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Effects of Vitamin E on Phostoxin-Induced Changes on the Hematological Parameters and Lungs of Adult Wistar Rats.

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ABSTRACT

The effects of Vitamin E on Phostoxin-induced changes on the hematological indices and the histology of the lungs in adult Wistar rats were studied. Thirty adult Wistar rats were obtained and acclimatized for three weeks and were randomly divided into six (6) groups of five rats each. Group 1, was the Control and was given distill water while Group 2 was exposed to Phostoxin for one and half hours and administered Vitamin E at a concentration of 112mg/kg body weight. Group 3 was exposed to phostoxin for three hours and was administered with Vitamin E at concentration of 112mg/kg body weight while, Group 4 was exposed to phostoxin only for three hours. Group 5 was exposed to phostoxin only for one and half hours while Group 6 was administered with Vitamin E only at the concentration of 112mg/kg body weight. The administration lasted for seven days after which the rats were sacrificed after anesthetizing them with chloroform. Blood was collected in containers for hematological analysis while the lungs were harvested and fixed in 10% buffered formalin. The lung tissues were processed and stained with hematoxylin and eosin, and Masson Trichrome stains. The results showed non-significant reductions in the hematological indices in phostoxin treated animals with reduced effects in rats co-administered phostoxin with Vitamin E. The results of histological observations revealed damages to the alveolar sacs, loss of lung cells and reduction in elastic fibers while rats co-treated with Vitamin E, showed reduced damages in the tissues of the lung. The results of the present study showed that Vitamin E have some level of protection on phostoxin-induced toxicity on the lung tissues and hematological parameters of the adult Wistar rats.

KeyWords: Vitamin E; Phostoxin; hematological indices; Lungs; Wistar rats

INTRODUCTION

The lungs as the organ of respiration are involved in gaseous exchange, and are the first to be affected by the aluminum phosphide gas, the major component of phostoxin through inhalation[1, 2]. In sufficient quantity, phosphine affects the liver, kidneys, lungs, nervous system and circulatory system. Inhalation can cause lung edema and hyperemia, small perivascular brain hemorrhages and fluid in brain while ingestion can cause lung and brain symptoms but damage to the viscera are more common[3,4,5].

Phosphine poisoning may result in pulmonary edema, liver elevated serum and alkaline phosphatase, reduced prothrombin, hemorrhage and jaundice, yellow skin color and kidney hematuria, blood in urine and abnormal lack of urination[6,7]. The pathology of phostoxin intoxication is characteristic of hypoxia or oxygen deficiency in body tissues. Frequent exposure to concentrations above permissible levels over a period of days or weeks may cause poisoning and treatment is symptomatic depending on the signs presented[8,9].

Antioxidants may protect cells from the damage caused by unstable molecules, the free radicals which may cause to cancer. Antioxidants interact with, stabilize free radicals, and may prevent some of the damage free radicals might otherwise cause. Examples of antioxidants include beta–carotene, lycopene, Vitamins C, E, and A[10].

Vitamin E is the collective name for a set of eight related tocopherols and tocotrienols, which are fat-soluble vitamins with antioxidant properties[11]. Of these, α-tocopherol has been the most studied as it has the highest bioavailability, with the body preferentially absorbing and metabolizing this form[12].

Phostoxin is a fumigant or pesticide used in storage of commodities such as grains, animals feed and ingredients, as well as non-food items[13, 1, 14]. It is a market name for chemical compounds containing aluminum phosphide as its active ingredient. Other trade names of similar compounds include; fastphos, fumitoxin, gastoxin, maxkill, phosfume, and weevilcide. Aluminum phosphide, the main reactive compound in phosphine reacts with moisture from the air, and many

other liquids to release phosphine gas[15,16]. Phosphine gas is a colorless gas with garlic or rotten fish smell. Aluminum phosphide comes into contact with the body basically though injection, orally or inhalational routes[17,18].

Aluminum phosphide when ingested orally reacts with water and stomach acids to produce phosphine gas, which may account for observed toxicity. Phosphine generated in the gastrointestinal tract is readily absorbed into the bloodstream, and it is readily absorbed through the lung epithelium[19, 20]. Phosphine may cause denaturing of oxy-hemeglobin, the carrier for systemic distribution of oxygen as well as enzymes important for respiration and metabolism, and may also have effects on cellular membranes[21, 22]. Inhaled aluminum phosphide dust undergoes the same reaction in the moist air sacs of the lung, although at a lower rate, resulting in similar local and systemic effects[19,23].

Symptoms of mild to moderate acute aluminum phosphide toxicity include nausea, abdominal pain, and tightness in chest, excitement, restlessness, agitation and chills[2]. Symptoms of more severe toxicity include diarrhea, cyanosis, difficulty breathing, pulmonary edema, respiratory failure, tachycardia, rapid pulse and hypotension, low blood pressure, dizziness and/or death. Convulsions have been reported in lab animals exposed to high concentrations of phosphine[19, 23, 18]. Severe exposure may also result in proteinuria or glucosuria, low molecular weight proteins or glucose in the urine indicating kidney damage[1]. The aim of the present study was to study the effects of Vitamin E on phostoxin-induced changes in the lung tissues and hematological indices of adult Wistar rats.

MATERIALS AND METHODS

Chemicals: Phostoxin tablet used was manufactured by D & D Holdings Inc. USA. The tablets weigh 3g and releases 1g of phosphine gas, was purchased from Agro Allied Store Kwangila, Zaria, Kaduna State-Nigeria. It takes an average of 3 days to completely decompose leaving a gray-white powder of aluminum hydroxide and inert ingredients of the ammonium carbarnate [16]. Vitamin E manufactured by Medizen USA was purchased from Beautiful Gate Pharmaceutical Store Samara, Zaria Kaduna State-Nigeria. Vitamin E used was soft gelatin capsules containing 100mg Vitamin acetate.

Experimental Animals: Thirty Wistar rats were obtained and acclimatized for three weeks in the animal house of Department of Human Anatomy, Faculty of Medicine, Ahmadu Bello University Zaria. The animals of average weight of 140gms and were randomly separated into six groups of five animals each and were fed with standard pellets and water was provided *ad-bilitum*.

Phostoxin was given through inhalation by using lightly suspended cotton wool in an enclosed box for one and a half hours and three hours respectively for a period of seven days. Vitamin E was administered at 800mg/kg body weight, orally by means of insulin syringe for animals in Groups 2, 3 and 6.

Experimental Protocol: The animals in Group 1, were used as the Control and given distill water, Group 2 was exposed to Phostoxin for one and half hours and administered Vitamin E at a concentration of 112mg/kg body weight, Group 3 was exposed to phostoxin for three hours and administered with Vitamin E at concentration of 112mg/kg body weight, Group 4 was exposed to phostoxin only for three hours, Group 5 was exposed to phostoxin only for one and half hours and Group 6 was administered with Vitamin E only at the concentration of 112mg/kg body weight. The administration lasted for seven days and on the ninth day, the rats were humanely sacrificed after anesthetizing them with chloroform and the blood was collected in properly labeled EDTA bottles for hematological analysis. The lung tissues were harvested and weighed with a Digital Weighing Balance and were fixed in 10% buffered formalin and were processed and stained for histological analysis using Haematoxylin and Eosin (H & E) for general tissue architecture, and Masson Trichrome stain to study the tissue fibers.
Oxidative Stress markers such as Catalase activity, Superoxide Dismutase Activity, Assessment of Lipid Peroxidation and Glutathione Concentration were studied using the respective assay methods according to the instructions of the manufacturers.

Statistical Analysis: Data was presented as mean ± standard deviation. For establishing significant differences between groups, data were analyzed using Student's T-test and One-Way analysis of variance (ANOVA), followed by the Turkey post hoc test. Values were considered statistically significant if P value is less than or equal to 0.05 ($P<0.05$). In order to simplify the presentation of data, all the analysis were carried out using Sigmastat 2.0 for Window.

RESULTS

Physical Observation: A little bit of sluggishness was observed after each period of exposure to phostoxin but after a moment, the rats became active again. The result showed that during the period of exposure to phostoxin, there was increase in food consumption by the experimental rats.

Hematological Analyses: The results from the present study showed a non-significant reduction in red blood cells (RBC) from the Control Group in all the experimental Groups except for Group 4 with a non significant increase in RBC value. Platelet count was non-significantly reduced in Groups 3 and 5 while increased in Groups 2, 6 and 4. The Mean corpuscular hemoglobin value (MCH) was non-significantly reduced in Groups 4 and 3 but was increased in Groups 2, 5 and 6. The Packed cell volume (PCV) values were all non-significantly increased. White blood cell

(WBC) count was non-significantly reduced in Groups 3 and 4 while increased in Groups 2, 5 and 6. The lymphocyte values were increased in all the Groups though the increase was not significant. The results of the hematological indices are presented in Table 1.

Table 1: Effect of Phostoxin on the Hematological Parameter of Wistar Rats

Group	RBC (10^{12}/L)	PLT (10^9/L)	MCH (pg)	MCHC (g/dl)	PCV (%)	WBC (10^9/l)	LYM (10^9/l)
Group 1 (Control)	6.55±1.11	441.0±198.4	18.86±1.09	30.53±1.17	0.40±0.06	9.20±1.35	121.4±146.3
Group 2 Low dose Phostoxin /Vit E	6.36±0.38	474.3±84.3	21.00±0.88	29.50±0.43	0.43±0.01	9.30±1.70	136.6±29.94
Group 3 high dose Phostoxin / Vit E	6.42±1.26	321.7±82.1	18.66±0.55	27.06±0.65	0.44±0.06	6.76±1.47	134.0±34.61
Group 4 high dose Phostoxin	6.90±0.50	591.3±69.2	18.43±0.75	26.33±3.23	0.48±0.08	6.70±2.36	136.4±34.62
Group 5 low dose Phostoxin	4.44±3.85	372.3±331.1	19.00±1.73	28.76±1.68	0.43±0.04	10.43±2.08	137.2±31.45
Group 6 Vit E	5.93±0.96	562.0±114.7	19.76±2.33	28.26±3.20	0.41±0.04	12.53±7.14	127.4±28.27

The result of the hematological indices showed a non-significant increase or decrease in all the parameters studied *P value <0.05

Weight changes

During the cause of the experiment, the initial weights of the animals were taken before the exposure of the animals to phostoxin and final weights were taken before the animals were sacrificed. The results showed a statistical significant increase in the body weight of the animals administered with Vitamin E only (Group 6) ($P \leq 0.05$), while a statistical significant decrease in the body weight of animals that were exposed to high concentration of phostoxin (Group 4) ($P \leq 0.05$) as shown in Table 2. The result also showed a statistical significant increase in the weight of the lungs in Groups 3 and 6 when compared to the Control group as shown in Table 2.

Table 2: Effect of Phostoxin on the Weight and Organ Weight of Wister Rat.

Groups	INITIAL (g)	FINAL (g)	DIFFERENCE (g)	Organ weight (g)
Group1 Control (Distil Water Volume /kg body weight)	121.40±14.63	136.20±20.24	14.8	1.28±0.08
Group 2 Low dose phostoxin/Vit E	136.60±29.94	148.20±32.87	11.6	1.57±0.64
Group 3 high dose phostoxin/Vit E	134.00±31.61	144.00±29.841	10.0	2.60±0.98*
Group 4 high dose phostoxin	136.40±34.62	145.40±25.82	9.0*	1.47±0.38
Group 5 low dose phostoxin	137.20±31.45	152.20±35.68	15.0	1.55±0.73
Group 6 VitE	127.40±28.27	151.20±28.96	23.8*	1.67±0.39*

The result of the body and organ weight changes showed a significant increase or decrease in some groups of the experimental animals studied *P value <0.05

Oxidative Stress Parameters

The study of effects of Vitamin E on phostoxin induced changes in adult Wistar rats was determined using the antioxidative markers namely Catalase, Superoxide Dismutase activity (SOD), Reduced Glutathione concentration (GSH) and Lipid Peroxidation (LPO) and the results compared to the control. The results showed a significant decrease in Catalase and SOD activities ($P \leq 0.05$) in Groups 4 and 5, while GSH activities showed significant decrease in Groups 3 and 4. The result of Lipid Peroxidation showed a significant increase in Groups 4 and 5 when compared to the Control ($P \leq 0.05$) as shown in Table 3.

Table 3: Effect of Phostoxin and Vitamin E on the Biochemical Parameters.

Groups	Administration (Mg/Kg bwt)	CATALASE (µmol/mg)	LPO (nmol TABRS/mg)	SOD (U/mg)	GSH (U/mg)
1	Distil water (Control)	12.90±4.82	42.52±4.85	346.60±16.41	26.75±3.11
2	1.21mg/kg bwt phostoxin 800mg/kg bwt of Vit E	9.46±1.86	50.37±6.10	287.25±82.38	19.32±5.78
3	2.42mg/kg bwt phostoxin 800mg/kg bwt of Vit E	10.37±8.87	46.21±18.55	254.75±154.5	18.87±12.34*
4	2.42mg/kg bwt phostoxin	7.59±0.45*	55.39±0.29*	140.95±6.44*	14.77±2.83*
5	1.21mg/kg bwt phostoxin	7.71±2.341*	53.11±17.32*	174.50±4.95*	19.00±11.5
6	800mg/kg bwt of Vit E	13.09±3.15	42.00±1.67	306.50±44.55	25.00±10.10

The result of Antioxidative analyses showed a significant increase or decrease in some of the parameters studied
*P value <0.05

Histological Observations of the tissue sections

The results from Histological observation of the Lung tissues showed normal structure of the Lungs in the Control Group and Group 6 as shown in Figures 1 and 6. The results of the Lungs of Phostoxin and Vitamin E treated animals showed lung tissues with minimal damages as shown in Figures 2, 3 and 5. While animals exposed with phostoxin only Groups, showed mark damages in the lung tissues as seen in Figures 7 and 9. The Masson trichrome staining showed fibers with deep brown coloration. The results showed that the tissue fibers were normal in Groups 1 and 6 as seen on Figures 8 and 12. However fewer fibers were observed in phostoxin and Vitamin E Groups as seen in Figures 4, 6 and 11, while on the Phostoxin only Groups, fibers were almost absent as shown in Figures 8 and 10.

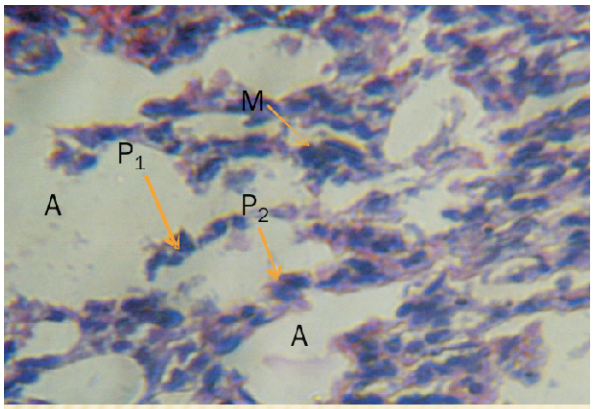

Fig.1: A Section of the Lungs from the Control Group 1 showing the alveolus (A) and its epithelial cells type I and type II Pneumocyte ($P_{1\&2}$), Macrophages (M), Neutrophels (N) and normal epithelium. H & E - 400.

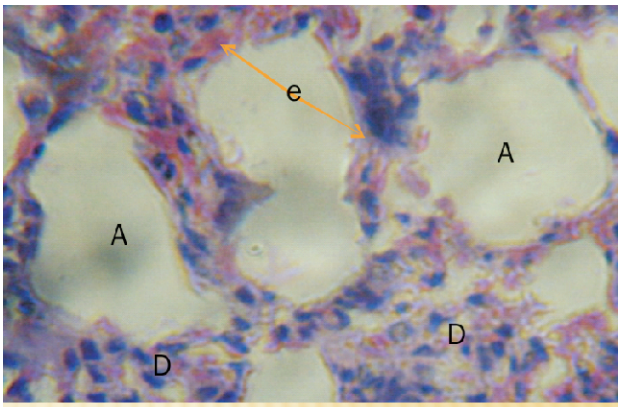

Fig. 2: A Section of the Lungs from Group 2, showing slight degeneration of endothelia (e) with alveoli (A) with some Alveoli with some degenerated cells (D). H & E - 400.

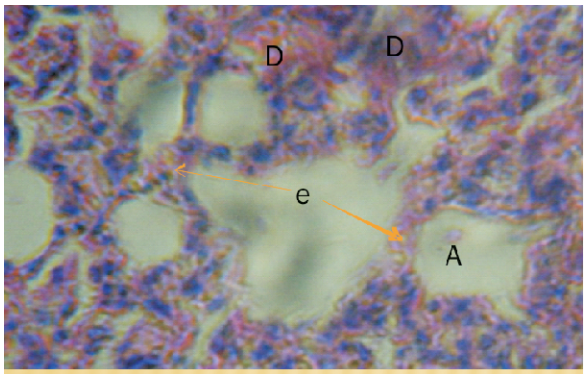

Fig. 3. A Section of the Lungs from Group 3 showing increased epithelial lining (e) of the Alveoli (A) with some degenerated cells (D). Group 3 (high dose phostoxin + 800mg/kg body weight t of vitamin E) H & E - 400.

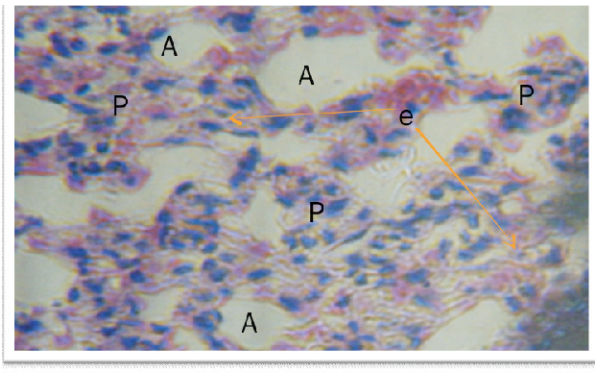

Fig. 4. A Section of the Lungs of Group 4 with Thickened epithelial lining (e) and proliferation of the cells (P), resulting in the reduction of the Alveoli volume (A) and Congestion of the alveoli spaces. H and E - 400

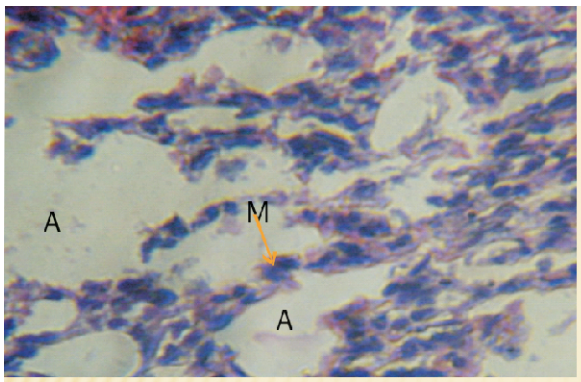

Fig.1: A Section of the Lungs from the Control Group 1 showing the alveolus (A) and its epithelial cells (type I and type II Pneumocyte (P_{IIC}), Macrophages (M), Neutrophels (N) and normal epithelium. H & E - 400.

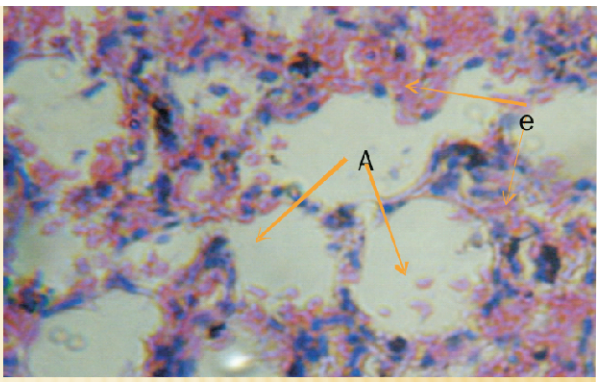

Fig.5: A Section of the Lungs from the Group 5, showing the alveolus (A) and its thickened epithelial Lining with degenerated epithelial cells (D) and Red Blood (RB) cells in the Alveoli. H & E - 400.

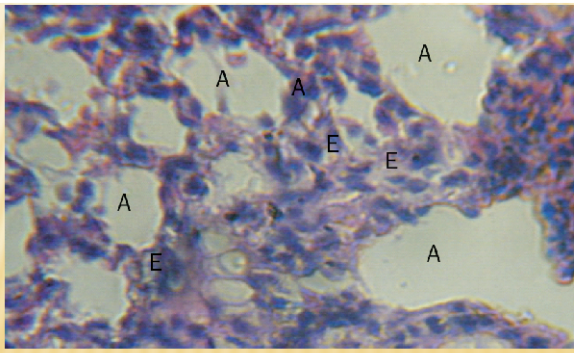

Fig .6: A Section of the Lungs of Group 6 with normal looking epithelial (E) cells and Alveoli (A). H and E - 400

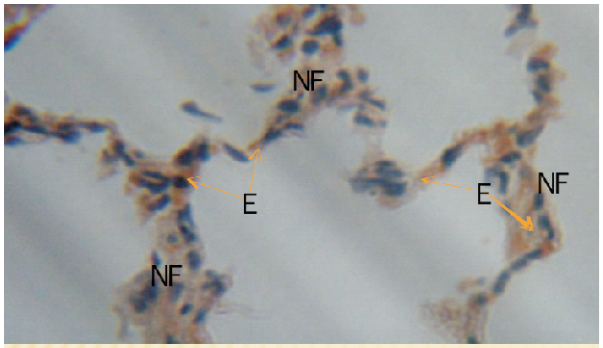

Fig. 7: A Section of the Lung of Group 1 with Mason Trichom stain with normal epithelial (E) cells and Normal elastic fibers (NF). MT - 400

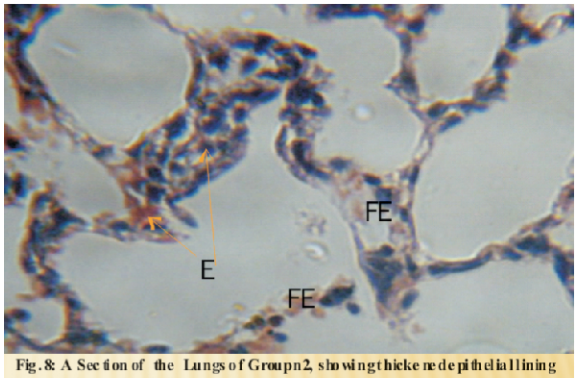

Fig. 8: A Section of the Lungs of Groupn 2, showing thickened epithelial lining (E) with fewer elastic fibers (FE). M.T. - 400

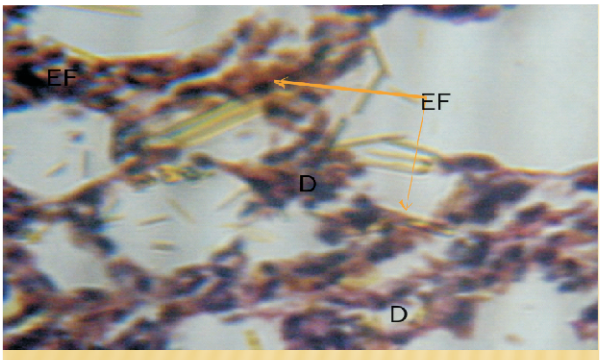

Fig. 9: A Section of the Lung of Group 3 showing degeneration (D) with few elastic fibers (EF). M.T. - 400

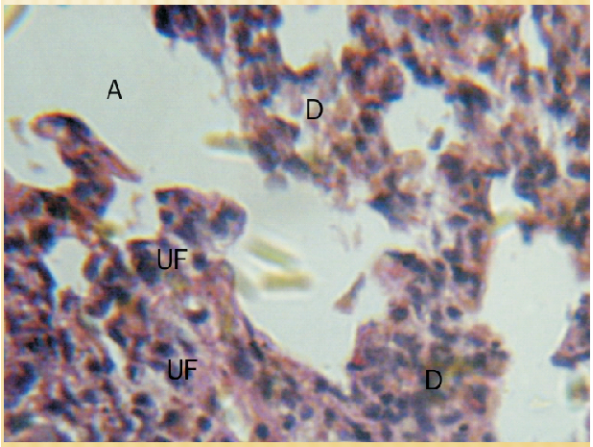

Fig. 10 : A Section of the Lung from Group 4 with Cellular degeneration (D) with undefined fibers (UF) and Alveoli (A). M.T. - 400.

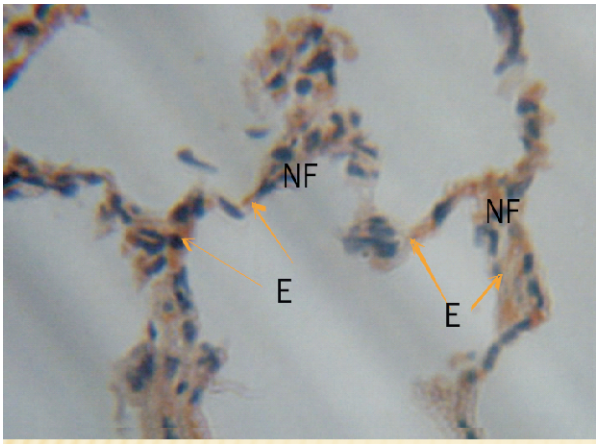

Fig 7: A Section of the Lung of Group 1 with Mason Trichom stain with normal epithelial (E) cells and Normal elastic fibers (NF). MT - 400

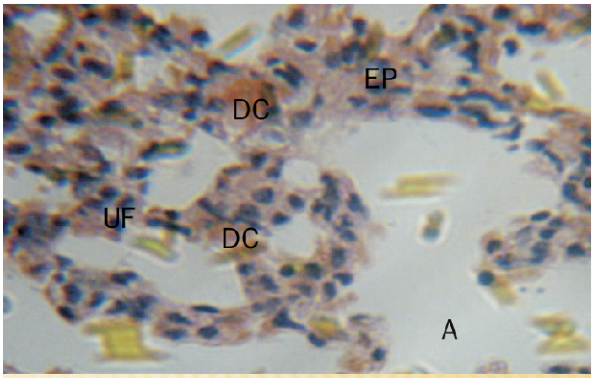

Fig. 11: A Section of the Lungs with Degenerated Cells (DC), Alveoli (A), thickened Epithelial Lining (EP) and undefined (UF) fibers. M.T. - 400.

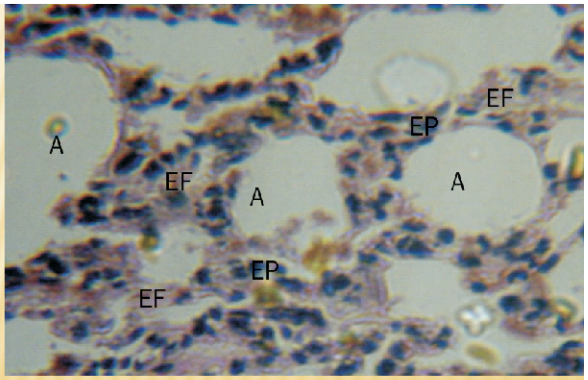

Fig.12: A Section of the Lungs from Group 6 with Normal Alveoli (A), Elastic fibers (EF) and Epithelial Lining (EP). M.T. - 400

DISCUSSION

The study of the effect of Vitamin E on phostoxin-induced changes on the hematological indices and the histology of the lungs of adult Wistar rats was undertaken and the results showed non-significant changes in the RBC, MCH MCHC and PCV with reductions in phostoxin treated rats and increases in rats treated with both phostoxin and Vitamin E. The reductions could be as a result of hypoxia or anemia leading to the reductions in red blood cell delivery to the tissues of the body[1]. The increase in Platelet count could indicate inflammation as observed in animals administered with high dose of phostoxin while the increase in WBC could be as a result of Vitamin E being immune buster which deals with immunity and could have led to the high values in the Vitamin E treated rats[24, 25]. Increases in the lymphocyte count could indicate that phostoxin could be an allergen resulting from the toxic substances present[26,27]. This is because the lungs serve as gaseous exchange which takes place between the air-blood barrier, the alveoli and blood capillary. Disorders in the respiratory tracts such as inhaled chemical toxins, bacterial prolonged activity or repeated actions could cause repeated damages on the respiratory epithelial cells which could lead to the death and replacement of the squamous epithelium [28, 29]. Hematoxylin and Eosin staining, show type I and II pneumocytes, macrophages and blood cells as blue while epithelial membranes were shown as pink. Type I pueumocyte matures to type II and the type two cells produces surfactants which reduces surface tension and enhancing breathing while deficiency of these leads to lung collapse[30, 31, 32]. Masson trichrome stain was used to indicate the presence of fibers, mostly elastic and collagen fibers. Elastic fibers were indicated by the presence of dark brown staining. Any condition or disease that reduces the amount of air in the alveoli or reduces the surface area of the alveoli makes the wall thick and impermeable to gases will lead to inadequate oxygenation of blood or hypoxia, carbon dioxide retention and breathlessness[33, 27]. This could explain the feeling of uneasiness and confusion by the animals when exposed to phostoxin during the experiment.

The major effects induced by phostoxin are in the loss of pneumocytes which are then replaced by the epithelium and the loss of elastic fibers. Pathological examination of the Lung tissues showed evidence of hypoxia with local trauma[19, 1]. These damages were observed more on the animals administered with high dose of Phostoxin compared to the animals administered with low dose[34, 20]. While the vitamin E treated groups showed mild forms of the damages as compared to the phostoxin only treated groups. This explains the symptoms of severe

poisoning which may occur within a few hours or days of exposure which may result in pulmonary edema which could lead to dizziness, cyanosis, unconsciousness and death as reported by Garry and others, and Turkez & Togar[21,1].

CONCLUSION

The present work showed that vitamin E has antioxidative effect on phostoxin induced changes according to the results from the hematological indices, antioxidative analyses and histological studies from the Vitamin E treated groups as compared to other groups. Emphasis on the level of caution that need to be exercised when handing the phostoxin and to stress the importance of healthy living by meeting the body's Vitamin demands which can help the body minimize the damages when accidentally exposed.

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