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*Original Research Article*

# Effects of Vitamin E on Phostoxin-Induced Changes in the Liver and Biochemical Parameters of Adult Wistar Rats

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## ABSTRACT

**The effects of antioxidant Vitamin E on phostoxin-induced changes in the liver and haemato-biochemical parameters in Wistar rats were studied. Thirty adult Wistar rats of both sexes were grouped into six groups of five rats each. Group 1 was the Control and was given normal saline, Group 2 was exposed to phostoxin for 3 hours with vitamin E and Group 3 was exposed to phostoxin for 1½ hours with vitamin E. Groups 4 and 5 were exposed to phostoxin for 3 hours and 1½ hours respectively and Group 6 was treated with vitamin E only. The rats were exposed to phostoxin through inhalational method for one week. At the end of the exposure period, the rats were sacrificed, the blood and tissues collected for analysis and procession for studies. The results showed increase in the body weight of the rats which could be as a result of the effect of phostoxin leading to increase in appetite of the rats. The results of biochemical parameters showed there was significant increase in AST and ALP ($P<0.05$), but the change in ALT in the serum of treated animals was not significant. The results from the histological observations of the Liver in the experimental rats showed changes in all the Groups that were treated with phostoxin of which the damage was dependent on the exposure time to phostoxin and Vitamin E may play a role as an antioxidant resulting in ameliorative effects in phostoxin-induced toxicity.**

**KeyWords: Vitamin E, phostoxin, biochemical parameters, oxidative enzymes, liver, Wistar rats**

## INTRODUCTION

Phostoxin is mainly used as a fumigant in pest control and is used for stored grains. Phostoxin provides an effective alternative to traditional control methods of rabbits, moles and rats (Sudakin, 2005). Inhalation of phostoxin may cause severe pulmonary irritation leading to acute pulmonary edema, cardiovascular dysfunction, CNS excitation, coma and possibly death. Gastrointestinal tract disorders, renal damage and leucopenia and hepatic damage may develop (Sudakin and Power, 2007; Turkez and Togar, 2013).

Phostoxin emits a colorless gas which is odorless when pure, but the technical product has a foul odor, like that of a fish or garlic, because of the presence of substituted phosphine and diphosphine (Proudfoot , 2009; O'Malley *et al.*, 2013). Phostoxin is flammable and explosive in air and can auto ignite at ambient temperatures. It is slightly soluble in water and soluble in most organic solvents and supplied in cylinders either as pure phosphide or diluted with nitrogen (Singh *et al.*, 1996; Shadnia *et al*., 2008; Bumbrahs *et al*., 2012). Aluminum phosphide which is really available as a fumigant for stored grains, is highly toxic when consumed from freshly opened containers, it is available in pellet and tablet form, and is also available in porous blister packs, sachets or as dusts (Shaheen, 1996; Shadnia *et al.*, 2009; Easterwood *et al.*, 2010).

Exposure to phostoxin must not exceed the 8hours Time Weighted Average of 0.3ppm or 15minutes Short Term Exposure Limit of 1.0ppm phosphine (USDHHS, 1994; Türkez and Toğar , 2013), and all persons are covered by this exposure. Phosphine exposure at a level of 11mg/$m^3$or 8ppm for 1-2 hours daily over a period of six weeks may result to death (Sudakin, 2005; Sudakin and Power 2007). Arsine and hydrogen sulfide exposures were considered to have been too low for the death while autopsy examination showed acute pulmonary edema (Furuno *et al.*, 1976; Curry *et al*., 2003).

Aluminum phosphide is an inorganic phosphide used to control insects and rodents in a variety of settings and phostoxin is one of the market names of aluminum phosphide. It is mainly used as an indoor fumigant at crop transport, storage or processing facilities for both food and non-food crops (Brautbar and

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Howard, 2002; Curry *et al.*, 2003; ). It may also be used as an outdoor fumigant for burrowing rodents and in mole control, or in bait for rodent control in crops (W.H.O, 1988; Moghadamnia *et al.*, 2012; Tehrani et al., 2013).

Vitamin E has oxygen sparing effect on heart muscle and helps to gradually breakdown blood clots in the circulatory system and helps to prevent more clothing from forming (Curry et al., 2003; Tehrani *et al*., 2013). Vitamin E encourages collateral circulation in the smaller blood vessels of the body. It seems to promote healing with the formation of much less scar tissue. Vitamin E helps to strengthen and regulate the heartbeat (Shadnia *et al*., 2011). It is indicated for use in cancer prevention, treatment and indicated for use before and after any surgery (Sulliva, 2002; Holden *et al.*, 2003).

Free radicals are molecules having an unpaired valence electron which are highly reactive. Antioxidants protect lipids from peroxidation by radicals. Examples of antioxidants include Vitamin A, Vitamin C, Vitamin E and mineral selenium (Alessio and Blasi, 1997; Shadnia *et al.*, 2011). The aim of the present study was to evaluate the effects of Vitamin E on Phostoxin-induced changes in haemato-biochemical parameters and the Liver of adult Wistar rats.

## MATERIALS AND METHODS

### *Chemicals*

Phostoxin tablet used was manufactured by D & D Holdings Inc. USA. The tablets weigh 3g that releases 1g of phosphine gas, was purchased from Agro Allied Store Kwangila, Zaria, Kaduna State-Nigeria. Each tablet takes an average of 3 days to completely decompose leaving a gray-white powder of aluminum hydroxide and inert ingredients of the ammonium carbarnate (Degesch, 2011). Vitamin E manufactured by Medizen USA was purchased from Beautiful Gate Pharmaceutical Store Samara, Zaria Kaduna State-Nigeria. Vitamin E used was a soft gelatin capsule containing 100mg Vitamin E 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 according to the methods of Valmas and Ebert (2006). 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 Biochemical analysis. The liver tissue was harvested, weighed with a Digital Balance and was fixed in 10% buffered formalin. The tissue was processed and stained for histological analysis using Haematoxylin and Eosin (H & E) for general tissue architecture, and Masson Trichrome special stain for detailed study of the tissue.

***Biochemical Assay***

The levels of biochemical parameters were determined by the use of an auto analyzer made by Roche-Hittachi, Japan, using commercial assay kits manufactured by Roche, Basel, Switzerland.

Liver secretes different serum enzymes, acting as biomarkers of liver cell damage and these enzymes include Alanine Amino Transferase (ALT) of which moderate increase from the normal may be seen in chronic liver disease such as cirrhosis, hepatitis and non-alcoholic steato-hepatitis (Vasudevan and Sreekumari, 2007). Aspartate Amino Transferases (AST) is moderately increased in liver diseases and a marked increase in AST may be seen in primary hepatoma (Vasudevan and Sreekumari, 2007).Alkaline Phosphatase (ALP)is a nonspecific enzyme which hydrolyses aliphatic, aromatic or heterocyclic compounds of which the upper level of Normal serum value may be more in children because of the increased osteoblastic activity in children (Vasudevan and Sreekumari, 2007).

***Estimation of Oxidative Parameters***

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. Catalase activity was determined using the method described by Sinha (1992). The absorbance was read at 570 nm and Standard cure was made using the absorbance obtained at various levels. Superoxide Dismutase (SOD) activity was determined by the method described by Fridovich (1989) and the absorbance was measured every 30s up to 150 s at 48Onm.Lipid peroxidation as evidenced by the formation of TBARS was measured by the modified method of Niehaus & Samuelson (1968) as described by Adhikari *et al*. (2009).The absorbance of the pink supernatant was measured against a reference blank using spectrophotometer at 535nm. Reduced glutathione (GSH) concentration measured according to the methods Ellman (1959) as modified by Rajagopalan *et al*. (2004) and Seiler *et al.* (2008). The absorbance was read at 412 nm.

***Statistical Analysis***

Data obtained were expressed as means ± standard deviation (SD). Differences between group means were estimated using Students' T-test and one-way analysis of variance (ANOVA) followed by Post-hock Turkey's test using SPSS 12.0 for windows. A P value less than or equal to 0.05 was considered to be significant.

## RESULTS

***Physical Observations***

During phostoxin exposure, the animals were observed to show signs of weakness and reduction in their physical activities. There were increase in body weight of the animals in Control Group and phostoxin exposure with or without Vitamin E administration although the increase was not statistically significance (Table 1).

The result showed that the mean change in body weight of animals in the Control Group and Groups 4, 5 and 6 were significant when compared to their initial mean body weights ($P \leq 0.05$). While the mean change in the body weight of animals in Groups 2 and 3 showed a non-significant increase as shown in Table 1. The result also showed a non-significant decrease in the liver weight of animals in all the experimental Groups when compared to the Control.

**Table 1.**Showing the mean change in the body weight and the liver weight.

| Grps | Administration (mg/Kg bwt) | Liver weight(g) | Initial body weight(g) | Final body weight(g) | Change in body weight(g) |
|---|---|---|---|---|---|
| **1** | Distil water (mg/kg bwt) | 5.90±1.22 | 121.4±14.64 | 136.2±20.26 | 14.8* |
| **2** | Low dose of phostoxin + 800mg/kg bwt of Vit E | 4.83±1.04 | 136.6±29.95 | 148.2±32.87 | 11.6 |
| **3** | High dose of phostoxin + 800mg/kg bwt of Vit E | 4.51±2.15 | 134.0±31.62 | 144.0±29.84 | 10.0 |
| **4** | High dose of phostoxin | 5.04±2.24 | 136.40±34.62 | 149.4±31.35 | 13.0* |
| **5** | Low dose phostoxin | 5.75±1.95 | 137.20±31.46 | 152.2±35.69 | 15.0* |
| **6** | 800mg/kg bwt of Vit E | 5.82±1.98 | 127.40±28.27 | 151.2±28.96 | 23.8* |

Table showing body weight change and kidney weight of experimental Rats *P≤0.05

***Biochemical Parameters***

The results of the effect of Vitamin E on phostoxin-induced changes on the liver in adult Wistar rats were evaluated. The results show different changes in serum levels of AST, ALT and ALP in phostoxin treated animals. The result of AST showed a significant increase in Group 5 animals only (P≤0.05), while a non-significant decrease in the levels of serum AST in all the Groups when compared with the Control. There was a statistical significant difference between Group 5 and Group 4, and between Group 5 and Group 2 respectively as shown in Table 2

There was a significant increase in the serum levels of ALT in Groups 5 and 6 animals compared to the Control (P≤0.05), while other Groups show non-significant changes in ALT serum levels when compared to the Control. The result showed a significant increase in serum levels of ALP in Group 6 animals as compared to the Control group (P≤0.05) while other Groups showed non-significant changes in serum levels of ALP when compared to the Control Group as shown in Table 2.

**Table 2**.Biochemical parameters (mean ±SD) in AST, ALT and ALP of Wistar Rats.

| Grps | Administration (Mg/Kg bwt ) | AST (IU/DL) | ALT (IU/DL) | ALP (IU/DL) | AST:ALT |
|---|---|---|---|---|---|
| **1** | Distil water(control) | 102.0±7.21 | 49.67±13.65 | 19.00±1.73 | 2.05 |
| **2** | 3g of phostoxin tablet (1½) hour + 800mg/kg bwt of Vit E | 100.3±3.22 | 50.00±14.00 | 20.67±3.06 | 2.01 |
| **3** | 3g of phostoxin tablet (3hours) + 800mg/kg bwt of Vit E | 104.7±3.06 | 47.00±10.82 | 18.00±1.73 | 2.23 |
| **4** | 3g of phostoxin tablet (3hours) | 94.33±5.51 | 39.00±6.93 | 20.33±3.51 | 2.42 |
| **5** | 3g of phostoxin tablet (1½) hour | 115.0±5.00* | 61.67±4.73* | 18.67±1.53 | 1.86 |
| **6** | 800mg/kg bwt of Vit E | 107.3±3.01 | 59.67±2.52* | 31.67±10.69* | 1.80 |

Table showing the values of biochemical parameters *p<0.05**.**

***Oxidative Parameters***

The results of oxidative stress analysis show changes in serum levels of Catalase activity, Lipid peroxidation (LPO), glutathione (GSH), Superoxide Dismutase (SOD) in the experimental groups. The results showed a statistical significant decrease in the level of Catalase activity in Groups 2, 3, 4 and 5 ($P \leq 0.05$), while Group 6 show a non-significant increase in Catalase activity when compared to the Control Group. The results of LPO activity show a statistical significant increase in Groups 2, 3, 4 and 5 ($P \leq 0.05$) while Group 6 animals showed no change when compared with the Control Group. The result of SOD activity, showed a significant decrease ($P \leq 0.05$) in all the experimental Groups when compared to the Control while the result of GSH activity showed a significant decrease in Groups 2, 3, 4, 5 ($P \leq 0.05$) and Group 6, show the same level of GSH activity when compared to the Control Group as shown in Table 3.

**Table 3**.Oxidative stress markers analysis in Wistar rats exposed to phostoxin**.**

| Grps | Administration | 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** | 3g of phostoxin tablet (1½hrs) + 800mg/kg bwt of Vit E | 9.46±1.86* | 50.37±6.10* | 287.25±82.4* | 19.32±5.78* |
| **3** | 3g of phostoxin tablet (3hrs)+ 800mg/kg bwt of Vit E | 10.37±8.9* | 46.218.55* | 254.8±154.5* | 18.87±12.3* |
| **4** | 3g of phostoxin tablet (3hrs) | 7.59±0.45* | 55.39±0.29* | 140.95±6.44* | 14.77±2.83* |
| **5** | 3g of phostoxin tablet (1½hrs) | 7.71±2.34* | 53.11±17.3* | 174.50±4.95* | 19.00±11.5* |
| **6** | 800mg/kg bwt of Vit E | 13.09±3.15 | 42.00±1.67 | 306.5±44.55* | 25.00±10.10 |

Table showing the levels of oxidative markers in experimental and Control Groups *$P \leq 0.05$

***Histological Observations***

The results of histological observation of the transverse section of the liver show changes in the experimental rats exposed to phostoxin with or without Vitamin E. The result from the Control Group showed normal hepatic lobule while liver of the rats in the treated Groups showed several histological changes. Group 1 showed normal the histology of liver with normal arrangement of central vain, sinusoid and hepatocytes as shown in Plate1. Group 2 animals showed congested central vain and distorted sinusoid as shown in Plate 2. Group 3 showed congested central vain, vacuolation and distorted sinusoid as shown Plate 3 while Group 4 animals showed congested central vain, necrotic hepatocytes and infiltrated inflammatory cells as shown in Plate 4. Group 5 showed slight congestion of central vain and distorted sinusoid as represented in Plate 6, while Group 6 animals show normal cyto-architecture of liver which are similar to the Control Group as shown in Plate 1.

## DISCUSSION

The present study showed significant increases in biochemical parameters namely the AST ALP and ALT levels in the serum of phostoxin exposed animals when compared to the Control. These increases in Serum Liver enzymes showed that the liver must have been destroyed leading to the leakage of these enzymes (Arora *et al.,* 1995; Saleki *et al.,* 2007). As such there was some degree of liver damage due to phostoxin toxicity. The present findings agreed with the work of Bai *et al.* (1980), and Atchabarov *et al.* (1984), who observed that the biochemical parameter changes were similar to those in Control Group treated with hydrogen fluoride of known toxic level (Sinha *et al.,* 2005). However, the overall mean values of the enzymes in Groups treated with Vitamin

E tend to be closer to the normal values thus indicating the effect of this antioxidant against phostoxin toxicity (Shadnia *et al*., 2011; Tehrani *et al.*, 2013).

The animals in the Control Group and those administered with phostoxin with Vitamin E, showed normal of the liver structure, but there was some cell damage in the liver of phostoxin, exposed animals showed histological changes in the liver (Saleki *et al.*, 2007; Tehrani *et al.*, 2013). This could be as a result of reducing agents that protect the cells against free radicals, peroxides and other toxic compound. Meanwhile, there was reduction in the levels of GSH in phostoxin exposed Groups which could have been due to the exposure to phostoxin which could consequently lead to some disorders. However, both SOD and Catalase levels were reduced in animals in high and low phostoxin exposure due to high oxidative stress caused by free radical generation but all other Groups treated with Vitamin E and the Control Group showed normal levels of the enzymes.

Vitamin E administration showed reduction in the effect on the liver which could be associated with ability of Vitamin E to ameliorate or inhibit the action of phostoxin. This could be as a result of removing the reactive oxygen species (ROS) once formed, via very rapid electron transfer chain that inhibit lipid peroxidation, thus preventing radical chain reaction by Vitamin E, which is in agreement with the findings of Alesico and Blasi (1997) and Saleki *et al.* (2007).

The liver of phostoxin showed congested central vain, focal accumulation of inflammatory cells and necrotic hepatocytes which could be due to the ability of phostoxin to activate signals that increase tumor necrosis factor-α, a substance in the liver that causes inflammation, malignancy and cell death (Arora *et al.*,1995; Sinha *et al.*, 2005; Saleki *et al.*, 2007; ). Cirrhosis, the end result of several conditions that affect the liver architecture, usually a consequence of sustained progressive injury to hepatocytes, produced by several agents, such as ethanol, drugs or other chemicals, hepatitis virus and autoimmune liver disease (Lall *et al.*, 2000; Saleki *et al.*, 2007). Moreso, the liver of the animals with only Vitamin E administration showed no changes and closely resembles that of the Control Group as vitamin E has been shown to play important role in preventing the oxidative damaged to the liver.

## CONCLUSION

The present study has demonstrated that phostoxin has effects on the body weight of the animals and the histology of the Liver. The significant changes were also observed in biochemical parameters in the experimental groups. Vitamin E has been shown to ameliorate the toxic effects of phostoxin on the biochemical parameters and also has been shown to play important role in preventing and protection against oxidative damage to the liver.

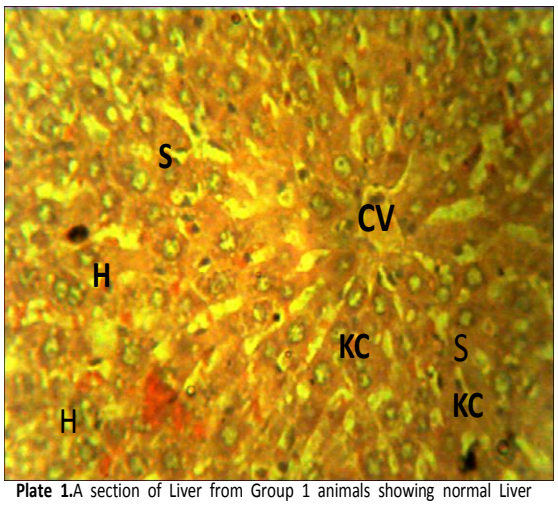


**Plate 1.**A section of Liver from Group 1 animals showing normal Liver architecture with CV- Central Vein; H-hepatic cells, S-Sinusoid; KC-Kupfer cell H & E Mg x250

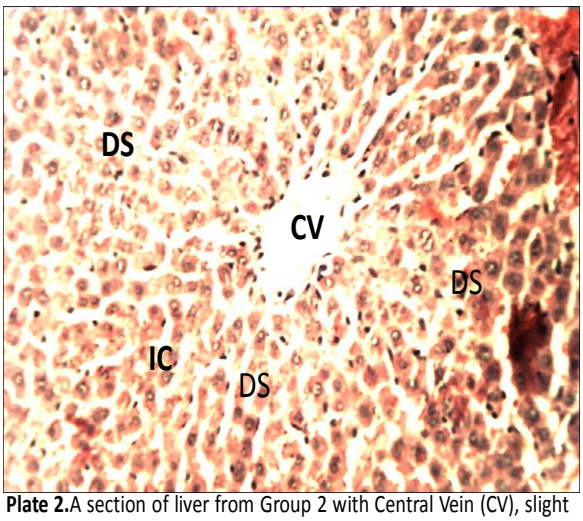


**Plate 2.**A section of liver from Group 2 with Central Vein (CV), slight distortion of sinusoids (DS) Few infiltrated Cells (IC) H & E, MgX 250

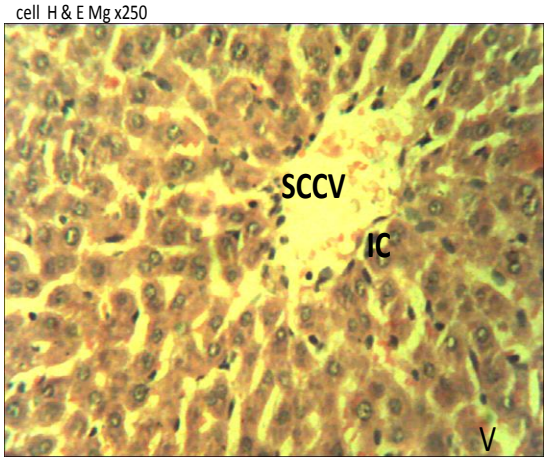


**Plate 3.**A section of a Liver from Group 3 animals with slight congested Central Vein (SCCV); Infiltrated Cells (IC) H& E Mg X 250

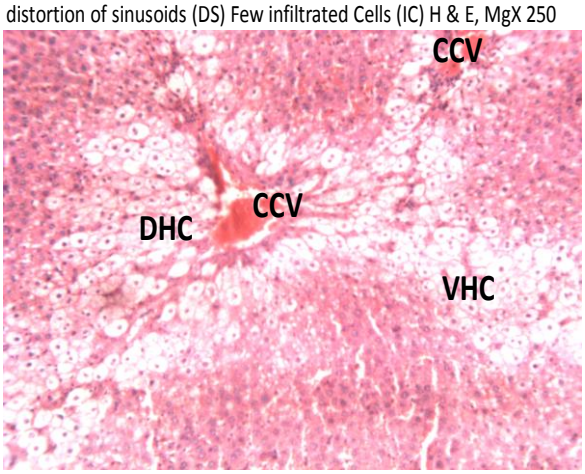


**Plate 4.**A section of Group 4 animals showing Congested Central Vein (CCV),Vacuolated Hepatic Cells (VHC); Degenerated Hepatic Cells (DHC) H& E Mg X 250

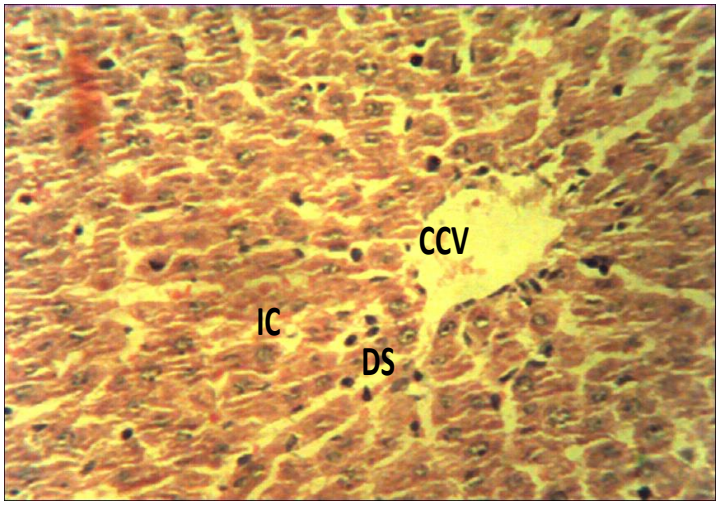


**Plate 5.**A section of the Liver from Group 5 animals showing slight Congested Central Vein (CCV), Distorted sinusoids (DS) and Slight infiltrated Cell (IC). H & E, Mg X250

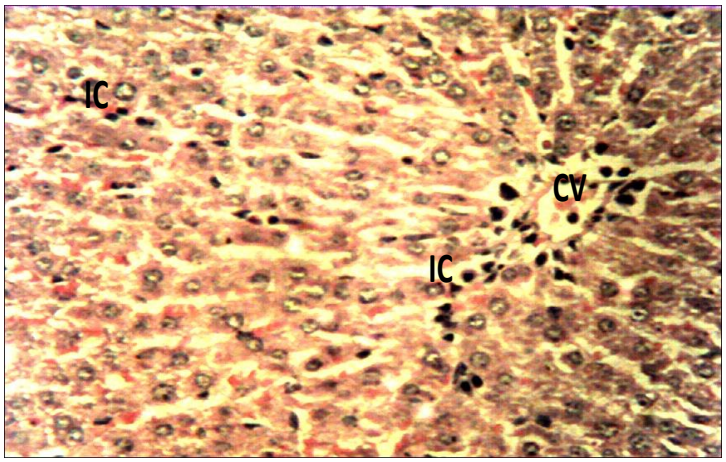


**Plate 6.**A section of the Liver of Group 6 animals showing normal histology of the Liver with Central Vein (CV),with some Infiltrated Cells (IC) H & E, Mg X250

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