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An Effects and impact of Intermitten Inhalation process of phostoxin on renal functions of albino rats

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Conflicts of Interest: None

Abstract

Occupational poisoning is major hazard in Agricultural industry and its affliates. Toxins meant to keep away pests or preserve the produce also affect man. One such agent is phostoxin, containing aluminum phosphide which gets into man acutely or chronically causing damages to the vital organs of man. Equisexes of Wister rats weighing 120-200g (mean 135 $\pm$ 2.5g) were exposed to intermittent inhalation of phostoxin (1000mg) for 30 and 60 days respectively after which the renal biomarkers were compared with the control. Results showed that Na^+, Cl^-values were elevated, while K^+, HCO_3^- values were lowered and were significant at $p<0.05$. The simultaneous rise and fall of Na^+ and K^+ respectively were sufficient to maintain equilibrium, delaying manifestations of severe toxic events until chronic stage.

Finally, the distortions in the renal architecture observed from the control, increasing from day 30 with mild hemorrhage, vacuolation, etc. to gross disruption of tabular cells by the 60th day. We conclude that toxicity by phostoxins builds up and accumulates to chronic toxicity.

Keywords: Phostoxi, Intermittent Inhalation, Chronic Toxicity

Introduction

Aluminum phosphide is a widely used inorganic compound that functions as pesticide and rodenticide for managing insect and rodent infestations across multiple environments. Its primary use is in indoor fumigation during the transportation and storage of crops, including

both food and non-food varieties, as well as in processing plants. It is also applied outdoors for controlling burrowing rodents and moles, and sometimes used as bait in agricultural fields for rodent management.[1,2] This compound is formulated in various forms, including tablets, pellets, blister packs, sachets, and powder or granules.[3]

Phostoxin is a commercial formulation of aluminum phosphide, which releases a colorless and nearly odorless gas when pure. However, the technical-grade formulation emits a strong, unpleasant odor.[3] This substance is highly reactive in air, capable of igniting spontaneously at room temperature, and is classified as being flammable and explosive.[3,4] It dissolves readily in water and most organic solvents and is often supplied in pressurized cylinders, either in its pure form or mixed with inert gases such as nitrogen.[5,6] Some formulations may contain about 55% active ingredient combined with aluminum carbamate and other inert substances.[7,8]

Insecticides and fumigants like aluminum phosphide have demonstrated toxic effects on multiple biological systems, due to non-selectivity of action, hence the liver, kidneys, nervous system, immune system, and reproductive system are usually affected.[9] One well-recognized mechanism of toxicity involves the inhibition of acetyl cholinesterase, which leads to an accumulation of acetylcholine and overstimulation of muscarinic and nicotinic receptors.[10,11] Additionally, certain insecticides exert their harmful effects by initiating electrophilic attacks on brain and liver cells, often accompanied by excessive production of reactive oxygen species.[1, 2] The toxicological evaluation of these compounds is typically based on changes in biochemical markers and microscopic damage to tissues and organs.[13,14] The kidney, in particular, is a major target organ in experimental toxicity studies involving the biomarkers.[12,14]

The kidney is a structurally complex organ essential for human survival since its embryonic development. Every cell in the renal parenchyma is highly specialized in maintaining electrolyte, volume, and waste homeostasis. [15] Renal pathologies can be grossly categorized depending on the affected segment of the nephron: the glomerulus, tubules, interstitium, or blood supply. Each one differs in clinical manifestations, making it vital for the clinician to integrate differential diagnoses.

As phostoxin (aluminium phosphide) is widely used as a fumigant for pest control in agricultural and storage settings due to its cost-effectiveness its non selective toxicity poses serious health risks to humans, where occupational inhalation is likely to occur.[16] Upon exposure to moisture, phostoxin releases phosphine gas, a highly toxic substance that induces multi-organ dysfunction,[17] the kidneys, being essential for filtration and excretion of toxins, are particularly vulnerable to damage following systemic exposure to phosphine gas.[18] Despite numerous reports on its acute systemic toxicity, limited studies have focused on the histopathological impact of whole body inhalation of phostoxin on the renal architecture. Given the prevalence of its agricultural use in Nigeria, and the increasing cases of pesticide poisoning, it is imperative to understand the implications of such exposure on the renal system.

The kidneys are two bean-shaped organs, with a tough, fibrous renal capsule surrounding each kidney and provides support for the soft tissue inside. Beyond that, two layers of fat reinforce the protection. The adrenal glands lie on top of the kidneys. Inside the kidneys are a number of pyramid-shaped lobes. Each consists of an outer renal cortex and an inner renal medulla. The kidney

is a structurally complex organ essential for human survival since its embryonic development. Every cell in the renal parenchyma is highly specialized in maintaining electrolyte volume, and waste homeostasis.[15]

The principal functional unit of kidney is the nephron, which has a filter, called the glomerulus, and a tubule, the glomerulus and the first portion of the tubular system, known as the proximal convoluted tubule (PCT), are located in the renal cortex. Following the PCT, the loop of Henle, a hairpin-like structure, penetrates the medulla and returns to the cortex to connect with the distal convoluted tubule (DCT). Finally, the nephron drains into the collecting duct via connecting tubules.[19] The glomerulus filters blood, which enters the kidneys through the renal arteries and leaves through the renal veins. The kidneys are relatively small organs, but they receive 20–25% of the heart's output. The tubule returns necessary substances to the blood and removes waste that then becomes urine. The kidneys excrete urine through the ureter, a tube that leads to the bladder.[19]

Glomerular filtrationis a passive process in which hydrostatic pressure pushes fluid and solute through a membrane. The outward and inward force from the capillaries determine how much water and solutes cross the filtration membrane. Hydrostatic pressure from the glomerular capillaries is the major filtration force with a pressure of 55 mmHg. [20]

There are four different tubular segments with each having a unique absorptive property. The first is the proximal convoluted tubule (PCT). The PCT cells have the most absorptive capability. In normal circumstances, the PCT reabsorbs all the glucose and amino acids as well as 65% of Na^+ and water. The PCT reabsorbs sodium ions by primary active transport via a basolateral Na^+-K^+ pump which drives the electrochemical gradient by passive para cellular diffusion. The PCT reabsorbs water by osmosis that is driven by solute reabsorption. It also reabsorbs lipid-soluble solutes via passive diffusion driven by the concentration gradient created by reabsorption of water. Reabsorption of urea also occurs in the PCT.[20]

From the PCT, the non-reabsorbed filtrates move on to the nephron loop which functionally divides into a descending and ascending limb. The descending limb functions to reabsorb water via osmosis. At the distal convoluted tubule (DCT), there is a primary active sodium transport at the basolateral membrane and secondary active transport at the apical membrane through Na^+-Cl^- symporter and channels.[21] This process is aldosterone regulated at the distal portion. There is also calcium reabsorption via passive uptake controlled by the parathyroid hormone. Aldosterone targets the cells of the distal portion of the DCT causing synthesis and retention of apical Na^+ and K^+ channel as well as the synthesis of Na^+-K^+ ATPase. Right after the DCT, there is a collecting tubule where the final stage of reabsorption occurs.[22]

The kidney performs the following function;

- Glomenular filtration, a passive process in which the hydrostatic pressure pushes fluid and solute through a membrane.
- Tubular reabsorption involving the four tubular segments that proximal convoluted tubule, the loop of Henle, the distal convoluted tubule and the collecting duct.

By excreting urine, the kidney plays a principal role in homestasis, maintains electrolyte balance and equilibrium. The kidney functions in the production of erythrocytes by secreting erythropoieten, a stimulating factor for erythropoiesis, it also secrets thrombopoieten which in turn stimulates the production of thrombocytes. The endo

crime function of the kidney includes erythropoieten, thrombopoieten, rennin, 1,25-dihydroxy cholecalciferol and protaglandin.[23] The kidney regulates blood pressure through regulating the volume of extracellular fluid and rennin-angiotensin mechanism.[24] Regulation of blood calcium by activating 1,25 -dihydroxy cholecalciferol into vitamin D, necessary for absorption of calcium from the intestine.

The kidney also syntheses glucose from amino acids and other precursors during prolonged fasting in the processes of gluconeogenesis.[24]

Kidney Function Markers

Biochemical markers play important roles in accurate diagnosis and assessing risk and adopting therapy that improves clinical outcome. As markers of renal function creatinine, urea, uric acid and electrolytes are for routine analysis whereas several studies have confirmed and consolidated the usefulness of markers such as cystatin C, β-Trace Protein.[25]

Creatinine is a breakdown product of creatine phosphate in muscle, and is usually produced at a fairly constant rate by the body depending on muscle mass. Creatinine is commonly used as measure of kidney function. The normal creatinine clearence test value is 110-150ml/min in male and in female it is 100-130ml/min. The creatinine clearance test is used to monitor the progression of renal disease. The diagnosis of renal failure is usually suspected when serum creatinine is greater than the upper limit of the "normal" interval. In chronic renal failure and uremia, an eventual reduction occurs in the excretion of creatinine by both the glomeruli and the tubules.[26]

The amount of creatinine produced per day depends on muscle bulk. Thus, there is a difference in creatinine ranges between males and females with lower creatinine values in children and those with decreased muscle bulk. Diet also influences creatinine values. Creatinine can change as much as 30% after the ingestion of red meat.[24] Creatinine values may alter as its generation may not be simply a product of muscle mass but influenced by muscle function, muscle composition, activity, diet and health status. The elevated values are observed in muscular dystrophy paralysis, anemia, leukemia and hyperthyroidism. The decreased values are noticed with glomerulonephritis, congestive heart failure, acute tubular necrosis, shock, polycystic kidney disease, and dehydration. [26]

Urea is major nitrogenous end product of protein and amino acid catabolism, produced by liver and distributed throughout intracellular and extracellular fluid. Inkidneys, urea is filtered out of blood by glomerulli and is partially being reabsorbed with water.[27] The most frequently determined clinical indices for estimating renal function depends upon concentration of urea in the serum. It is useful in differential diagnosis of acute renal failure and pre renal condition where blood urea nitrogen–creatinine ratio is increased.[26] Urea clearance is a poor indicator of glomerular filtration rate as its overproduction rate depends on several non-renal factors, including diet and urea cycle enzymes. Increased blood urea nitrogen is seen associated with kidney disease or failure, blockage of the urinary tract by a kidney stone, congestive heart failure, dehydration, fever, shock and bleeding in the digestive tract.Low levels are also seen in trauma, surgery, opioids, malnutrition, and anabolic steroid use.[27]

The Cystatin Cprotease inhibitor is a non-glycosylated low molecular weight protein which has been proposed to be a marker, produced by all nucleated cells at a constant rate and freely filtered by the glomeruli and completely catabolized in the proximal tubules. [25]

The concentration of serum Cystatin C is mainly determined by glomerular filtration, which makes Cystatin can endogenous marker of glomerular filtration rate. Cystatin C has been found to be an effective marker for glomerular filtration rate in patients with cirrhosis following liver transplantation. Cystatin C has been found more useful in detecting early renal impairment in both type 1 and type 2 diabetic patients.[26]

Albuminuria and Proteinuria

Clinically the appearance of significant amount of protein in urine is one of the earliest sign of almost all renal diseases. Estimation of proteinuria helps in differentiating between tubule-interstitial and glomerular diseases and also to follow the progress of renal disease and to assess the response to therapy. [27]

Albuminuria refers to the abnormal presence of albumin in the urine. Albuminuria is used as a marker for the detection of incipient nephropathy in diabetics. It is an independent marker for the cardiovascular disease since it connotes increased endothelial permeability, and it is also a marker for chronic renal impairment. [23]

Electrolyte

Electrolyte panel is frequently used to screen for an electrolyte or acid-base imbalance and to monitor the effect of treatment on a known imbalance that is affecting bodily organ function. The test for electrolytes includes the measurement of sodium, potassium, chloride, and bicarbonate for both diagnosis and management of renal, endocrine, acid-base, water balance, and many other conditions.[27]

Potassium is used as a most convincing electrolyte marker of renal failure. The combination of decreased filtration and decreased secretion of potassium in distal tubule during renal failure caused increased secretion potassium. Hyperkalemia is the most significant and life-threatening complications of renal failure.[24]

Materials and Methods

Materials

Chemical Used: Phostoxin (aluminum phosphide) used in this study was sourced from the Ministry of Agriculture Abakaliki, Ebonyi State, Nigeria. It was supplied as a pack containing twenty 3-gram tablets sealed in a tube and were considered analytical grade. The animals were allowed to inhale the phostoxin tablets for a period of 30 - 60 days.

Equipments

a) Microtome machine,
b) Microscope.
c) Automatic tissue processor,
d) Spectrophotometer (Sp 300), Optima, Japan.

Methodology

Experimental Animals: Forty equisex albino rats with weights, ranging from 130-200g were procuredfrom the Animal House Ebonyi State University, Abakaliki, Ebonyi State. They were allowed to acclimatize in the Departmental Animal House for 14 days before being used for the study, having access to standard feed and water adlibitum. They were weighed using electric weighing balance before assigning them to groups. The cages were cleaned every 2 days to prevent the rats from being infected. Care and treatment were conducted in compliance with the International guidelines of the National Institute of Health.[28]

Experimental design

The albino rats were randomly divided into four groups, with each group containing ten (10) rats of equal sexes. The animals were housed in the Histopathology Laboratory of the university, where the study was conducted and having adequate ventilation, controlled

lighting, moderate temperature, sufficient humidity and feeding water ad libitum.

Group 1 served as the control and consisted of equisex male and female rats. They were fed with standard animal feed with clean drinking water ad libitum throughout the study.

Group 2 served as experimental group exposed to phostoxin for 30 days.

Group 3 also served as an experimental group and was exposed to phostoxin for a 60-day period. After the treatment period, all animals were fasted for 12 hours prior to sample collection. Kidney tissues were harvested for further his to pathological analysis.

Experimental groups	Treatment Received	Duration of treatment (Days)
Control T1 (10)	Distilled water and feed	60
T2(10)	Feed + 30 days exposure to phostoxin	30
T3(10)	Feed + 60 days exposure to phostoxin	60

Samples Collection

The animals were anaesthetized using chloroform and the kidneys were harvested for histological studies and fixed in 10% buffered formal saline.

Laboratory Analysis

The tissues were processed using conventional paraffin wax methods and automatic Tissue Processor and embedded using standard embedding center, sectioned and stained using both routine (H and E) stains and special stains

The following studies were undertaken:

- Measurement Of Urea by Urease – Berthelot Enzymatic Colorimetric End Point Method

Reagent1 (R_1): Urease Enzyme made up of Urease Substrate 1a, Sodium Nitroprusside, EDTA and phosphate buffer.

Reagent2 (R_2): Phenol concentrate diluted with distilled water.

Reagent3 (R_3): Sodium hypochlorite**.**

Principle: Urea is hydrolysed by urease into ammonia and carbon dioxide. The ammonia generated reacts with sodium hypochlorite and phenol in the presence of sodium nitroprusside as coupling agent/catalyst to yield a blue chromophore. The intensity of the colour formed is proportional to the concentration of urea in the sample at 546nm.[29]

Test Procedure for Urea

1. Pre-incubate working reagent, samples and controls to reaction temperature

2. Ensure R_2 and R_3 working solution is reconstituted in the right volume using distilled water.

3. Set the spectrophotometer to 0 absorbance with distilled water

4. Pipette into test tubes as in Randox Kit:

	BLANK	STANDARD	SAMPLE
Reagent R_1	100ul (0.1ml)	100ul(0.1ml)	100ul (0.1ml)
Standard	---------	10ul (0.01ml)	------
Sample	---------	--------	10ul (0.01ml)
Mix, incubate for exactly 10mins at 37 °C in a water bath.			
Reagent R_2	1ml	1ml	1ml
Reagent R_3	1ml	1ml	1ml

Mix immediately and incubate for 15mins at 37 °C.

Read the absorbance of the samples and standard against the reagent blank at 546nm.

Colour is stable for at least 8 hours.

Expected Value: Serum 2.0 – 8.0µmol/l.

- **Creatinine Estimation by Jaffe-Slot Alkaline Picrate Colorimetric & Kinetic Method**

Principle: Creatinine is measured as a fixed timed chemical reaction using picrate (Jaffe reaction) in an alkaline environment to form an orange- red product. The increase in absorbance at 500nm due to the orange-red

complex is proportional to the creatinine concentration in the sample.[29]

	Standard	Test
Working Reagent:		
R_1Picrate	500 µl	500 µl
R_2Sodium hydroxide.	500 µl	500 µl
Standard (S)	100 µl	
Sample		100 µl

Mix gently and insert cuvette into the photometer immediately.

Record the reading at 500nm

Normal value: 44 – 130µmol/l.

- **Electrolyte estimation**

Electrolyte estimation was done using the procedure in the test kit manual used for this study.

a) Sodium (Na^+) assay by Enzymatic method

Principle: Sodium activates the enzymatic reaction between sodium-dependent β-D-galactosidase and its substrate, ONPG (O-nitrophenyl-β-D-galactopyanose), converting it to O-nitrophenyl and galactose. The rate of product formation at 500nm corresponds to the amount of sodium present in the sample.

Procedure

The following were pipetted into a clean dry test tube or cuvette labeled as Blank (B), Standard (S), and Test (T).

Additional sequence	Blank	Standard	Test
Working Reagent	1ml	1ml	1ml
Standard	-	20 µl	-
Sample	-	-	20µl
Distilled water	20 µl	-	-

It was well mixed and incubated for 5 minutes at 37°C. The absorbance was read against the reagent blank.

Calculation: $\text{Sodium [mmol/L]} = \frac{\Delta A \text{ Sample}}{\Delta A \text{ Std/Cal}} \times \text{Conc. of Std/Cal [mmol/l]}$

Reference ranges: 135 – 150 mmol/L.

b) Potassium (K^+) Assay by Enzymatic Method

Principle: Potassium is determined via enzymatic reaction, in which phosphoenolpyruvate is converted to pyruvate by the action of a potassium-dependent pyruvate kinase. The pyruvate produced is converted to lactate in the presence of NADH (nicotinamide dinucleotide), in a reaction which is catalyzed by lactate dehydrogenase. The oxidation of NADH to NAD and subsequent decrease in optic density at 380 nm is proportional to the amount of potassium present in the sample.

Reaction: Phosphoenolpyruvate + ADP $\xrightarrow[\text{Pyruvatekinase}]{K^+}$ Pyruvate + ATP

Pyruvate + NADH $\xrightarrow[\text{Lactate dehydrogenase}]{}$ Lactate + NAD

Procedure:

The following were pipetted into a clean dry test tube or cuvette labeled as Blank (B), Standard (S), and Test (T).

Addition sequence	Blank	Standard	Test
Working Reagent	1ml	1ml	1ml
Standard	-	10 µl	-
Sample	-	-	10µl
Distilled water	10 µl	-	-

It was well mixed and incubated for 5 minutes at 37°C. The absorbance was read against the reagent blank.

Calculation: $\text{Potassium [mmol/l]} = \frac{\Delta A \text{ Sample}}{\Delta A \text{ Std/Cal}} \times \text{Conc. of Std/Cal [mmol/l]}$

Reference range: 3.5 – 5.6 mmol/l.

c) Chloride (Cl^-) assay by Enzymatic method

This assay method is based on the following reaction sequences:

$6\ Cl^- + 3\ Hg(SCN)_2 \longleftrightarrow 3\ HgCl_2 + 6\ (SCN)^-.$

$6\ (SCN)^- + 2\ Fe^{3+} \longleftrightarrow 2\ Fe(SCN)_3.$

Chloride ions in the sample react with mercuric thiocyanate releasing equivalent quantities of thiocyanate. Free thiocyanate ions then react with Fe^{3+} ions forming a red coloured complex whose absorbance at 500 nm is proportional to the chloride concentration in the sample.

Procedure

The following was pipetted into a clean dry test tube or cuvette labeled as Blank (B), Standard (S), and Test (T).

Addition sequence	Blank	Standard	Test
Working Reagent	1ml	1ml	1ml
Standard	-	10 µl	-
Sample	-	-	10µl
Distilled water	10 µl	-	-

It was well mixed and incubated for 5 minutes at 37°C. The absorbance was read against the reagent blank.

Calculation: $\text{Chloride [mmol/l]} = \frac{\Delta A \text{ Sample}}{\Delta A \text{ Std/Cal}} \times \text{Conc. of Std/Cal [mmol/l]}$

Reference range: 95 – 110 mmol/l.

- **Histopathological Examination**
- **Tissue Processing**

The fixed kidney underwent a routine procedure for processing; Grossing, fixing, dehydration, alcoholization, clearing, infiltration, and embedding in paraffin wax. Using a microtome, the tissues were sectioned at a thickness of five microns (5μm). They were then ready for staining. All stained slides were photomicrographed at × 400 Magnification.

- **Haematoxylin and Eosin Staining.**

Principle

The principle is based on the chemical interaction between tissue and dye. Haematoxylin, a basic dye imparts blue-purple contrast on basophilic structures, primarily those containing nucleic acid moieties such as chromatin, ribosomes and cytoplasmic regions rich in RNA. An acidic eosin counterstain the basic elements such as RBCs, cytoplasm, muscle and collagen in varying intensities of pink, orange and red.

Procedure

The tissue sections were heated in a hot air oven at 40°C for 5 minutes before being deparaffinized in three changes of xylene. Each section was also soaked in increasing concentrations of alcohol (absolute, 90%, 70%, and dipped in water). Tissues were differentiated and then stained progressively in Mayar's haematoxylin for 15 minutes before being blued in tap water for 10 minutes. All stained sections were counterstained with Eosin for 2 minutes each, rinsed in water, and dehydrated in ascending grades of alcohols (70%, 90%, absolute 1, absolute 2) before being dried completely in an oven, cleared in xylene, and mounted with DPX mountant before being examined microscopically.

Statistical Analysis

Data obtained were analyzed using SPSS version 25.0 (IBM Corp, USA). Results were expressed as mean ± standard deviation (SD). One-way analysis of variance (ANOVA) was used to compare the means among the different groups, followed by Tukey's post hoc test for multiple comparisons. A p-value < 0.05 Was Considered Statistically Significant.

Results

Table 4: Effects of Phostoxin exposure on electrolyte Parameters of Male Albino wistar rats used for this study.

	Na^+ (mmol/l)	K^+ (mmol/l)	Cl^- (mmol/l)	HCO_3^- (mmol/l)
Control	133.47 ± 1.34	4.47 ± 0.18	94.89 ± 1.05	25.77 ± 0.97
30 Days Exposure	136.47 ± 2.52	4.14 ± 0.15*	98.76 ± 2.12*	22.85 ± 1.02*
60 Days Exposure	140.23 ± 1.80^{*,a}	3.49 ± 0.14^{*,a}	107.39 ± 2.75^{*,a}	20.13 ± 1.21^{*,a}

Values are expressed as mean ± SD, n = 6.

* = significantly different from Normal Control at $p<0.05$;

a = significantly different from 30 Days Exposure at $p<0.05$;

b = significantly different from 60 Days Exposure at $p<0.05$.

Table 5: Effects of Phostoxin exposure on electrolyte Parameters on Female Albino wistar rats used for this study.

	Na^+ (mmol/l)	K^+ (mmol/l)	Cl^- (mmol/l)	HCO_3^- (mmol/l)
Normal Control	132.37 ± 0.86	4.69 ± 0.20	95.41 ± 1.34	25.59 ± 1.38
30 Days Exposure	135.25 ± 1.39*	4.40 ± 0.12	99.65 ± 2.02*	23.02 ± 1.25*
60 Days Exposure	140.63 ± 1.64*,a	3.63 ± 0.15*,a	108.27 ± 1.63*,a	19.45 ± 1.13*,a

Values are expressed as mean ± SD, n = 6.

* = significantly different from Control at $p<0.05$;

a = significantly different from 30 days Exposure at $p<0.05$.

Discussion

The kidneys are highly vascularised and metabolically active organs which are also vulnerable to oxidative, traumatic and chemical injuries. Histological examination in experimental animals has shown that phostoxin inhalation results in notable renal distortion and pathology. Phostoxin inhalation results in oxidative damage to renal tissues, primarily through increased production of reactive oxygen species, lipid per oxidation and impaired antioxidant defences. These changes compromise renal activity and functions. [30]

In the present study, result showed that phostoxin exposure to the animals had significant effects on the electrolyte levels or concentrations both male and female rats.

K^+ is one of the major intracellular cations that plays a role parallel to Na^+ in this extracellular fluid that is impatant in muscle contraction, nerve impulse conduction, enzyme action and cell membrane function by causing changes in the concentration of extracellular K^+ muscle excitability, conductance and rhythm are affected.

In the present study, the concentration of K^+ decreased progressively (0.15%) after exposure to the toxin for 30 days and 0.23% after 60 days from the control thereby increasing the pH. This level of decrease might not raise any alarm because the increase in Na^+ concentration (0.02%) for 30-days exposure and 5.12% after 60-days exposure was near sufficient to maintain equilibrium i. e Na^+K^+ maintains equilibrium and delays the manifestation of toxicity.

Low K+ in the extracellular fluid results in impaired neuromuscular function with profound weakness of skeletal muscle leading to impaired ventilation and smooth muscle producing ileus.[29] Loss of K^+ results in metabolic acidosis as the pH rises and bicarbonate concentration developes as a result of K^+ deficit which is accompanied by renal excretion of H^+ and reabsorbtion of HCO_3^-, movement of Na^+ and H^+ from extracellular fluid into the cells as K^+ is lost. A defect of water reasorbtion occurs, producing polyuria, hyposthenuria and paralytic ileus.[30]

HCO_3^- is one of the principal buffering agents in the body which provides cations and removes H^+ which is ultanately excreted by the kidney and in the present study HCO_3^-had decreased (falls) of 0.11% for 30days and 0.22% for the 60-days duration, during which its buffering capacity was compromised.

The effects of phostoxin inhalation on serum urea and creatinine showed similar trends of peaking at 60-days exposure ($p<0.05$) when compared with control. The result of this study isin tandum with the earlier results of Abdelaziz and Ali and Al-Eissa et al.[30,31]

We concluded that phostoxin inhalation induces significant renal toxicity irrespective of gender in Wistar rats as evidenced by significant rise in serum and creatinine levels, disruption in the serum electrolyte

levels, i.e. K^+, Na^+, HCO_3^-, Cl^-, etc. Histopathological evaluation of renal tissues also revealed vast or extensive glomerular atrophy, tabular necrosis, interstitial hemorrhage, etc. as hall-mark of nephrotoxicity.

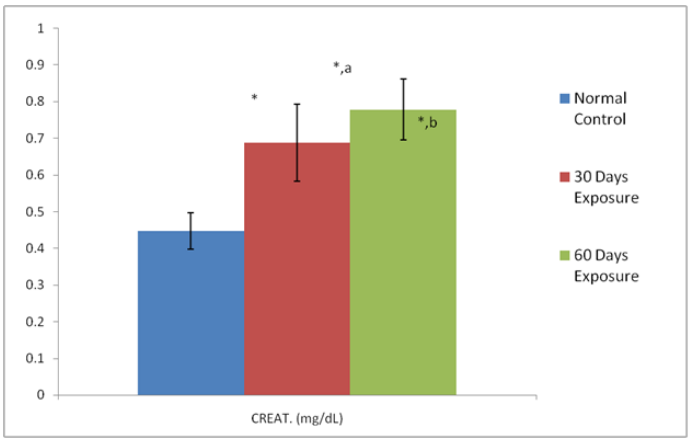

Figure 1: Effects of Phostoxin exposure on Creatinine Levels of Male Albino wistar rats used for this study.
Values are expressed as mean ± SD, n = 6.
* = significantly different from Normal Control at p<0.05;
a = significantly different from 30 Days Exposure at p<0.05;
b = significantly different from 60 Days Exposure at p<0.05.

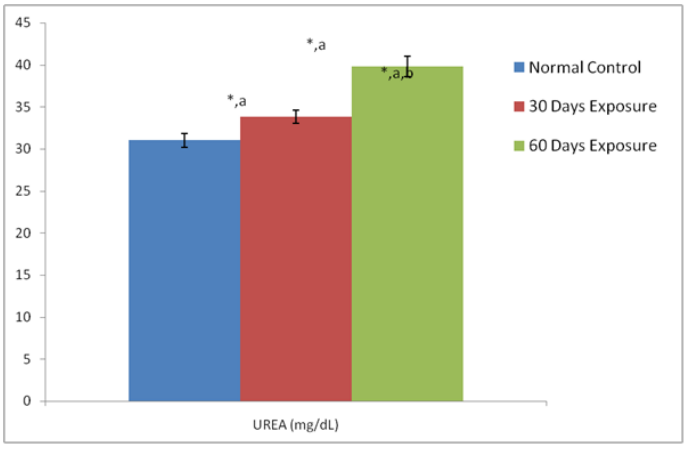

Figure 2: Effects of Phostoxin exposure on Urea Levels of Male Albino wistar rats used for this study
Values are expressed as mean ± SD, n = 6.
* = significantly different from Normal Control at p<0.05;
a = significantly different from 30 Days Exposure at p<0.05.
b = significantly different from 60 Days Exposure at p<0.05.

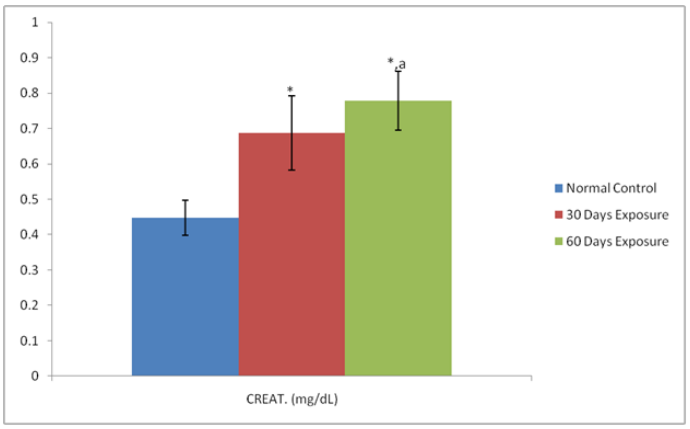

Figure 3: Effects of Phostoxin exposure on Creatinine Levels of Female Albino wistar rats used for this study.
Values are expressed as mean ± SD, n = 6.
* = significantly different from Normal Control at p<0.05;
a = significantly different from 30 Days Exposure at p<0.05.

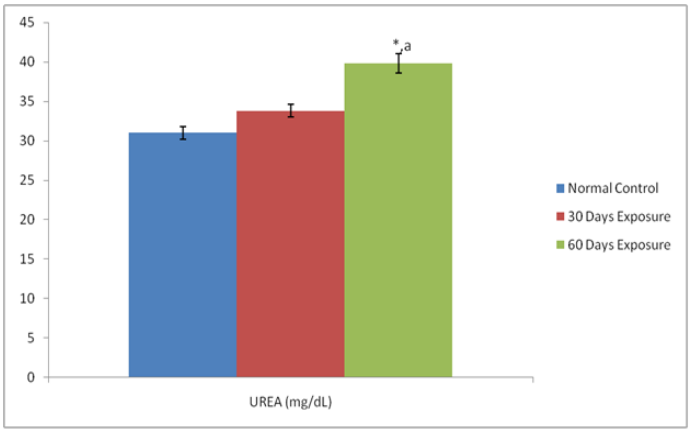

Figure 4: Effects of Phostoxin exposure on Urea Levels of Female Albino wistar rats used for this study.
Values are expressed as mean ± SD, n = 6.
* = significantly different from Normal Control at p<0.05;
a = significantly different from 30 Days Exposure at p<0.05.

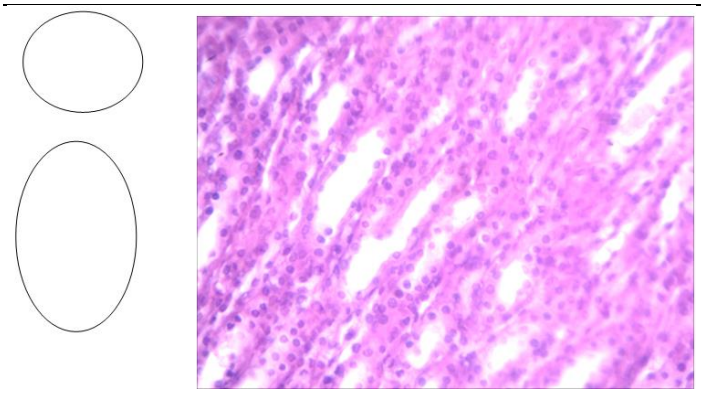

Figure 5: Photomicrograph of tissue section of kidney (Group1) (control group) (X400) (H/E) shows normal structures with well-arranged collecting ducts

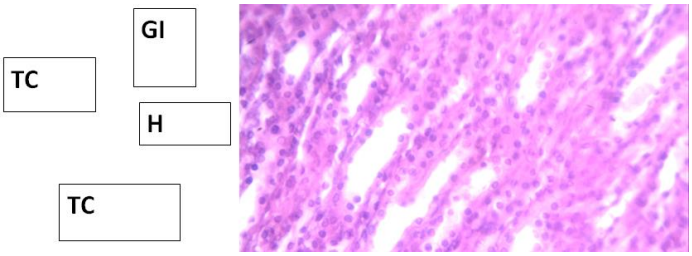

Figure 6: Photomicrograph of tissue section of kidney exposed to phostoxin inhalation for 30 days (Group2) (X400) (H/E) shows mild hemorrhage (H), glomerular infiltration (GI) and gross disruption of tubular cells(TC)

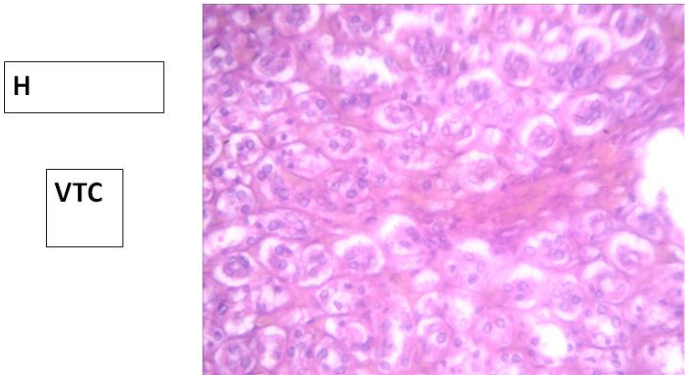

Figure 7: Photomicrograph of tissue section of kidney exposed to phostoxin inhalation for 30 days (Group2) (X400) (H/E) shows mild hemorrhage (H) and gross vacuolation with mild disruption of tubular cells (VTC)

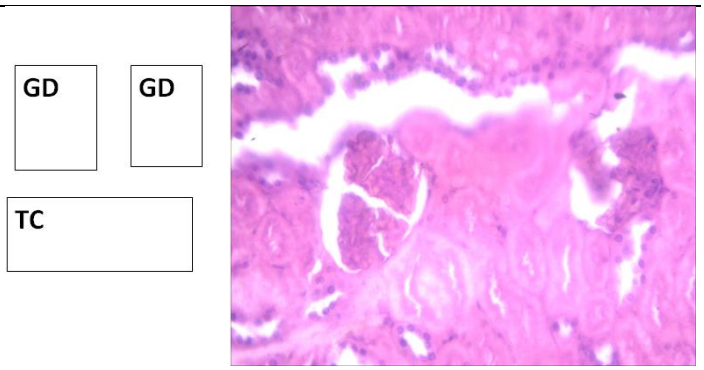

Figure 8: Photomicrograph of tissue section of kidney exposed to phostoxin inhalation for 60 days (Group3) (X400) (H/E) shows glomerular distortion (GD) and gross disruption of tubular cells(TC)

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