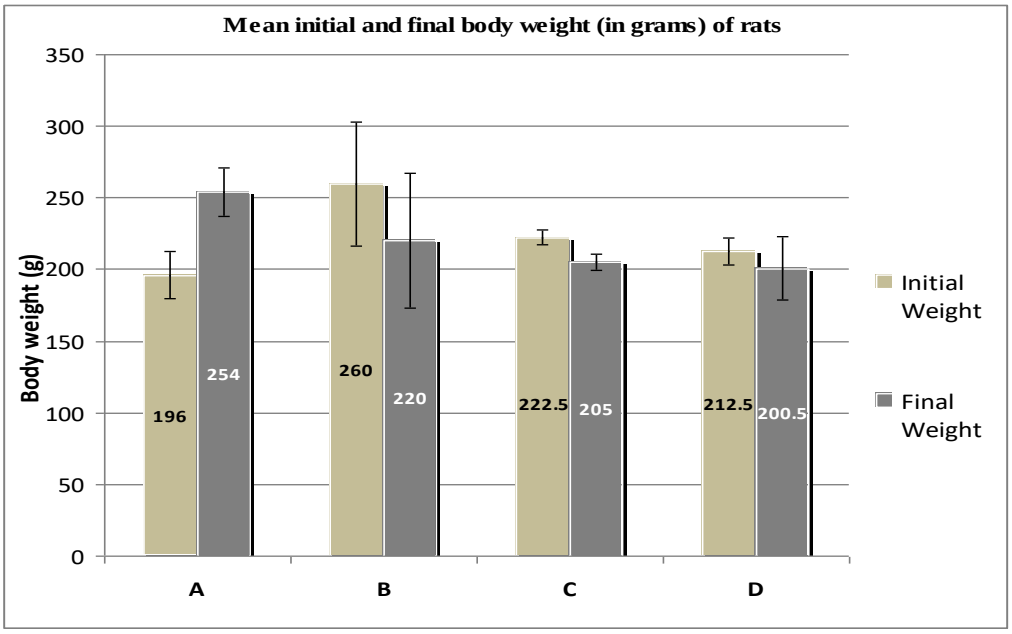

Figure 1: The Chart above Depicts the Mean Initial and Final Body Weights of the Rats in Groups A, B, C and D
[* Indicates Significant Increase in Body Weight; # Indicates Significant Decrease in Body Weight]

Analysis of the Organ Weight

Table 2: Liver Weight of the Rats

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

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

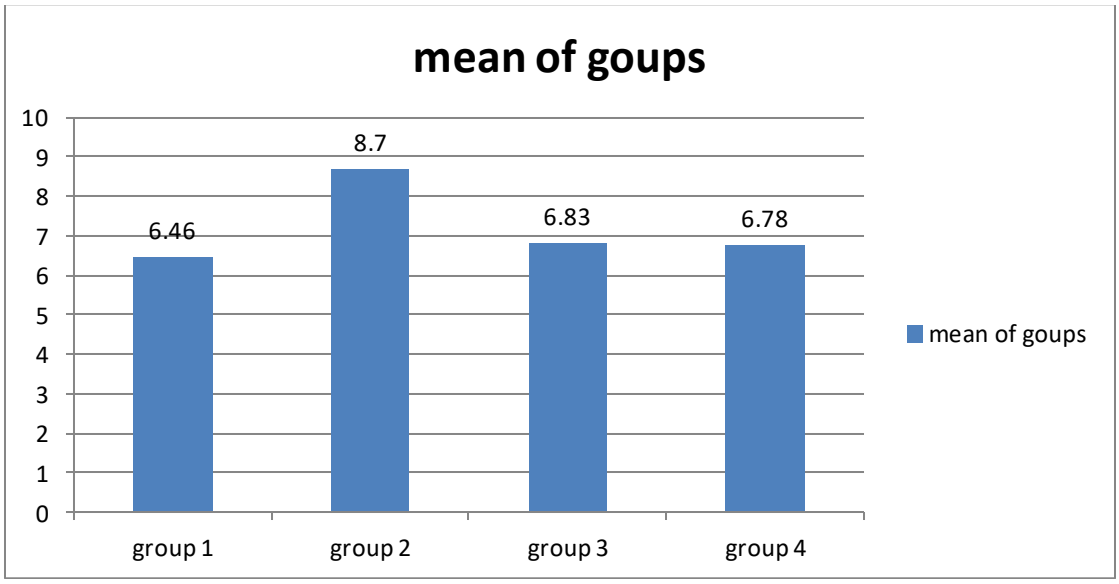

Figure 2: The Chart above Depicts the Mean Liver Weights of the Rats in Groups A, B, C and D

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

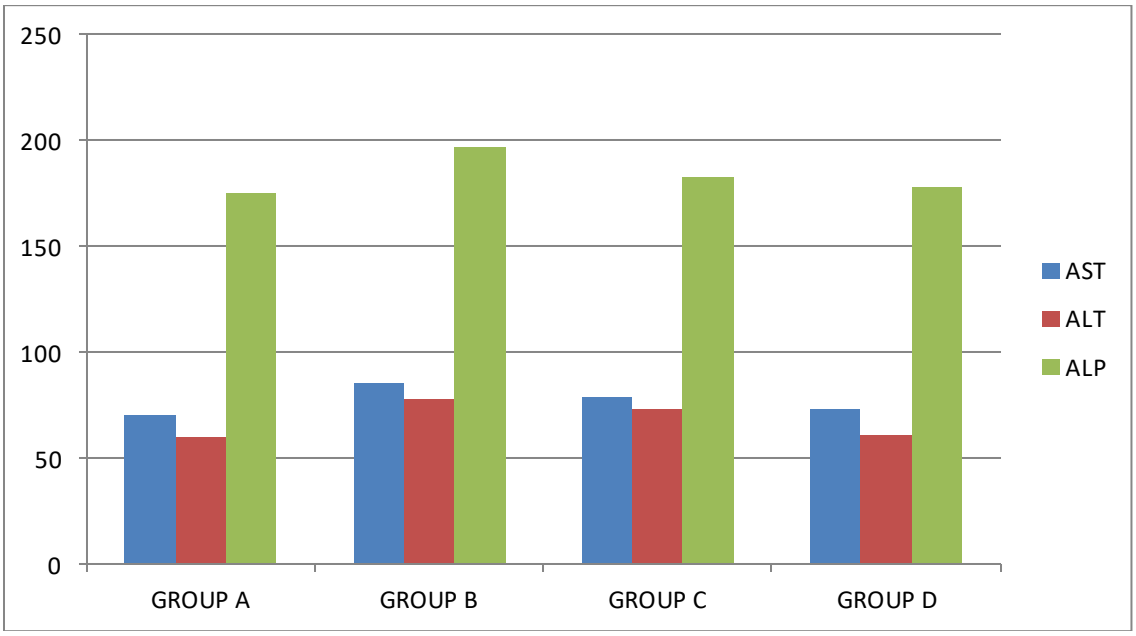

Figure 3: Bar Chart Showing the Comparison of Activities of Serum Level of AST, ALT & ALP in all the Groups

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 induced liver damage. 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).

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.

The comparison of activities of serum level of the enzymes (ALT), (AST) and (ALP) were used to measure the protective effect of *Ocimum gratissimum* on mercury induced hepatotoxicity in wistar rats, and it showed a significant increase in the level of the enzymes recorded in group B ($p< 0.01$) when compared to the control group.

Furthermore, significant differences ($p<0.01$) was equally observed in the serum and tissue level of AST ALT and ALP in groups C and D which were exposed to *Ocimum gratissimum* alone and group D treated with both mercury and extract of *Ocimu gratissimum* respectively when compared to the mercury exposed. Therefore, these result indicated that the extract of *Ocimum gratissimum* did not bring about cellular damage in the liver during the experimental period. This is in agreement with (Orafidiya *et al.*, 2001) and (Egesie *et al.*, 2006) that *Ocimum gratissimum* has proved to be an effective anti-microbial, hypoglycemic and hepatoprotective agent.

Serum AST, ALT and ALP are the most sensitive markers employed in the diagnosis of hepatic damage because these are cytoplasmic in location and are released into the circulation after cellular damage (Sallie *et al.*, 1991) ALP is a membrane bound enzyme (Wright and Plummer, 1974). While ALT and AST are cytosolic enzymes (Christen and Merzler, 1985). These enzymes are higly concentrated in the liver and kidney and are only found in serum in significant quantities when the cell membrane become leaky and even completely ruptured (Cotran *et al.*, 1989; Ngaha, 1981).

In addition, significant reduction in the serum total protein levels may be due to decline in protein synthesis by hepatic cells reflecting the hepatic dysfunction that accompanied by mercury treatment (Sankar *et al.*, 2009).

Conclusion

In this work, mercury showed it degenerative changes on the liver and also causes severe decrease in the final body weight and elevates the organ weight as well as increase in the serum activities of liver enzymes (ALT, AST and ALP) on the mercury exposed animals. From this work, it was observed that aqueous extract of *Ocimum gratissimum* has an appreciable ability to prevent damage to the liver enzymes exposed to mercury.

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