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Investigation of Acute Toxicity Effect of Zinc Phosphide in Male Albino rat

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ABSTRACT

Zinc phosphide was used as rodenticide since more than one hundred year. Acute toxicity occurs through ingestion where converted to phosphine gas and passes to the blood stream through digestive tract and attracted by the lung and the liver where inducing various toxic effects by phosphine gas. There is no specific antidote and mortality rate from toxicity reach to 100 %. The aim of our present study was to investigate the effect of acute toxicity of zinc phosphide on biochemical and haematological changes in male albino rats. Results showed that zinc phosphide was found to induce damage in liver, lung, and kidney by significant increase in zinc and phosphorous in these tissues and significant increase in serum of phosphorous, zinc, chloride, sodium(Na), potassium (K), urea, red blood cells (RBC), and platelets. Whereas a significant decrease in white blood cells (WBC), haemoglobin (Hb), and haematocrit (HCT). In conclusion, acute toxicity of zinc phosphide causes haemolytic anaemia through early haemolysis and alteration in kidney, liver, and lung.

Keywords: Zinc phosphide, acute toxicity, haematological studies, biochemical finding.

INTRODUCTION:

Zinc Phosphide "is an inorganic chemical with formula (Zn3P2), dark grey compound, smell similar to garlic, low price used as a rodenticides" that is used to control rat, mice, vole, and other rodents' populations. It is also used as a tracking powder for the control of house mice. It is used on non-crop areas and crop areas and including golf areas, lawns, highway medians, and areas adjacent to wetlands [2]. A mixture of food and zinc phosphide leaves where it can be for rodents to eat. Acid in the digestive system of the rodents react with the phosphide and hence generates toxic gas, phosphine.

In spite of the fact that rodents play important function in nature, they may on occasion need to be control. There is serious risk of using rodenticides as they may cause toxicity for all vertebrates in regarding to their fate in the environment. Chemical rodenticides are considered as toxins have detrimental effect on the vital functions of living organisms of various kinds. However, the toxic effect of these rodenticides may reach all the components of the environment, and to the man himself. Statistics worldwide in 1992 showed that, rodenticides caused poisoning cases for nearly twenty five million people in developing countries, die of whom nearly 20 thousand people per annum. Toxicity origin comes from the spread of this product and easy gets it.

Firstly, Soil acidity tends to smash this compound down setting free phosphine, a highly toxic gas. There is potency for movement of this compound into neighbouring, lightly acidic waters, where it can imperil fish populations. Also, they can hurt harvested crops, violate housing rules, convey illness and sickness, and in some cases cause ecological damage. Secondly, Exposing to zinc phosphide through suicide or accident through oral toxicity, it transformed into phosphine gas and absorbed from digestive tract to the blood stream into liver and lung causing mortality rate reach to 100% [1]. Rodents, humans, dogs and cats are all mammals; which implies a similar body functioning. Rodenticides have the same effect when eaten by any mammal. They also can affect birds.

Rodenticides are often prepared as baits, which are designed to attract animals. Flavourings include fish oil, sugar cane molasses or peanut butter. Baits used in agriculture and natural areas on the other hand, may contain ground meat, vegetables, grains, or fruits. These may be attractive to children and pets and so should be kept out of their reach. Tamper-resistant bait stations decrease the probability of accidents occurring. Accordingly, these compounds have the ability to eliminate rodents, pets and even human if misused and mishandled.

The present research study was executed to estimate the severe virulent effect of zinc phosphide at different concentrations and to evaluate the changes that occur in haematology, kidney and liver tissues at varied time intervals. The test species selected for the current experiment are male Sprague-Dawley rats. The reason for selection is that, they possess many biochemical and

physiological similarities with those of human beings.

MATERIALS AND METHODS

1. Animals

Twelve male Sprague-Dawley rats of weight (200-220g) were obtained from the Experimental Research of Princes Noura University, Riyadh, Saudi Arabia. Maintaining of rats was done under standard laboratory conditions in an air conditioned room and housed in stainless steel cages one per cage at temperature 22±3°C and relative humidity 30-70 %. The ad libitum was the animal diet. Acclimation of animals was done for one week prior to the experimental work.

2. Zinc phosphide Standard Toxicant and Experimental Protocol

Zinc phosphide powder (80%) was purchased from commercial market.The active ingredient is zinc phosphide. Twelve adult male rats were separated into two groups of six rats each and given the following treatments: Control (G1) was the control and given 0.5ml of saline solution by gastric gavage route single administration. Zinc phosphide (G2) acute oral toxicity group consists of 6 rats. A single (1 day) oral dose of zinc phosphide was given 21 mg/kg. At the end of the day of acute experimental toxicity (after 8 hours), rats were sacrificed under slight halothane anaesthesia.

3. Sampling and Parameters

Using the cardiac puncture procedure, blood was collected. Centrifugation at 860 xg for 20min, was used to separate serum and determination of sodium(Na), potassium (K),urea, creatinine, chloride, in addition to Alanine Aminotransferase(AST) and Aspartate aminotransferase(ALT) that were analysed in serum samples using the Reitman and Frankel (1957) method, and using a commercially available test kit (Egyptian Company for Laboratory Services, Egypt). Haematological finding for determination of red blood cells (RBC), haemoglobin (Hb), haematocrit (HCT) white blood cells (WBC) and platelets.

4. Statistical Analysis

All data are presented as mean ±SEM. Differences were only considered to be significant at $p<0.05$. A one-way ANOVA and post-hoc test were used to determine the differences between the two groups. The SPSS/PC program (version 17; SPSS, Chicago, Illinois, USA) was used for statistical analysis [6].

RESULTS

The biochemical parameters' results proved that the rats treated with Zinc

phosphide caused significant toxicant damage ($P \leq 0.05$) as indicated by liver enzymes, AST, and ALT as well as zinc and phosphorous in hepatic cells in comparable with control group (Table. 1). The outcome of Serum Zn, PO4, Chloride, Sodium, Potassium, and Urea were significantly ($P \leq 0.05$) increase in zinc phosphide treated group in comparing with the control group (Table. 2).

Table (1): Serum Creatinine, ALT and AST

Groups	Serum ALT (U/L)	Serum AST (U/L)	Creatinine (mg/dl)
Control (G1)	50.13±3.46	76.67±1.54	0.22±0.06
Zn3P2 (G2)	141.1±70.87*	224.83±27.72*	0.63±0.03*

Each value represent the mean ± SD (n=6), * significantly different from control at $p \leq 0.05$.

Table (2): Serum Zn, PO4, Chloride, Sodium, Potassium, and Urea.

Groups	Zn (Ug/dl)	PO4 (mg/dl)	Chloride (mmol/L)	Sodium (mmol/L)	Potassium (mmol/L)	Urea (mg/dl)
Control (G1)	81.48±1.71	4.42±0.11	106.9±34.15	129.0±0.44	3.60±0.11	19.80±0.62
Zn3P2 (G2)	116.73±2.00*	7.67±0.46*	110.92±0.40*	147.70±1.04*	3.93±0.03*	31.37±0.61*

Each value represent the mean ± SD (n=6), * significantly different from control at $p \leq 0.05$.

The results of RBCs and Platelets were significantly ($P \leq 0.05$) increase in zinc phosphide treated group whereas the finding of WBCs, Hb, and HCT were significantly ($P \leq 0.05$) decrease in zinc phosphide treated group as compared with the control group (Table. 3).

Table (3): Hematological findings for RBCs, WBCs, Platelet, Hb, and HCT.

Groups	RBCs	WBCs	Platelets	Hb (g/dl)	HCT (%)
Control (G1)	5.22±0.26	7.86±1.01	309.1±750.66	13.35±0.29	40.60±1.20
Zn3P2 (G2)	8.34±0.21*	3.24±0.33*	601.1±767.22*	10.43±0.27*	35.75±0.37*

Each value represent the mean ± SD (n=6), * significantly different from control at $p \leq 0.05$.

Results of zinc and phosphorous in tissues of kidney and lung were significantly ($P \leq 0.05$) increase in zinc phosphide treated group as compared with control group (Table. 4).

Table (2): Zn (Ug/dl) and phosphorous (mg/dl) in kidney, liver and lung in control and treated group

Groups	Zn (Ug/dl)		PO4 (mg/dl)	
	Control	Treated	Control	Treated
Kidney	87.00±0.89	110.33±0.56*	2.68±0.16	5.10±0.30*
Liver	100.00±0.58	108.67±0.71*	17.97±0.22	23.73±0.48*
Lung	101.83±0.65	108.67±0.67*	19.28±0.24	24.28±0.33*

Each value represent the mean ± SD (n=6), * significantly different from control at $p \leq 0.05$.

DISCUSSION

Due to the recent intensification of agriculture and increasing availability of agro-chemicals in low and middle income countries, the phenomenon, which is acute rodenticide poisoning, has gained large

momentum worldwide, with as much as 300,000 deaths per annum [10]. Moreover, the ease of access to these types of products has increased the rate of suicide by agro-chemicals since they have a high mortality rate associated with them [10]. In rural communities, they make extensive use of phosphide tablets especially in the storage areas of grains. When the storage sites are sealed, the phosphine is released. The gas accumulates, causing the toxicity of the sealed air to increase. When the sites are unsealed later on, the inhalation of the gas by people can lead to death [11]. rodenticide poisoning often causes damages to internal organs, and the extent of these damages can be observed by the amount of damage markers present in the organs or tissues. The mechanism of action of the rodenticide, is more often than not, based on free radical production [12].The main effects of zinc phosphide poisoning include metabolic abnormalities, electrolyte imbalances, metabolic acidosis, respiratory alkalosis, hypokalaemia and acute renal failure [13].Other effects of respiratory toxicity include dyspnea, Tachypnea, rhonchi and crepitation [14].

As mentioned earlier, zinc phosphide can be categorised as an acute rodenticide as it has the ability to eliminate its target in a single feeding. The zinc phosphide is normally found in a powdered form or as pellets. In humans, the mechanism of action of zinc phosphide is as follows: orally-ingested zinc phosphide reacts with the water and acid found in the stomach to produce phosphine gas. This phosphine gas has a very high toxicity rate, thus leads to severe systemic toxicity, and is highly irritating to the respiratory tract. The phosphine blocks the action of cytochrome C oxidase and thus disrupts mitochondrial functions. When the mitochondria are unable to function properly, the energy levels in the cells decrease, limiting cellular functions. Not only does phosphine de-energises the cells, it also increases the production of free radicals, causing lipid peroxidation. The process of lipid peroxidation is the damaging of the lipids in the cell membranes thus decreasing the functionality and viability of cells. Lipid peroxidation has also been associated with atherosclerosis, asthma, Parkinson's disease, kidney damage, preeclampsia, among others. Zinc phosphide and other phosphides have a high and rapid rate of toxicity, usually within 30 minutes of ingestion and death may occur within 6 hours following ingestion [3].

Zinc phosphide is a broad-spectrum rodenticide with a high toxicity for several

species of rodents [5]. However, there are some differences in the susceptibility to zinc phosphide depending on the animal species. For example, zinc phosphide has high oral toxicity for small mammals and other small rodents 2 – 15 times more toxic to them than to large mammals [5].

As such, for that to happen, the LD50 values for oral toxicity of zinc phosphide to some mammal species: Brushtail possum (*Trichosurus vulpecula*) was 9.6 mg/kg, Ferret (Mustela *putorius furo*) was 16.4 mg/kg, Black rat (*Rattus rattus*) was 21.3 mg/kg, Polynesian rat (*Rattus exulans*) was 23.1 mg/kg, House mouse (*Mus musculus*) was 32.7 mg/kg, Norway rat (*Rattus norvegicus*) was 40.5 mg/kg, Dog (*Canis familiaris*) was Approximately 40 mg/kg, Cat (Felis domesticus), Approximately 40 mg/kg, Cattle (*Bos taurus*), sheep, goats, pigs was 30-40 mg/kg, Sheep (*Ovis aries*) was 60-70 mg/kg, and Rabbit (*Oryctolagus cuniculus*) was 75 mg/kg.

Zinc phosphide poisoning after ingestion causes the following symptomsin humans: nausea, vomiting, agitation, chills, chest tightness, dyspnoea, cough, pulmonary edema, systemic toxicities such as hepatic failure with jaundice and haemorrhage, delirium, convulsions and coma (from toxic encephalopathy); tetany from hypocalcaemia and anuria from renal tubular damage [4]. On the other hand, symptoms of phosphine poisoning via inhalation include: pulmonary edema, myocardial injury and multisystem involvement [4].

Zinc phosphide has been used for the past 70 years as an effective deterrent and control measure for rodents such as rats, prairie dogs, ground squirrel, etc. The efficacy of the zinc phosphide mainly depends upon the probability of the rodent eating or ingesting the bait. Prebaiting has been shown to increase the efficacy of the rodenticides. However, the efficacy is influenced by factors such as the time of the year, the geographic location, the habitat treated, the species of rodent, number of rodent, amount of bait laid out, positioning of the baits, etc.

As it can be observed from the results' section, administering zinc phosphide to the twelve male Sprague-Dawley rats resulted in an increase in serum ALT, AST, liver zinc level and liver phosphorus level as compared to the control group. The increase in ALT andAST, was nearly three-fold, while the increase in zinc and phosphorus levels was not as much significant. ALT and AST are largely used in the assessment of liver and heart damage by drugs or any other hepatotoxin

[16]. It should be noted that ALT is more specific to the liver and its concentration or level is a better indicator of extent of liver damage [16].

In this case, this increase in ALT and AST levels may be due to acute toxic hepatitis. Toxic hepatitis is caused by the damaging actions of toxins on the liver. Usually, in the initial stages of the disease, if exposure to toxin is stopped, then the disease can be reversed. However, exposure to toxins is continuous and the damages done to the kidney are irreversible, then even preventing exposure to toxin will not revert to a healthy kidney. This toxic hepatitis may lead to hepatocellular failure and ultimately death. This increase in ALT and AST levels was also observed by Karanth and Nayyar, 2003 [15].

Electrolyte disturbances were observed. The levels of zinc and phosphorus in the liver increased for the male rats. Since zinc has the ability to maintain the levels of hepatic elements and plays an important role in the regulation of liver functions by maintaining the activities of marker enzymes during metal toxicity, it can be concluded that the rise in zinc levels in the liver must have been a sort of regulatory measure to try and bring down the levels of toxins in the liver back to a more normal level which could then be safely excreted from the body [17]. The levels remained high throughout the experiment which would lead to the conclusion that the zinc was unable to carry out its function and could further lead to the conclusion that liver function was impaired [17].

The Hyperphosphatemia observed in the male rats, represented by an electrolyte disturbance in which an abnormally elevated level of phosphate in the blood was observed, is an indicator of liver failure. Indeed, hyperphosphatemia leads to damaged oxygen transport and tissue hypoxia, abnormal leucocyte function, lowplatelet numbers and function, generalised muscle weakness, and disruption of the central nervous system; all of which are complications associated with acute liver failure [18].

Hyperphosphatemia is also an indicator of poor recovery, which means that even if the rats were no longer exposed to the toxic substances, if their phosphate levels remained high, their chance at recovery will be minimal, if any [18].

A high serum urea level is called uraemia and in indicative of renal failure and/or renal damage [19]. It usually

develops as a result of chronic kidney disease but may also develop as a result of acute kidney injury [19]. In this case, the uraemia developed in the male rats is a result of acute kidney injury due to the zinc phosphide toxicity. It should be noted that when uraemia develops, other endocrine abnormalities may crop such. These include: "decreased thyroid hormone excretion, changes in carbohydrate metabolism and abnormal sexual hormone regulation" [19].

The hypernatremia observed in the male rats is indicative of kidney disease/failure [20]. As the kidney becomes damaged, it loses its ability to regulate water balances or water homeostasis of the body. This increases the probability of developing hypo- and/or hyper-natremia. Hypernatremia may lead to fatal brain edema or osmotic demyelination syndrome and thus has been linked with an increased risk of mortality [20]. Along with hypernatremia, hyperchloraemia can also be observed in the male rats. As with the case with the hypernatremia, hyperchloraemia is also due to kidney disease/failure [21]. If both conditions occur in tandem, as is the case here, there is an extremely high probability of the kidney being unable to concentrate the urine and produce large amounts of dilute urine [21].

An increase in the levels of potassium is termed as hyperkalemia and is associated with significant morbidity and mortality [21]. The kidney is responsible for the maintenance of potassium homeostasis in the body [21]. Any damage or disease affecting the kidney will automatically lead to an imbalance in the potassium levels. Hyperkalemia usuallyoccurs when there the platelet count is greater than 600,000, WBC greater than 200,000 or significant hemolysis [21].

The high red blood cells count, or Polycythaemia, observed in the male rats may be due to dehydration or excessive production of erythropoietin by the kidney [7]. In both cases, it is the kidney which regulates the level of red blood cells and as such, any imbalances, disease or damage to the kidney will automatically lead to dehydration and or an increased production of erythropoietin, causing polycythaemia [7].

The low white blood cell count, or leucopoenia, is associated, in this case, with renal failure. This is because kidney failures may lead to a weakened immune system and thus poor immunity. This renders them more vulnerable to other opportunistic diseases [8]. The high

platelet count, also known as thrombocytosis, observed in the male rats, can be said to be due to nephritis, which is the inflammation of the kidneys and can be attributed to increased production and secretion of thrombopoetin the primary regulator of platelet production [9]. The low haemoglobin, Hb, and haematocrit, HCT, levels observed in the male rats are due to increased iron losses. Iron is necessary for haemoglobin and haematocrit production, thus kidney disease, damage or failure, which leads to increased iron losses, will ultimately lead to a decrease in haemoglobin and haematocrit production [22].

From the above arguments, it can be concluded that the lethality of the zinc phosphide in male rats is mostly due to kidney damage and/or failure, and in case of ingestion, the mortality is due to presence of toxins in the blood.

In order to reduce the incidence of zinc phosphide poisoning, along with other rodenticide poisoning, the current agricultural policies must be changed. The core change should be to reduce the dependency of the agricultural sector on rodenticides and pesticides to the lowest possible levels. If complete independence from these products can be achieved, then it should be pursued. Decreasing the usage of rodenticides will not only decrease their production but also their availability. Thus, this would cause a chain reaction, ultimately leading to a decrease in the number of deaths due to suicide attempts via rodenticides. Not only that, but decreasing the usage of rodenticides would also decrease the probability of getting into accidental contact with them and thus accidental poisonings; decrease the number and probability of work related poisoning cases, and, decrease human exposure to such toxic chemical products. These can be achieved by promoting farming systems and practices with a decreased use of rodenticides. Moreover, human health concerns should be taken into consideration when providing aid to the agricultural sectors of low income countries. There should be an increased sharing of knowledge, tools and technologies internationally, with an increased focused on the low income countries and countries which grow and produce large amounts of foods.

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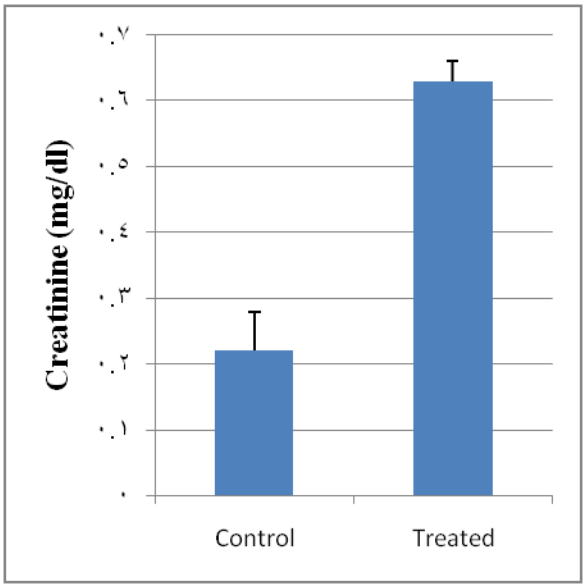

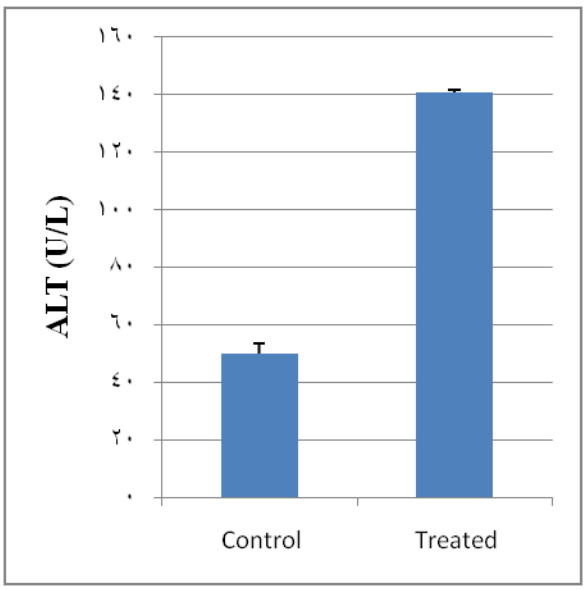

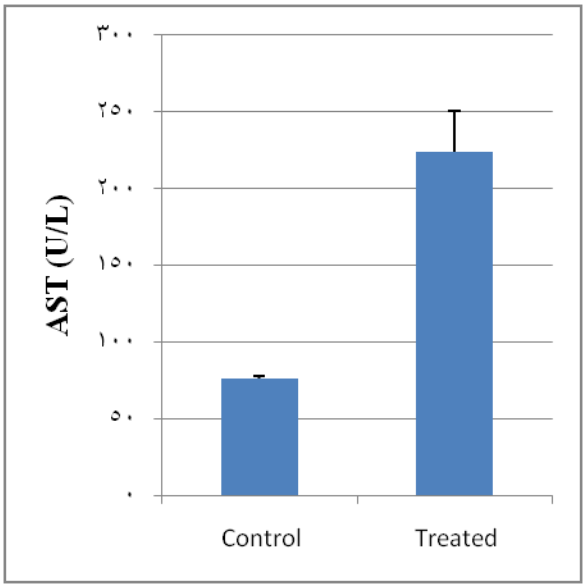
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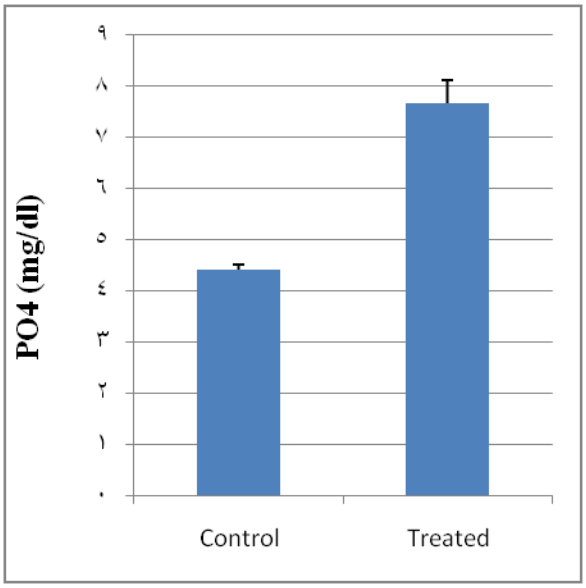
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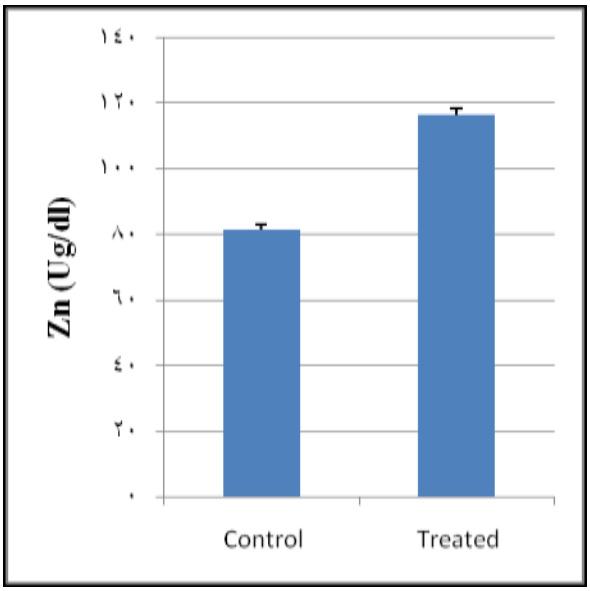
Zn (Ug/dl)
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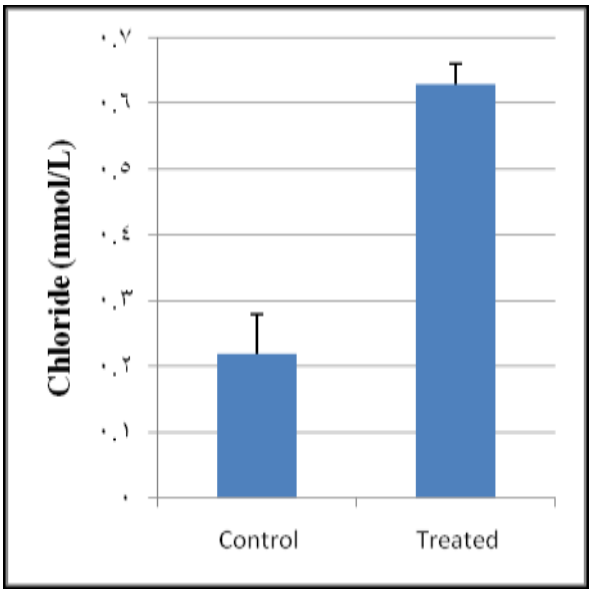
Chloride (mmol/L)
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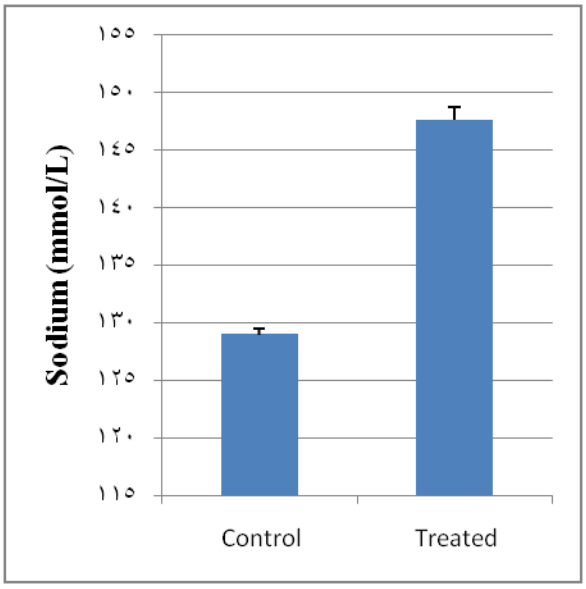
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