

## **Hepatoprotective and Hematological Effects of *Solanum melongena* (Garden Egg), *Solanum lycopersicum* (Tomato) and *Daucus carrots Subsp. Sativus* (Carrot) Extracts against Lead Induced Toxicity in Wistar Rats**

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### **Authors' contributions**

*This work was carried out in collaboration among all authors. Author DA designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors IPE and EDE managed the analyses of the study. Author MCM managed the literature searches. All authors read and approved the final manuscript."*

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### **ABSTRACT**

**Background of the Study:** Lead effects were assessed by analyzing the impacts of the extract on the liver enzyme concentrations and hematology parameters.

**Materials and Methods:** Thirty five male wistar rats weighing 85-110 g were distributed into five groups consisting of seven rats each. Group I served as control group, group II served as the test group, groups III, IV and V served as treatment groups. Lead acetate solution was given to the rats orally at a dose of 50 mg/kg body weight and 200 mg/kg of fruit extracts for 14 days. On day 15, biochemical analysis were carried out.

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**Results:** Effects of extracts showed that ALT,AST and ALP concentration in group II was observed to be significantly ( $p<0.05$ ) higher than the control and treatment groups with values. The hematology results showed that lead did not cause a significant reduction in the packed cell volume, white blood cell and red blood cell counts. However, the group treated with carrot and garden egg showed slight increase in RBC and WBC count when compared with the positive and negative control groups.

**Conclusion:** From the results above, it showed that the fruits extract have hematopoietic potentials and hence aid in the hepatoprotection of the liver of lead induced Wistar rats.

**Keywords:** Lead acetate; toxicity; liver enzymes; poison and diseases.

## 1. INTRODUCTION

In relation to public health and the number of people exposed to it, lead is among the biggest medical problems affecting the environment on a large scale [1]. It is one of the metals found since the beginning of mankind and it is rampant in different regions of the world. It is discovered as an ore and in minute amounts, similar to metals such as Copper, Silver and Zinc [2]. Lead poisoning can also be known as *plumbism* or *colica pictonium*. This is as a result of an increase in levels of lead within the body [1]. As a result of its vast usage in various industries, the appearance of lead in the ecosystem of biological organisms alongside their environment is in large concentrations and rising, affecting the operations of the biological ecosystems [3]. Heavy metals enters the body through different routes and are then toxic when the body does not metabolize them and they gather up in delicate tissues. This mostly affects people that work in the sectors of industry, agriculture and mining [4].

When found present in the body, lead affects approximately all the organs in the body which results in it being very toxic. Vital clinical signs of lead poisoning at sub-acute levels include loss of weight, weakness of muscles, severe pain as well as gastrointestinal problems [1]. Diverse chronic diseases have given life on earth signs of threat such as respiratory diseases such as corona virus, cancer which affects different parts of the body such as lungs, breasts etc, and strokes and so on. The one and only way is to rely and make use of nature's resources such as fruits and vegetables in fighting of these dreaded diseases and illnesses.

Fruits and vegetables are important for the proper functioning of the body such that they provide essential nutrients and minerals which perform various functions in different parts of the body and are derived from plant origin. Eating

fruits and vegetables, as well as grains, have been linked to minimal risk of cancer, diabetes, Alzheimer disease, cataracts, and diseases related with increased ageing. Other diseases include liver disease (Cirrhosis), stress, kidney diseases (such as kidney stones). These fruits and vegetables possess unique chemicals that fights against microorganisms, viruses, and inflammation alongside malaria and worms which makes it essential in drug production and to sustain proper health.

Over the course of time, plants were used to carry out different roles before the evolution of numerous scientific techniques and methods being hired in this modern day. Most plants have medicinal properties which increases their valuableness and ready for use. Plant species of different varieties are spread across the globe in the exception of those rare and indigenous plants found in only certain countries. Parts of the plant such as its roots, barks, stems or leaves serves as special storage units where the pharmacological and medicinal qualities of the plant are found. Fruits and vegetables preserves vast varieties of essential nutrients such as Vitamins A, D, E, K, C and Bs and minerals as sodium, potassium and calcium and so on. They help to prevent diseases resulting from lack of vitamins and improper acquisition of nutrients. Fruits and vegetables are essential parts of a food regime as they are elevated in fiber, vitamins and minerals which cannot be easily obtained . The fiber found in plants aid in digestion, adequate water absorption and easy removal of waste products. The nutrients provided by fruits and vegetables aids in stability the human body and ensures its proper functioning [5].

They possess different phytochemicals (also known as bioactive components) which are known for their pharmacological effects and health benefits. They help in preventing heart-related diseases because of the antioxidants

they possess, inflammation reduction functions and properties responsible for the modification of plasma lipids [6].

Hematology refers to the study of the numbers and morphology of the cellular components of the blood and the use of these results in the diagnosis and monitoring of disease. Hematological studies are useful in the diagnosis of many diseases as well as investigation of the severity of damage to blood [7,8]. Hematological studies are of ecological and physiological interest in helping to understand the relationship of blood characteristics to the environment [9] and so could be useful in the selection of animals that are genetically resistant to certain diseases and environmental conditions [10,11].

Haematological parameters are good indicators of the physiological status of animals [12]. Haematological parameters are those parameters that are related to the blood and blood forming organs [13]. Blood act as a pathological reflector of the status of exposed animals to toxicant and other conditions [14]. As reported by [11] animals with good blood composition are likely to show good performance. Laboratory tests on the blood are vital tools that help detect any deviation from normal in the animal or human body [15]. The examination of blood gives the opportunity to investigate the presence of several metabolites and other constituents in the body of animals and it plays a vital role in the physiological, nutrition and pathological status of an organism [16,17]. According to [14] examining blood for their constituents can provide important information for the diagnosis and prognosis of diseases in animals. Blood constituents change in relation to the physiological conditions of health [8]. These changes are of value in assessing response of animals to various physiological situations [12]. According to [18], changes in haematological parameters are often used to determine various status of the body and to determine stresses due to environmental, nutritional and/or pathological factors.

Natural toxins may also be present in food plants because of natural selection and new breeding methods that enhance these protective mechanisms. Plants are usual cause of medical dilemma, generally due to the phytochemicals, which are naturally found in plants and are biologically active and function to protect plants against invasion, disease, and infection. Secondary metabolites are produced by plants to

protect themselves against various threats such as bacteria, fungi, insects and predators . Phytochemical substances are not only to aid animal pollinators and seed distributors, but also to protect them from animals, which pose a risk. However, some phytochemical or secondary metabolites produced by plant are toxins like substances, which are alike to extracellular bacterial toxins in their properties and may cause problems in humans. They are both useful and harmful effects in human beings and animals. Plant toxins may enter the body either by inhalation, swallowing or by contact. The action is mainly dependent on their Phyto-constituents like alkaloids, glycosides, proteins, tannins, volatile oils, terpenes, and steroids.

The aim of this research is to investigate liver enzymes and hematology parameters in response to damage caused by lead intoxication in albino wistar rats using the extracts of garden egg, tomato and carrot. The extracts of garden egg, tomato and carrot have been observed to help protect liver from acute injury by the toxic effects of environmental chemicals. Different studies have shown that these fruits possess phytochemicals that protect the liver from diseases and severe damages. It is essential to discover the hepatoprotective tendencies of these fruits for the control of lead poisoning.

## 2. MATERIALS AND METHODS

### 2.1 Identification and Preparation of Fruits Juice

Garden eggs (*Solanum melongena*), Carrots (*Datura carota subsp Sativus*) and Tomatoes (*Solanum lycopersicum*) were obtained from Bwari market, Federal Capital Territory, Nigeria. The fruits were validated by a botanist from the department of Microbiology, Veritas University Abuja. Using deionized water, sliced into pieces with sterile knife. The samples juice was extracted individually using juice extractor. The extracts were placed in a storage vessel and preserved in the refrigerator. The extract was refrigerated at 2–5<sup>0</sup>C until when used [19].

### 2.2 Handling of Experimental Animals

Thirty five healthy male rats were used for this study. The albino rats were 8-10 weeks old, with weights varying from 85-110 g. They were obtained from an animal farm in Kaduna state, Nigeria and housed in cages. The rats were

divided into five groups with seven rats each, as follows:

Group I (Control group receiving feed and distilled water).

Group II (Test group receiving 50 mg/kg of lead acetate, feed and distilled water).

Group III (Treatment group receiving 200 mg/kg of carrot and garden egg extract juice per average body weight).

Group IV (Treatment group receiving 200 mg/kg of carrot and tomato extract juice per average body weight).

Group V (Treatment group receiving 200 mg/kg of carrot, tomato and garden egg extract juice per average body weight).

Each group was given 120 g of feed and 300 ml of water daily until start of the experiment.

### 2.3 Induction of Experimental Lead Acetate

They were allowed to acclimatize for a week to the climate of the animal house, in the Veritas University, before the beginning of the experiment. During this period, they were fed with rat feed and distilled water *ad libitum* and kept at a standard laboratory condition of 12 hour light and 12 hour dark time alternations at a temperature range of 22-28°C and 40-50% relative humidity.

Good hygiene was maintained by proper cleaning, changing of beddings and removal of faeces and spilled feed from cages daily. The rats were induced lead acetate solution,  $Pb(CH_3COO)_2 \cdot 3H_2O$  which was prepared by dissolving 5 g of salt in a 500ml Erlenmeyer flasks, adding 2ml of water, and shaking vigorously.

### 2.4 Collection and Treatment of Samples

All the animals were anaesthetized with chloroform vapor, forty-eight (48) hours after the last day of extract administration and dissected for blood collection. Blood was collected from the heart by cardiac puncture using a 2 ml syringe and needle into a set of labelled plain bottles and EDTA bottles to obtain the serum plasma respectively. Each sample of blood was centrifuged at 3,000 rpm for 15 minutes, the serum was collected and distributed into labelled plain bottles. The serum was refrigerated and was used to carry out biochemical analysis.

The biochemical tests carried out were Liver enzymes: Alanine aminotransferase and Aspartate aminotransferase according to the method described by Reithman and Frankel (1957), serum alkaline phosphatase using Randox test kits. Hematology parameters were analysed as follows:

#### 2.4.1 Packed cell volume

The EDTA-treated blood samples were centrifuged in a capillary tube (microhematocrit tube) at 15,000 RPM for ten (10) minutes and the packed cell volume percentage was read using a microhematocrit reader [20].

#### 2.4.2 Total leucocytes count

The blood was filled to 0.5 in the pipette, then the TLC solution was filled to point 11, the rubber tubing was removed and both ends sealed, then the pipette was shook for 1 minutes which is important before filling the Neubauer chamber, after thorough mixing, the first few drops were discarded and was filled again until the platform was filled. The chamber was kept on the microscope stage for 2-3 minutes for the cells to settle, then 0.02 mL of blood was mixed with the diluting fluid and 0.38 mL of the TLC dilution fluid was mixed well with the blood. The blood was diluted with a fluid that causes the hemolysis of the RBCs but WBCs remain intact and then was counted in the Neubauer chamber. The gentian violet lightly stained the leucocytes and allows it to be counted. Neubauer chamber. The gentian violet lightly stained the leucocytes and allows it to be counted.

### 2.5 Red Blood Cell

#### 2.5.1 Preparation of RBC (Red Blood Cells) solution

Hayme's solution was used as the RBC counting solution which consists of NaCl = 1 g (Isotonic solution),  $Na_2SO_4 = 5$  g which will prevent rouleux formation,  $HgCl_2 = 0.5$  G acts as antiseptic and Distilled  $H_2O = 200$  mL.

#### 2.5.2 Procedure

A dilution of 1:200 was made with a diluting solution, and then the red bulb pipette was filled up to 0.5 marks with the solution drawn to the mark 101 of RBC pipette while the blood mixed. The first few drops (4 to 5) were discarded and then Neubauer chamber free from air bubbles

was filled and allow the cells to settle for 2 minutes. Then the RBC in the corner 4 squares and one central square chamber which fell on the left and top border of the squares was counted using 40 X microscopic lenses. Then the counting was repeated twice and divided by 2 to get the average.

### 2.5.3 Statistical Analysis

The results obtained from this study were analyzed by one-way analysis of variance (ANOVA), followed by Student's T-test to evaluate the significance of the difference between the mean value of the measured parameters in the respective control and test groups using SPSS windows. A significant change was considered acceptable at  $P < 0.05$ .

## 3. RESULTS

### 3.1 Serum Liver Enzyme Concentrations

Effects of extracts showed that ALT concentration in group II was observed to be significantly ( $p < 0.05$ ) higher than the control and treatment groups with values  $63.50 \pm 6.81$  U/l. AST concentration in group II was observed to be significantly ( $p < 0.05$ ) higher than the control and treatment groups with values  $46.75 \pm 2.25$  U/l. ALP concentration in group II was observed to be significantly ( $p < 0.05$ ) higher than the control and treatment groups with values  $156.63 \pm 3.46$  U/l.

### 3.2 Hematology Results

PCV = Packed Cell Volume, WBC = White Blood Cell Count, RBC = Red Blood Cell Count The hematology results showed that lead did not

cause a significant reduction in the packed cell volume, white blood cell and red blood cell counts. However, the group treated with carrot and garden egg showed slight increase in RBC and WBC count when compared with the positive and negative control groups.

## 4. DISCUSSION

Lead is considered as a medical hazard and environmental pollutant that affects biological systems when found in the air, water or food. Lead is poisonous in whatever form it is in and route of entry into the body. It is in the environment in three major forms; Metallic lead, salts of lead and carbon-containing organic lead [4]. Mechanisms of toxicity by lead include bioelements interaction, apoptosis inhibition and alteration of DNA structure and DNA repair inhibition. Lead mostly binds to hemoglobin in red blood cells than the cell membrane. It affects the liver by injuring hepatocytes which leads to an increase in serum enzyme levels and lowered production of proteins. Lead as shown harmful effects especially in the hepatic, cardiovascular, nervous and reproductive systems, having been found in large concentrations in the bones [21]. The liver is the major organ in the body responsible for carrying out different metabolic roles for the body's functioning. The liver serves as the primary organ for the detoxification of xenobiotics and foreign beings, thus purifying the other organs from harmful chemicals and compounds. Xenobiotics undergo phase I and phase II reactions for the breakdown of harmful compounds to their least potentially toxic sub-compounds which can easily be excreted through the kidneys or through the faeces. Amongst the cells of the liver, the hepatocytes are the major cells found in the liver and carry out different functions.

**Table 1. Showing serum liver enzyme concentrations**

Group	ALT (U/l)	AST (U/l)	ALP (U/l)
Group one (control)	$39.75 \pm 2.14^a$	$18.00 \pm 3.54^b$	$55.20 \pm 8.94^b$
Group two (test group)	$63.50 \pm 6.81^e$	$46.75 \pm 2.25^e$	$156.63 \pm 3.46^e$
Group three (treatment group1)	$39.75 \pm 3.47^b$	$22.50 \pm 6.45^c$	$55.18 \pm 11.44^a$
Group four (treatment group 2)	$45.50 \pm 1.44^d$	$17.00 \pm 2.74^a$	$59.32 \pm 16.50^c$
Group five (treatment group 3)	$44.50 \pm 2.18^c$	$31.25 \pm 1.843^d$	$75.89 \pm 15.60^d$

Values are expressed as Mean  $\pm$  SEM at significant level of  $p < 0.05$ . Values on the same column with the same superscript are statistically not different, whereas values on the same column with different superscript are significantly different. Values are presented  $\pm$  standard error of mean (SEM)  $P \geq 0.01$   $n=3$

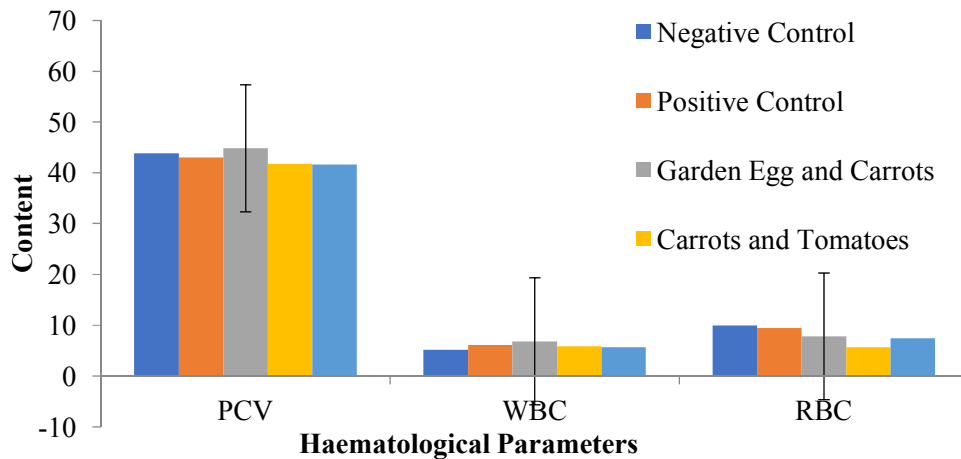
<sup>a</sup>Values = values are significantly lower values than values down or within the treatment column

<sup>b</sup>Values = values are significantly higher than the values before or within it in the treatment column

<sup>c</sup>Values = Values are significantly higher values than values before it or with in the treatment column

<sup>d</sup>Values = Values are significantly higher values than values before it or with in the treatment column

<sup>e</sup>Values = Values are significantly higher values than values before it or with in the treatment column



**Fig. 1. Effect of garden egg and carrots, carrots and tomatoes, and carrot, garden egg and tomatoes on packed cell volume, white blood cells and red blood cell counts on lead intoxicated rats**

Lead activates the synthesis of reactive oxygen species (ROS), which attacks the cell membrane of hepatocytes. When hepatocytes are injured by these species, they release enzymes, mostly Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST), and this makes their serum concentration levels higher than normal [22].

The effects of lead on liver enzyme levels was assessed by analyzing the concentration of serum liver enzymes which serves as key markers for liver damage and injury. Hepatocyte injuries are as a result of the generation of reactive oxygen species caused by the accumulation of lead in the liver. The rise in the concentration of AST, ALT and ALP in the groups administered with lead acetate can be considered that the chemical is indeed harmful to the liver. The levels of AST ( $46.75 \pm 2.25\text{U/l}$ ), ALT ( $63.50 \pm 6.81 \text{ U/l}$ ) and ALP ( $156.63 \pm 3.46 \text{ U/l}$ ) in the serum of group 2 rats appears to be higher than the normal level of the enzymes in comparison to the normal range of AST (10-40U/l), ALT (20-50U/l) and ALP (20-140U/l). Accumulation of lead in the liver caused injuries to the hepatocytes and the liver causing abnormalities in liver function.

Groups 3, 4 and 5, administered with fruit extracts after intoxication with lead acetate showed decreased levels in the various hepatic parameters. Group 3 has levels of AST ( $22.50 \pm 6.45\text{U/l}$ ), ALT ( $39.75 \pm 3.47\text{U/l}$ ) and ALP ( $55.18 \pm 11.44\text{U/l}$ ) administered with carrot and garden

egg extracts, group 4 levels are AST ( $17.00 \pm 2.74\text{U/l}$ ), ALT ( $45.50 \pm 1.44\text{U/l}$ ) and ALP ( $59.32 \pm 16.50\text{U/l}$ ) administered with carrot and tomato extracts and group 5 levels are AST ( $31.25 \pm 1.843\text{U/l}$ ), ALT ( $44.50 \pm 2.18\text{U/l}$ ) and ALP ( $75.89 \pm 15.60\text{U/l}$ ) administered with carrot, tomato and garden egg extracts, compared to the elevated levels in group 2 AST ( $46.75 \pm 2.25\text{U/l}$ ), ALT ( $63.50 \pm 6.81 \text{ U/l}$ ) and ALP ( $156.63 \pm 3.46 \text{ U/l}$ ) administered with only lead acetate. This is as a result of the active compounds of the extracts that help to reverse lead intoxication and help in healing the liver cells and the liver as an organ.

It was observed from this study that the plant extracts showed their potential to reduce serum liver enzyme levels and played a role in the generation of liver cells damaged by lead acetate. This showed that the fruit extracts contains bioactive compounds that inhibits the action of lead acetate on the liver such as the degeneration of reactive oxygen species that cause hepatocellular injuries. This study was essentially carried out to show the hepatoprotective effects of *Solanum melongena* (Garden Egg), *Solanum lycopersicum* (Tomatoes) and *Daucus carota Subsp. Sativus* (Carrot) extracts on the liver of albino wistar rats induced with lead acetate. This was showed by the analysis of the effects of the extracts on Alanine aminotransferase, Aspartate aminotransferase and Alkaline phosphatase enzyme concentration in the blood. The study shows that the plant extracts inhibits the action of lead acetate on the liver. It also showed the

poisonous effects of lead acetate by increasing ALT and AST levels which serves as indicators for heptaocellular injuries and ALP that acts as a biomarker for cholestasis.

The toxicity of lead depends on its chemical form, the route of administration, concentration, frequency and duration of administration [23]. The earlier findings of [24,25] showed that absorbed lead following oral ingestion is carried via blood to soft tissues and 95% of blood lead is transported on the erythrocyte as lead diphosphate. Accumulation of lead produces damaging effects in the haematological, haematic, renal and gastrointestinal systems [26].

There was no significant difference ( $p \leq 0.05$ ) between the PCV and WBC count of the rats in the positive control groups when compared with the treatment groups although the group treated with garden egg and carrot had the highest packed cell volume and WBC count. This could imply that 50 mg/kg of lead administered for 14 days did not have serious adverse effect on the PCV and WBC count of experimental rats. This finding is in line with the study conducted by [27] that showed the administration of 75 mg/kg of lead for 42 days did not significantly affect the PCV and WBC count of rats when compared with the negative control. Also, [28] reported that PCV is not altered in birds after supplementation of 50 mg/Kg of lead in their feed. [29] also reported no changes in PCV in calves after supplementation of 100 mg/kg of lead in their diets. However, the group treated with carrot and garden egg showed slight increase in RBC and WBC count when compared with the positive and negative control groups suggesting that carrot and garden egg may have hematopoietic potentials.

Carrots helps in Lowering Blood Pressure as they are power-packed with potassium and ample amounts of potassium in the body not only relaxes the arteries and blood vessels, but also helps in improving blood circulation, thus bringing down the blood pressure [30].

Garden egg is rich in iron and could be a good source of blood components as reflected in the increase in some of the haematological parameters in this study. Therefore, garden egg can aid in fighting against anaemia or deficiency in iron. It is also rich in copper [31] another important component of red blood cells.

There was no significant difference in the value of RBC in the treatment group when compared with the positive control group although the animals in the positive control group showed slight increase in RBC which was not significant. This was in accordance with the study of [32] who found that subchronic lead intoxication caused a slight increase in the red blood cell count of the rats and it was suggested that the tissue hypoxia is a possible mechanism for high production of Red blood cells in moderate lead poisoning, an increase in red blood cell (erythrocytosis) can result from severe dehydration.

The plant extracts showed the potential of recuperating the damaged organ.

## 5. CONCLUSION

The plant extracts possess hepatoprotective potential which is dependent on its bioactive compounds. This study has shown that the used plants has the ability to regulate and reduce elevated liver biomarkers in individuals that are in constant exposure to Lead, in different forms. It may therefore be assumed that any of these plants is equally effective in hepatoprotective functions against lead intoxication in albino wistar rats. Tomatoes, carrot and garden egg are potential source of haematinics which are very useful in the treatment of anaemia or iron deficiency problems which are symptoms of lead toxicity.

These plant extracts may be used in the future in the management and treatment of individuals exposed to toxic chemicals and ameliorating illnesses associated with anaemia.

## ETHICAL APPROVAL

The experimental animals used were handled and managed according to the guidelines of handling of experimental animals.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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