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Original Research Article

Combination of Hydration and Ascorbic Acid Synergistically Prevents Organophosphate Pesticide Toxicity of Vital Organs in Experimental Rat Model

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ABSTRACT

Triazophos, an organophosphate acetylcholinesterase inhibitor and synthetic organic thiophosphate molecule, is employed as a pesticide. It is a yellowish-brown oily liquid, and exposure can happen through inhalation, ingestion, or touch. This study evaluated the toxic effects of organophosphate pesticide exposure on several vital organs, and the therapeutic potential of ascorbic acid **(**vitamin C) or water hydration alone or their combination. Random grouping of 25 adult male Wistar rats into five (A-E) was done. A-D Groups received pesticide (0.5mL/kg, i.p) for 3 days. B and D groups were treated with Vitamin C (200mg/kg, oral) only. Groups C and D received oral hydration with distilled water only (15mL/kg) for 3 days. Only pesticide was given to group A (negative control); while group E received no administration. The functions of the kidneys, liver, testicles and heart respectively were determined by measuring serum levels of urea**,** creatinine, $K^+$and Na^+; Alanine transaminase (ALT), Aspatate transaminase (AST), Alkaline phosphatase (ALP) And total bilirubin; testosterone; and lipid profiles (Total cholesterol (TC), Triglycerides (TG), and Low density lipoprotein cholesterol (LDL-C) and by histopathological examination of the kidneys, liver, testes and heart using standard methods. The pesticide exposure alone caused a marked toxicity to the vital organs. Vitamin C alone showed significant ameliorative effect on the organs ($p<0.05$). Distilled water alone indicated a non-significant difference improvement ($p>0.05$). Interestingly, there was very marked restoration after coadministration of vitamin C and distilled water ($p<0.05$ or $p<0.01$). The findings of histopathology matched the biochemical findings. The combination of water hydration and ascorbic acid synergistically protected these vital organs against toxic chemicals.

Key words: Organ toxicity, Organophosphate pesticides, Vitamin C, Water hydration

Introduction

The world produces and uses a large number of harmful chemical compounds. These compounds are important economically, but they also have the potential to contaminate the environment and/or injure human vital organs. Pesticides are harmful substances commonly used to control living organisms including disease causing organisms, unwanted plants and animals in a typical agricultural farming.[1]

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These chemical substances and their remnants in the soil have been linked with various health issues as a result of their toxicity.[2] Therefore, monitoring the amount in food crops by regulating pesticide use in an agricultural setting is key.[1]

Punch® is an organophosphate pesticide which consists of 35% Triazophos, 1% Deltamethrin and other heterogeneous compounds that share a common chemical structure. Just like other pesticides, it protects food crops from infestation with pathogens and vectors. It equally has deleterious side effects on health of humans who are accidentally exposed. According to studies, organophosphates stimulate the synthesis of reactive oxygen radicals capable of causing damage to vital organs including the testes.[3,4] Other researchers have noted that organophosphate pesticides inhibit acetylcholinesterase which is an enzyme that catalyzes the breakdown of acetylcholine (a neurotransmitter) causing an increased acetylcholine level.[5]

The use of pesticides has a significant economic impact. Pesticides have greatly enhanced the productivity of agricultural products over the years, but this does not rule out the possibility that they have harmful impacts on human health. The effects on various organs of the body, particularly the hepatorenal tissues, cardiovascular and reproductive systems, must be investigated in order to determine and

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ensure the safety of farmers, who are frequently exposed to pesticides. The aim of the research is to assess the effects of organophosphate pesticide (Punch®) on the kidneys, liver, heart and testes and to investigate for possible remedy using vitamin C or water hydration alone or their combination.

Materials and Methods

Chemicals and reagents

The organophosphate-containing chemical used in the study was Punch® (Gujarat Pesticides Ahmedabad, India) to serve as the toxicant (Figure 1). Distilled water was used for oral hydration. Vitamin C (Alpha Pharmaceuticals, Enugu, Nigeria) was employed as a treatment drug. Reagents: Serum testosterone assay ELISA kits (Elabscience, Texas, USA). The Urea, Creatinine, total cholesterol, triglyceride, HDL-C, ALT, ALP, AST and bilirubin laboratory kits from Randox Laboratory LTD, UK.

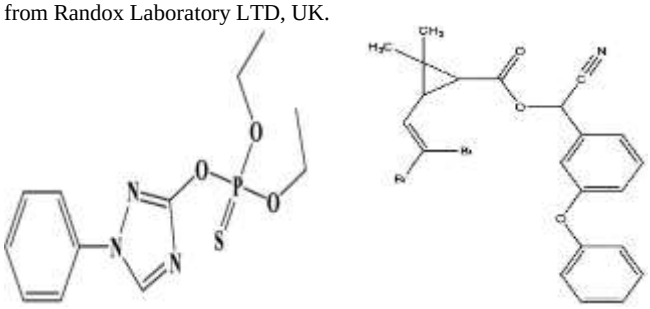

Figure 1: Active compounds in organophosphate pesticide, Punch® Triazophos, $C_{12}H_{16}N_3O_3PS$ Deltamethrin, $C_{22}H_{19}Br_2NO_3$

Punch® is an organophosphate pesticide which consists of 35% Triazophos, 1% Deltamethrin and other heterogeneous compounds that share a common chemical structure. Triazophos is an extensively used broad-spectrum insecticide, nematicide, and acaricide; nevertheless, because to its high toxicity and protracted half-life, it is prohibited in agriculture in Europe and other countries. Due to high residues and dietary risks, its use is prohibited in many crops, including fruits and vegetables. It was nevertheless frequently found to surpass the maximum residual limits in food and was nevertheless regularly discovered. Deltamethrin is found in numerous places, particularly in soil and water, and it can have a harmful effect on people and other living things. As a hydrophobic substance, deltamethrin has a low mobility in soils. This characteristic results in significant soil organic matter sorption and minimal groundwater leaching.

Animals

Twenty-five (25) matured Wistar rats weighing 60-70 g were collected from the animal house of the University of Nigeria's College of Veterinary Medicine. The animals were kept in a metal cages kept at animal house of the Department of Anatomy at a temperature of 22±3 ^{o}C and on a cycle of 12 hours of light and 12 hours of darkness. Commercial rat pellets were fed to them in sufficient quantities. To achieve acclimatization, for a period of 14 days' time, the animals were maintained under observation prior till the start of the experiment. The experimental procedure was approved by the institution's animal ethics committee at the University of Nigeria Teaching Hospital (UNTH/CSA. 712/VOL. 21).

Acute toxicity of Triazophos

The aim of the Chandra *et al*.[6] investigations was to determine the LD_{50} of the organophosphorus insecticide triazophos in Wistar rats. Experimental animals received a single oral dosage of triazophos dissolved in dimethyl sulfoxide (DMSO) at concentrations of 35, 55, 90, and 100 mg/kg, respectively. The triazophos in DMSO was determined to have an LD_{50} of 72.44 mg/kg.[6]

Experimental design

The twenty-five rats were divided into five groups (A-E). Before and after the experiment, the animals were weighed.

Group A: (Negative Control): received Punch® agrochemical (0.5 mL/kg, i.p) only for 3 days.
Group B: received co administration of Punch® and vitamin C (200 mg/kg, oral) for 3 days.
Group C: received distilled water (15 mL/kg, oral), waited for 30 minutes before receiving Punch® agrochemical, for 3 days.
Group D: received distilled water, waited for 30 minute before receiving co administration of Punch® and vitamin C, for 3 days.
Group E: (Normal Control): Did not receive any treatment.

Sample collection and analyses

Samples of blood were carefully collected from the heart's left ventricle under chloroform anesthesia for electrolyte, urea, creatinine, total bilirubin, total cholesterol, triglyceride, HDL-C, ALP, AST, and ALT determination, and Histological investigations were performed on excised kidneys, liver, heart, and testes.

Biochemical analysis

For renal function was evaluated by measuring the levels of electrolytes, urea, and creatinine in the blood: serum K^+ and Na^+ were assessed using a Perlong Medical PL1000A Electrolyte Analyzer. The diacetylmonoxime procedure with protein precipitation, as reported by Natelson *et al*.[7], was used to determine serum urea concentration, and the Jaffe Reaction, as described by Fabiny and Ertingshausen[8], was used to estimate serum creatinine concentration. The colorimetric approach described by Reitman and Frankel[9] was used to assess serum ALT and AST activity for liver function tests. The colorimetric method for assessing ALP activity was developed by Kind and King[10]. Total bilirubin was measured using the colorimetric method published by Malloy and Evelyn[11]. Testosterone determination was by ELISA technique as described by Zhang *et al*.[12] The cholesterol oxidase method was used to determine total cholesterol (TC) as described by Fredrickson *et al*.[13], the precipitation method was used to estimate HDL-C,[14] and the glycerol phosphate oxidase method was used to calculate triglyceride (TG).[15]

Histopathological analysis

The removed testicles, liver, kidneys, and heart were sectioned at 5 microns, embedded in paraffin wax, and stained using the Hematoxylin and Eosin method. An Olympus TM light microscope was used to examine the histological sections.[16]

Statistical analysis

The data was analyzed using GraphPad prism version 7.0. The results of the biochemical assays were presented as Mean ± SEM. To establish the degree of significance, ANOVA was utilized, followed by a Tukey post hoc analysis. It was considered significant for probability level less than 0.05 ($p<0.05$).

Results and Discussion

Effects of treatments against Punch® agrochemical on body weight of wister rats

Effects of treatments with Punch® agrochemical on the body weight of Wistar rats are shown in Figure 1. It was noted that rats in the Punch® agrochemical group had increased appetite, although they were lethargic and took a long time to respond to stimuli. In comparison to the other groups, the Punch® agrochemical group (negative control) had the highest mean increase in body weight.

Biochemical results

The function of the kidneys was assessed by evaluating the biochemical markers at different levels in the blood, such as creatinine, blood urea nitrogen (BUN), potassium (K^+), and sodium (Na^+) (Table 1). From the results, combination of water and vitamin C and/or vitamin C alone showed significant nephroprotective effects ($P< 0.05$) when compared to negative control (punch agrochemical only). In addition, it was discovered that the combination of hydration (water) and ascorbic acid (vitamin C) demonstrated substantially stronger nephroprotective effects than either ascorbic acid (vitamin C alone) or hydration (water only).

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Estimating the blood level of liver biochemical markers was used to determine the liver's functioning: alanine transminase (ALT), aspartate transminase (AST), alkaline phosphatae (ALP) and total bilirubin (TB) (Table 2). The results indicate that combination of water and vitamin C and/or vitamin C alone showed significant hepatoprotective effects (P< 0.05) when compared to negative control (punch pesticide only). In addition, it was discovered that the combination of hydration (water) and ascorbic acid (vitamin C) demonstrated substantially stronger hepatoprotective effects than either ascorbic acid (vitamin C alone) or hydration (water only). Although its specific function in shielding cells from oxidative stress is unclear, vitamin C is a well-known antioxidant. The protection offered to the tissues against the toxic pesticide could be due to its antioxidant property. The enhance protection observed in the presence of hydration could be as a result of the water-soluble nature of vitamin C; hence its increased delivery to sites for tissue protection. The testis' functioning was determined by estimating the serum level of testosterone concentrations in the various experimental groups (Table 3). Based on the findings, oral administration of distilled water. Vitamin C alone showed a non-significant restoration of serum testosterone levels (P > 0.05) in comparison with negative control (punch pesticide only). It was also noted that the combination of hydration (water) and ascorbic acid (vitamin C) showed much better restoration of testosterone levels in the rats than either ascorbic acid (vitamin C alone) or hydration (water only).

The levels of total cholesterol (TC), triglycerides (TG), and high-density lipoprotein-cholesterol (HDL-C) in the different experimental groups are shown in Table 4. Based on the findings, combination of water and vitamin C and/or vitamin C alone showed significant antidylipidaemic effects (P < 0.05 or P < 0.05) when compared to negative control (punch pesticide only). It was also discovered that the combination of hydration(water) and ascorbic acid (vitamin C) showed much better antidylipidaemic effects in the rats than either ascorbic acid (vitamin C alone) or hydration (water only).

Table 1: Effects of Punch® on the kidneys of different experimental animal groups

Groups	BUN (mg/dl)	Creatinine (mg/dl)	K^+ (mmol/l)	Na^+ (mmol/l)
A- Punch® only	37.51 ± 4.12	1.43 ± 0.09	9.01 ± 0.02	134.23 ± 2.37
B- Punch® +Vit C	22.91 ± 3.72*	1.01± 0.17	7.15 ± 0.21	138.18 ± 0.23
C- Water +Punch®	27.37 ± 3.26	1.28 ± 0.24	8.70 ± 0.18	135.09 ± 0.19
D- Water +Punch® +Vit C	22.82 ± 1.08*	0.91± 0.29*	6.04 ± 0.19*	141.99 ± 0.26*
E- Normal Control	20.65 ± 1.41*	0.89 ± 0.17*	5.75 ± 0.07*	143.60 ± 1.31*

Values are Mean ± SEM. *$P < 0.05$ is significant when (Punch only) is compared with all other groups.

Table 2: Effects of Punch® on the liver of different experimental animal groups

Groups	ALT (IU/L)	AST (IU/L)	ALP (IU/L)	TB (mg/dl)
C- Punch® only	28.45 ± 1.94	41.23 ± 5.41	395.71 ± 27.67	1.29 ± 0.31
D- Punch® +Vit C	23.73 ± 3.91	28.21 ± 5.41*	272.71 ± 35.65*	1.18 ± 0.20
C- Water +Punch®	33.92 ± 3.46	32.8 ± 7.27	290.92 ± 35.45	1.09 ± 0.54
D- Water +Punch® +Vit C	22.53 ± 2.83*	25.61± 2.06*	251.82 ± 24.19*	0.99 ± 0.27*
E- Normal Control	22.42 ± 2.73*	24.65 ± 4.57*	231.42 ± 29.28*	0.95 ± 0.23*

Values are Mean ± SEM. *$P < 0.05$ is significant when (Punch only) is compared with all other groups.

Table 3: Effects of Punch® on the testosterone levels

Groups	Serum testoterone ng/mL
E- Punch only	1.68 ± 0.15
F- Punch +Vit C	2.65 ± 0.31
C- Water +Punch	1.86 ± 0.37
D - Water +Punch +Vit C	2.72 ± 0.22*
E- Normal Control	2.90 ± 0.17*

Values are Mean ± SEM. *p < 0.05 is significant when (Punch only) is compared with all other groups

Histopathological results

Normal control rats' renal nephrons (Group E) looked functionally and anatomically normal. The nephrons had a well-preserved shape, with typical glomeruli and tubules. The nephron of Punch® agrochemical group (negative control, group A) showed Some constricted glomeruli, there was an area of significant inflammation. Moreover, in the nephrons of Vitamin C-treated rats (group B), the glomeruli and the tubules appeared normal. However, there was interstitial cellular infiltration, while in the rats administered with water only before administering with Punch (group C), the glomeruli were enlarged and there wass mild interstitial cellular infiltration; the tubules show features of autolytic degeneration. In addition, a photomicrograph of a kidney segment taken from Water + Punch + Vitamin C (group D), showed normal glomeruli and normal tubules (Plate 1).

Normal control rats (Group E) had an anatomically and functionally normal liver. The morphology of the hepatocytes in the liver was retained. The liver of Punch® agrochemical group (negative control, group A) showed moderately normal hepatocytes appearance; there is significant periportal inflammation. Moreover, the hepatocytes of Vitamin C-treated rats (group B) appeared normal, however the central veins appeared congested; sinusoidal cellular infiltration was also seen. While in the rats administered with water only before administering with Punch (group C), The hepatocytes are in good condition. The venules of the portal arteries look dilated and occluded. Furthermore, photomicrograph of liver section from Water + Punch + Vitamin C (group D), showed normal hepatocytes, central vein (CV) appeared dilated and there was mild periportal cellular infiltration and focal inflammation (Plate 2).

Normal control rats' testes (Group E) looked to be anatomically and functionally normal. The seminiferous tubules appear normal with normal distribution of germ cells in the germinal epithelia and mature spermatids in the lumen. The testis of Punch® agrochemical group (negative control, group A) showed dilated appearance of seminiferous tubules and marked reduction of germ cell population in the germinal epithelia. Moreover, majority of the seminiferous tubules of Vitamin C-treated rats (group B) appear normal with normal distribution of germ cells in the germinal epithelia and mature spermatids in the lumen while some have mildly eroded germinal epithelia. While in the rats administered with water only before

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administering with Punch (group C), majority of the seminiferous tubules (*) appear normal while some have eroded germinal epithelia. Furthermore, in the photomicrograph of testis section from Water + Punch + Vitamin C treated rats (group D), the seminiferous tubules appear normal with normal distribution of germ cells in the germinal epithelia and mature spermatids seen in the lumen (Plate 3).

Normal control rats' hearts (Group E) looked to be anatomically and functionally normal. The morphology of the cardiac fibres was largely preserved. The heart of Punch® agrochemical group (negative control, group A) showed abnormal changes; transverse section shows an area of necrotic myocardial fibres with mononuclear cellular infiltration. Moreover, the cardiac fibres of Vitamin C-treated rats (group B) have normal histological features. While in the rats administered with water only before administering with Punch (group C), transverse section of heart shows some degenerated fibres with loss of cellular and nuclear detail. In addition, a photomicrograph of a heart section taken from Water + Punch + Vitamin C (group D), the appearance of cardiac fibers was normal. (Plate 4).

Humans are exposed to various potentially toxic agents in their natural and occupational environments. Because of its concentrating and excretory functions, the kidney is extremely sensitive to the effects of environmental pollutants.[17-19] The specific etiology and processes of environmentally induced kidney damage remain a challenge that will require collaboration from a variety of scientific areas.[20] Researchers are challenged with the seemingly unlimited variety of toxins, their reciprocal interaction, the body's handling/metabolization, methods of exposure, and so on. Identifying, mechanistically unraveling, and combating environmental toxin–induced diseases require interdisciplinary efforts and perseverance.[21]

The liver, as the principal site of xenobotic metabolism, is susceptible to chemical/toxins-induced injury. The liver plays a prominent role in the bioactivation and detoxification of xenobiotics. Liver injury arises when these toxic chemicals overwhelm the protective defenses of the liver.[22,23] Acute liver injury is a condition which shows the rapid deterioration of liver functions due to the alterations in the liver biochemical markers, and this occurs as a rapid damage to the liver.[24] When the liver is exposed to toxic doses of some xenobiotics/chemical toxins, adverse reactions lead to the liver injury.[25]

The testes are vital organs of reproduction in males and thus should be protected from harmful substances that could interfere with this biologic function.[26] The testicular toxicity of toxic chemicals has been reported.[27-29]

Pesticides are widely utilized in agriculture, which is the most common occupation in developing nations, according to.[30] Pesticides are frequently used by farmers, usually at higher quantities than are recommended.[31] Despite the use of such a vast number of insecticides, pests alone are predicted to cause a 10-30% loss.[32] Organophosphates are irreversible cholinesterase inhibitors that disrupt glucose, lipid, and protein metabolism;[33] pesticide toxicity may be attributed to its stress-inducing action.[34] The aim of this study is to investigate the effect of the Punch® pesticide (an organophosphate) on selected vital organs, and the therapeutic potential of vitamin C or water hydration alone or in combination.

Table 4: Effects of Punch® on the serum lipid profile

Groups	Serum TC, mmol/l	Serum HDL-C, mmol/l	Serum TG, mmol/l
G- Punch only	6.147 ± 0.337	1.427 ± 0.101	2.605 ± 0.319
H- Punch +Vit C	5.316 ± 0.198*	2.349 ± 0.111*	1.649 ± 0.021
C- Water +Punch	5.863 ± 0.075	1.405 ± 0.091	2.078 ± 0.403
D- Water +Punch +Vit C	4.906 ± 0.083**	2.206 ± 0.120*	1.503 ± 0.102*
E- Normal Control	4.50 ± 0.131**	2.248 ± 0.115*	1.491 ± 0.136*

Values are Mean ± SEM. *p < 0.05 or **p < 0.01 is significant when (Punch only) is compared with all other groups

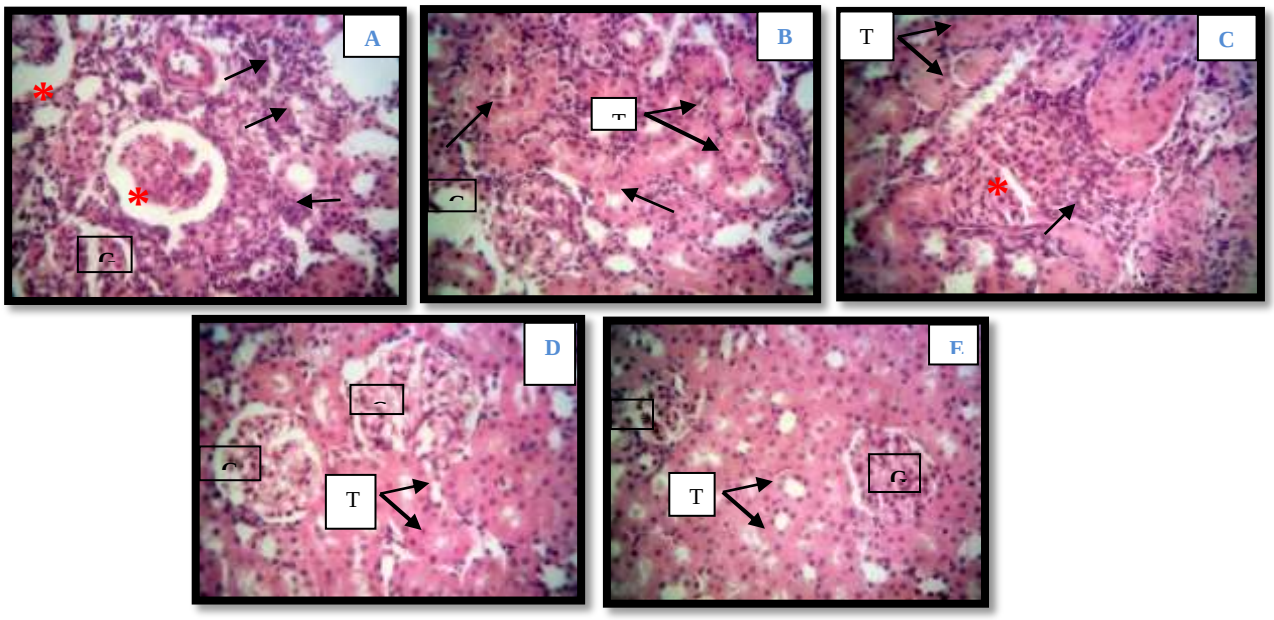

Plate 1: Photomicrograph of kidney section **A)** Punch®-treated, negative control group. Feature: Some glomeruli are constricted (*), there is an area of inflammation (arrows). **B)** Punch® + Vitamin C-treated, negative control group. Features: The glomeruli (G) and tubules (T) appear to be in good condition. There is interstitial cellular infiltration (arrows). **C)** Water + Punch®-treated, negative control group. Features: The glomeruli are enlarged (*) and there is mild interstitial cellular infiltration (arrow). The tubules (T) show features of autolytic degeneration. **D)** Water + Punch® + Vitamin C-treated rates group. Feature: The glomeruli (G) and tubules (T) appear normal. **E)** Normal control group. Feature: The glomeruli (G) and tubules (T) look to be in good condition. Stain: Haematoxylin and eosin. Magnification: X100.

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Organophosphate-induced tissue injury can be caused by acute or chronic exposure to excess organophosphate, which most often results in oxidative stress that is mitigated by antioxidants, as well as alterations in organ function and morphology.[34] Organophosphates can be absorbed through the skin, ingested, or inhaled. They attach to the enzyme acetylcholinesterase in red cells and render it inactive once ingested. The key feature is acetylcholinesterase (AChE) inhibition, which is present in both the central nervous and peripheral systems and plays an important role in the breakdown of the neurotransmitter acetylcholine. They phosphorylate the enzyme's serine hydroxyl group, rendering it inactive, resulting in acetylcholine buildup and receptor overstimulation.[35]

Several studies have confirmed the liver tissue as the principal target organ in organophosphate toxicity studies. Although, the mechanism of action in the liver and metabolism is still unknown, organophosphates disrupt the antioxidant defense system in the liver, causing oxidative stress and metabolic, biochemical, ultrastructural, and mitochondrial damage, as evidenced by changes in hepatic biomarkers and histomorphology.[36-39] From the results of the current study, we observed that intraperitoneal administration to rats with Punch® only (0.5 m/kg) for 3 days caused significant increase ($p< 0.05$) in the levels of the serum enzyme markers of liver damage (ALT, AST, ALP and total bilirubin levels). This finding is suggestive of hepatic injury; and agrees with the findings in the study by Karami-Mohajeri *et al*.[40] which reported on the adverse effect of organophosphorus pesticides in the liver. The rise in serum concentration of tissue liver enzymes maybe attributed to the leakage of cytosolic enzymes from the hepatocytes due to the oxidative stress imposed on them by the organophosphates through the generation of free radicals and oxygen reactive species.[41] The treatment with water hydration alone against the organophosphate showed a non-significant decrease in the levels of serum enzymes. There is scant and inconsistent information about the role of water in tissue function. It is well recognized that chronic tissue dehydration can lead to damage and dysfunction; as a result, maintaining tissue moisture may not have been enough to provide adequate defense against hazardous pesticides.

Water may have increased the harmful pesticide's hepatic metabolism and renal removal from the system, but this may not have provided much protection from the pesticide.

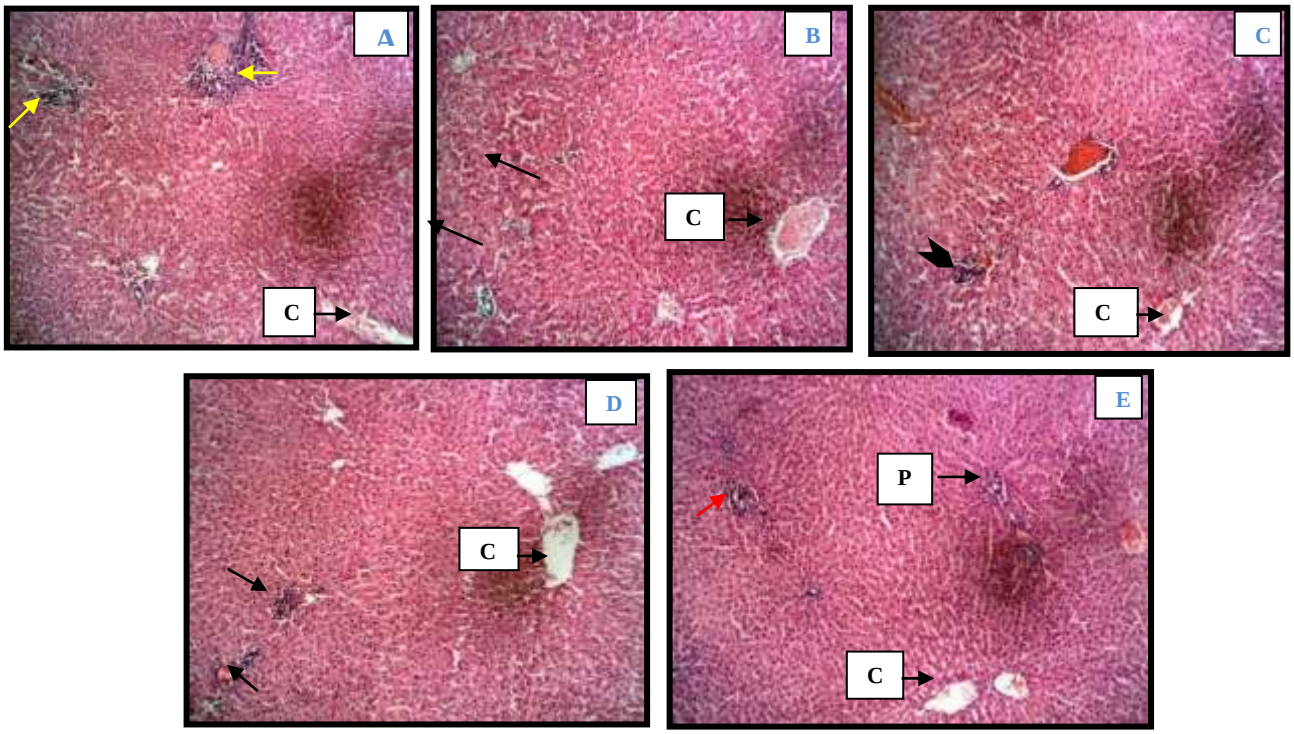

Plate 2: Photomicrograph of liver section**A**) Punch®-treated, negative control group. Features: Hepatocytes are moderately normal; there is significant periportal inflammation (arrows). CV– central vein. **B**) Punch® + Vitamin C-treated, negative control group (B). Features: The hepatocytes are normal. The central veins (CV) are congested; sinusoidal cellular infiltration is also seen (arrows). **C**) Water + Punch®-treated, negative control group. Features: Hepatocytes appear to be in good condition. The portal venule is dilated and congested (arrow). A small area of inflammation is seen (black arrow head). **D**) Water + Punch® + Vitamin C-treated rats. Feature: Hepatocytes appear to be in good condition, central vein (CV) appears dilated and there is mild periportal cellular infiltration (red arrow) and focal inflammation (black arrow). **E**) Normal control group. Feature: Hepatocytes are normal; an area of focal inflammation is seen (arrow). CV- central vein; PT –portal triad. Stain: Haematoxylin and eosin. Magnification: X100.

The treatment with vitamin C only against the organophosphate showed a significant antihepatotoxic effect. Interestingly, we observed that the combined treatment with water (hydration) and ascorbic acid (vitamin C) against the organophosphate showed a much better hepetoprotective effect in the rat than that of ascorbic acid (vitamin c alone) or hydration (water only). This was observed in all the vital organs studied. The broadly tissue protective effects observed could be due to the antioxidant property of ascorbic acid in scavenging of the free radicals and reactive oxygen species produced by the organophosphates, thereby ameliorating the oxidative stress on the liver, kidney, heart and the testes tissues. Vitamin C is a water-soluble antioxidant with a powerful reducing ability (high redox potential), together with glutathione, nicotinamide adenine dinucleotide phosphate (NADP) to protect the cells from oxidation damage via scavenging reactive oxygen species. As such, the hydration of the rats by the administration of distilled water enables a better absorption and availability of vitamin C (a water soluble vitamin) in the tissues, thereby, giving a higher antioxidant effect by reduction of the oxidative stress levels in the tissues studied.[42] Also, there was significant weight gain after three days of intraperitoneal administration of Punch® when the post-weight was compared with the pre-weight. This could be due to increased appetite in the rats, since organophosphate are lipophilic; binding to adipose tissues which are potential site of toxicant accumulation.[43,44] This binding is believed to inhibit the production of leptin by the adipose tissue.

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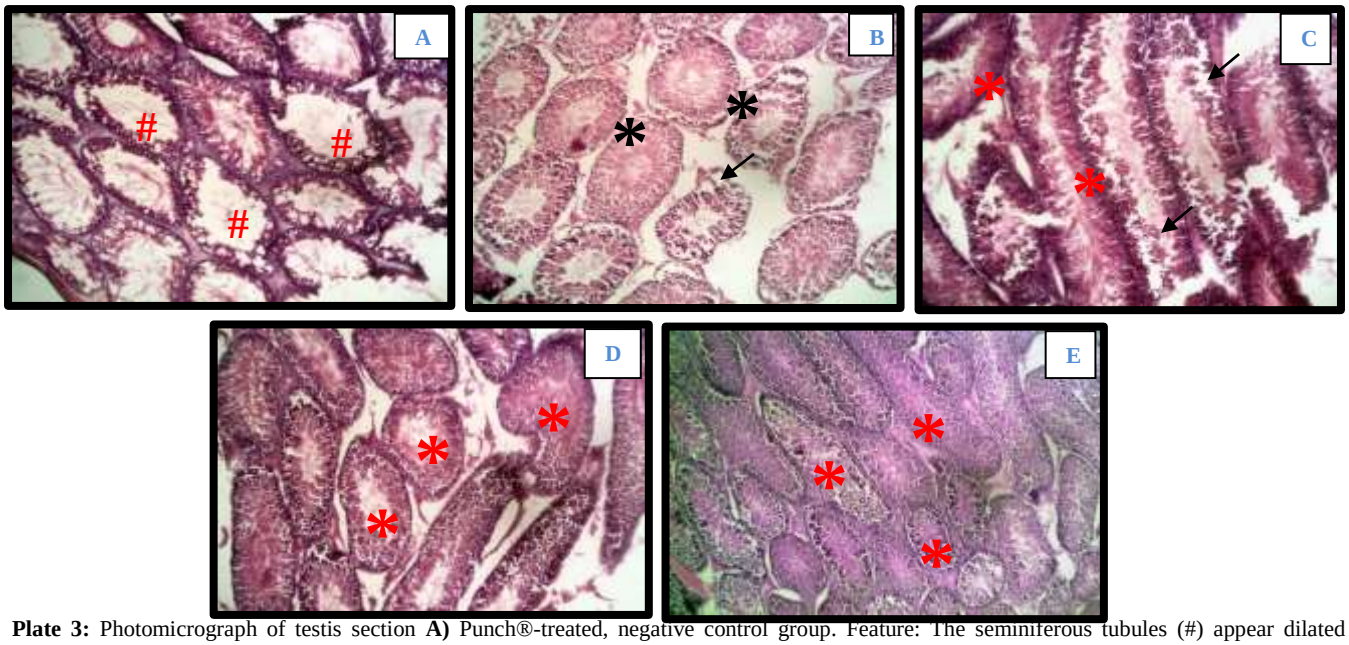

Plate 3: Photomicrograph of testis section **A)** Punch®-treated, negative control group. Feature: The seminiferous tubules (#) appear dilated with marked reduction of germ cell population in the germinal epithelia. **B)** Punch® + Vitamin C-treated, negative control group (B). Features: Majority of the seminiferous tubules (*) appear normal with normal distribution of germ cells in the germinal epithelia and mature spermatids in the lumen while some have mildly eroded germinal epithelia (arrow). **C)** Water + Punch®-treated, negative control group. Features: Majority of the seminiferous tubules (*) appear normal while some have eroded germinal epithelia (arrow). **D)** Water + Punch® + Vitamin C-treated rats. Feature: The seminiferous tubules (*) appear normal with normal distribution of germ cells in the germinal epithelia and mature spermatids in the lumen. **E)** A section of the testis from a normal control group was photographed under the microscope. Feature: The seminiferous tubules (*) appear normal with normal distribution of germ cells in the germinal epithelia and mature spermatids in the lumen. Stain: Haematoxylin and eosin. Magnification: X100. **AS**

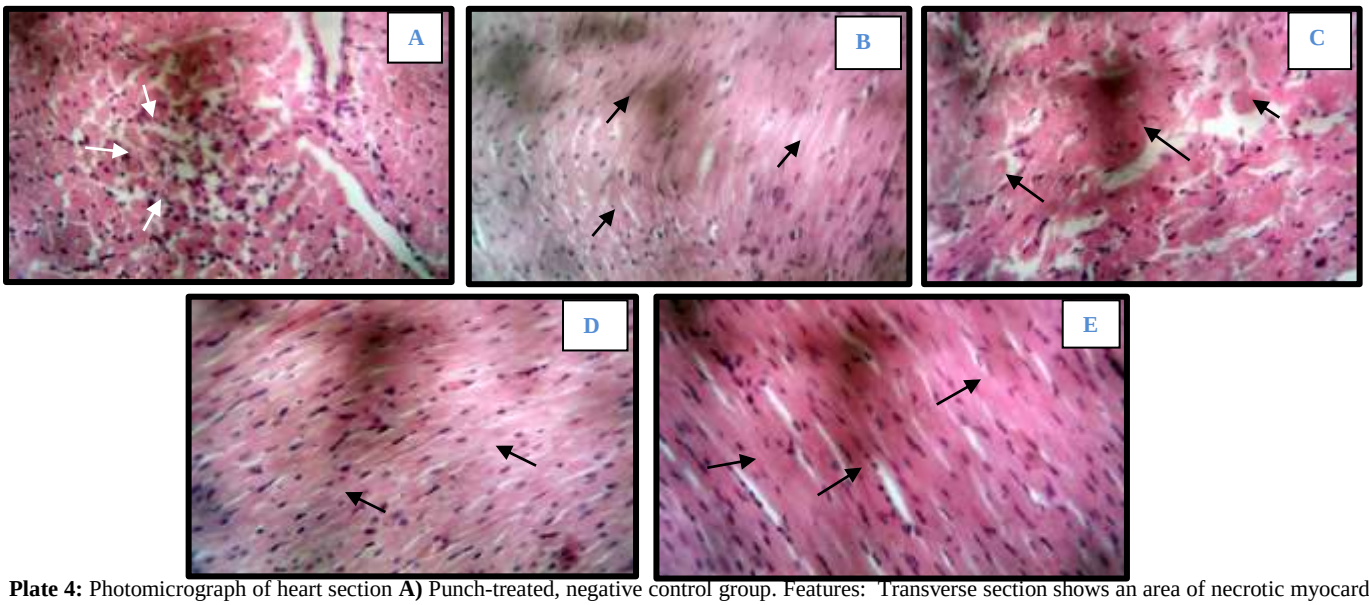

Plate 4: Photomicrograph of heart section **A)** Punch-treated, negative control group. Features: Transverse section shows an area of necrotic myocardial fibres with mononuclear cellular infiltration (arrows). **B)** Punch + Vitamin C-treated, negative control group. Features: The myocardial fibres have normal histological features (arrows). **C)** Water + Punch-treated, negative control group. Features: Transverse section of heart showing some degenerated fibres with loss of cellular and nuclear detail (arrow). **D)** Water + Punch + Vitamin C-treated rates. Feature: The myocardial fibres have normal histological features (arrows). **E)** Normal control group. Feature: The myocardial fibres have normal histological features (arrows). Stain: Haematoxylin and eosin. Magnification: X100.

Leptin acts by inhibiting hunger: The decreased level of leptin hormone causes increase in hunger and appetite. The accumulation of organophosphates in the adipose tissue depot suggests a disruption in their function which can promote abnormal weight gain.[45,46] The sluggish movement by the rats could be attributed to overabsorption of acetylcholine caused by the organophosphates' suppression of acetylcholinesterase.[47]

Conclusion

Organophosphate pesticides caused damage to key organs in rats and significantly altered the histoarchitecture of those organs, according to biochemical and histological results. This suggests that they may also cause harm to humans. The use of vitamin C and appropriate hydration can lessen or even reverse these effects.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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