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BIOCHEMICAL ASSESSMENT OF SELENIUM AND VITAMIN E CO-ADMINISTRATION ON MALATHION SUBACUTE TOXICITY ON MALE WISTAR RATS

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ABSTRACT

The need to alleviate hunger globally has deliberately introduced the use of pesticides such as organophosphates (Ops), which are highly and widely adopted, agriculturally. This study was delineated to evaluate coadministration of selenium and α-tocopherol on malathion induced toxicity. Twenty-five (25) male Wistar rats (weighing between 220g - 250g) were sub-divided into 5 groups of 5 rats each. Group I (control) received 0.5 ml of Carboxyl Methyl Cellulose (CMC) as placebo once daily for 30 days. Once daily and for 30days, rats in Group II, III, IV and V were exposed orally to1/50 of LD_{50} value (27 mg/kg b.wt) malathion dissolved in 0.5 ml CMC. With the exception of Group II which got toxicant alone, Groups III and IV were treated with selenium as sodium selenite (Na_2SeO_4) (0.1 mg Se/kg b. wt.) and α-tocopherol (100 mg α-tocopherol/kg b.wt.) respectively, while in the same manner and same duration rats in Group V were cotreated with selenium (0.1 mg Se/kg b. wt.) and α-tocopherol (100 mg α-tocopherol/kg b.wt.). Body weight change as decreased by malathion exposure was significantly improved upon selenium and α-tocopherol administration. Nevertheless, cotreatment with selenium and α-tocopherol administration further increased weight reduction by the toxicant than other groups. Malathion caused a significant ($p<0.05$) increase in serum ALT, ALP, AST when compared with control. This elevation indicated a remarkable decrease upon coadministration with selenium and α-tocopherol and was supported by histological examination. Interestingly, the effect of selenium or α-tocopherol on lipid profile showed no significant ($p>0.05$) difference as against the toxicant

group, yet cotreatment of selenium and α-tocopherol revealed significantly ($p<0.05$) a decreased LDL, TAG, Total cholesterol with an elevated HDL. Conclusively this study demonstrated that coadministration of selenium with α-tocopherol was able to attenuate the induced toxicity as generated by malathion pesticide most effectively than selenium and α-tocopherol alone.

Key words: Malathion (organophosphates), Selenium, α-tocopherol, lipid profile, serum Biomarkers

INTRODUCTION

Local farming or crude agricultural operations is an unavoidable, inevitable and integral avenue for sustainability and economic growth in developing nations like Nigeria. Such operations have graduated from the crude methods of ancient farming to semi-improved systems that culminate into the use of pesticide or insecticides to obtain high crop productivity and yield. These pesticides are highly toxigenic to biosystems, hence, generating debilitating health consequences as it relates their disposition to both occupational and environmental conditions [1, 2]. The myriads of environmental toxicants like malathion have received reviews in scientific documentations for deleterious effects on the liver, kidneys, brain and haematopoiesis [1, 2]. Malathion has its classification as an organophosphate (OP) agent, which is utilized as an industrial chemical and agriculturally, as an insecticide [3]. Primarily, malathion base pesticide is highly neurotoxic with neurosymptoms associated with the peripheral and central nervous system (CNS) [4, 5]. Several literatures have revealed that the major mechanism of malathion (OP) toxicity in brain cells, is by acetylcholinesterase (AChE) inhibition [6, 7]. This inhibitory mechanism causes acetylcholine accumulation in the synaptic cleft ensuing activation of nicotinic, cholinergic, muscarinic receptors [4].

In developing nations where agricultural practices are rampant, indiscriminately and crudely performed, the consequences of malathion toxicity on the biotic and abiotic lives is abysmal. Studies have demonstrated myriads of complications such as haematological deterioration, renal and hepatic dysfunctions associated with pesticide discharge and exposure [7, 8]. Furthermore, related symptoms with malathion toxicity include: eye and skin irritation, diarrhoea, profuse

sweating, cramps, nausea, seizures and even death [9, 10]. Malathion, upon absorption undergoes biotransformation into malaoxon, a product which is substantially and materially more detrimental with biotoxicity estimated to be about 61 times higher than malathion [11].

Postulations from toxicological studies have connected Ops with oxidative stress via reactive oxygen species formation (ROS) [12, 13]. Op toxicity as it relates oxidative stress is based on their "redox cycling" activity as well as alteration in the normal antioxidant homeostasis that culminates in antioxidant depletion [14, 15].

Micronutrients are required in minute amounts and are essential for certain metabolic reactions occurring in animals and plants. Selenium is fundamentally a trace element nutrient acting as a cofactor/prosthetic group in many antioxidant enzyme reactions [16-18]. Such cascade of antioxidant system of which selenium performs its radical quenching and scavenging principle includes iodothyronine deiodinase (IDD), glutathione peroxidase (GPx) and thioredoxin reductase (TrxR) [19]. In addition, unusual amino acids such as selenomethionine and selenocysteine have selenium as a prosthetic component of the holoprotein thereby enhancing their functionality in metabolic reactions [20]. By analytical procedures, the organic and inorganic forms of selenium, functions essentially in the elimination of sugar molecules (forming selenosugars) from the body by phase II reaction [[21, 22].

Although some experimental studies with rodents have revealed the propensity of selenium supplementation alone exhibiting chemo-preventive principles for some categories of cancer, [23], HIV/AIDS [24], diabetes [25], heart disease, hypothyroidism, and a weakened immune system [16, 26] , other related studies have reported the uncertainty of selenium deficiency on health especially with diseases like Kashin-Beck disease [27]. Further postulations show that the efficacy of selenium treatment is associated with its interaction with other minerals like zinc and copper or vitamins like vitamin E [28]. The essentiality of selenium as a cofactor in most antioxidant cascade system cannot be over flogged; however, its functionality becomes overwhelmed under additional stress such as heavy metal exposure e.g. mercury or oxidative stress from α-tocopherol deficiency. It is on this hypothesis that this study attempts to evaluate

malathion subacute toxicity in animal model and similarly, the coadministration of both selenium and vitamin E in the alleviation of some malathion related biochemical disturbances.

MATERIALS AND METHODS

Experimental Animals: Twenty-five (25) inbred male Wistar rats (weighing 220g - 250g) at the Animal Unit of Basic Medical Science, Delta State University, Abraka, Nigeria, were experimental subjects used for the study. At 28 ±5°C, a 12-hour light & dark cycle was maintained and concomitantly the animals were fed with standard rat chows (Top Feed, Nigeria Ltd) *ad libitum*. The animals had access to H_2O while allowed to acclimatize for 10 days before the experiment.

Ethical Consideration: Ethical regulation was followed sequel to the national and institutional guidelines as stipulated for laboratory animal protection and safety during experiments. Approval to proceed with experimental protocol was endorsed by the Faculty of Basic Medical Sciences, Delta State University ethical committee.

Preparation of Reagents and Chemicals

Malathion was product of Fischer- Chemie Co. Ltd, Germany while the sodium selenite used was a product of Nice Chemicals Ltd, India. All other chemicals were of analytical grade.

- **Carboxyl-Methyl-Cellulose (CMC) Buffer:** Carboxyl-Methyl-Cellulose 0.5% was prepared by dissolving 0.5g of CMC into 100ml of distilled water.
- **Sodium Selenite (Na_2SeO_3) Preparation:** 0.0001g of Sodium selenite was weighed and dissolved in 1 ml of 0.5% CMC solution to yield 0.1mg/kg of Selenium solution.
- **Malathion:** The malathion was prepared by dissolving 0.0027mg in 1 ml of 0.5% CMC solution and the animals exposed to 1/50 of LD_{50} (27mg/ kg of bodyweight).
- **α- tocopherol preparation:** The α-tocopherol was prepared by diluting 0.1g in 1 ml of 0.5% CMC solution to get the appropriate concentration that was administered to the animals.

Study design and Protocol

The rats were allotted into 5 groups consisting of 5 animals each. Group I served as normal control, which received 0.5 ml of CMC as placebo once daily for 30 days. Once a day rats in Group II were given an oral dose of malathion 1/50 of LD_{50} value (27 mg/kg of body weight) dissolved in 0.5 ml CMC. Group III rats were treated with selenium as sodium selenite (Na_2SeO_4) (0.1 mg/kg b.wt) dissolved in water and 30 min later, malathion dissolved in CMC (27mg/kg body weight) was administrated to this group. In the same manner and same duration animals in group IV were given α-tocopherol dissolved in CMC (100 mg/kg b.wt) and malathion dissolved in CMC (27 mg/kg body weight) 30 minutes later. Once daily, Group V rats was cotreated with selenium (0.1 mg Se/kg b. wt.) and α-tocopherol (100 mg α-tocopherol/kg b.wt.) with 27 mg/kg body weight malathion dissolved in CMC which ensued 30 minutes later.

Collection of Specimen

Twenty-four (24) hours after the last treatment, the rats were placed under ether anaesthesia and whole blood collected via the retroorbital venous plexus into plain bottles. The rats were dissected, the liver tissues were cautiously excised and rinsed in some portions of ice-cold formyl saline for microstructural examination. At a speed of 3500 rmp for 10 min, the whole blood sample was separated using macro centrifuge. With the aid of Pasteur pipette the clear serum supernatant was obtained and further subjected to biochemical and haematological analysis.

Clinical Chemistry Assays

Serum supernatant was used to conducted the following biochemical assay; Alkaline phosphatase (ALP) as described by Tietz and Shuey [29], aspartate aminotransferase (AST) by Bergmeyer *et al.*, [30], alanine aminotransferase (ALT) method of Klauke *et al.*, [31]. Total serum triglyceride was assayed by peroxidase couple method [32], total serum cholesterol was measured by the Allain enzymatic method [33]. High density lipoprotein (HDL) cholesterol was determined after precipitation of LDL, VLDL and chylomicrons using the $MgCl_2$ and dextran sulphate. The LDL-cholesterol concentration was calculated from the Friedewald formula, [34]. The K^+

concentration was calculated using the standard calibration method of Tietz, [35]. Bicarbonate (HCO_3^{-}) and Chloride (CI^-) anions were measured as described, respectively by Tietz [35, 36] andSchales and Schales [37]. Flame photometry was adopted to estimate plasma sodium (N^+), potassium (K^+) and phosphate (PO_4^{2-}) ions.

Microstructural Examination

Malathion toxicity and selenium ameliorative ability on tissue architecture was evaluated by histological studies. The isolated organs were submerged in 10% formalin and inserted into an automatic tissue processor for 24 hours. Thereafter the sample was processed by molten wax solidification and sectioned with an automatic tissue sectioner. Tissue sectioning utilized Hematoxylin and Eosin (H & E) stains before fixed on slides. Fixation of stained slides with mountant was allowed to dry and microscopically viewed (Microscope CXSZ – 1075N, Techmel & Techmel USA).

Statistical Analysis

Experimental data were statistically represented as Mean±SD with associated significance were placed as $p<0.05$. A One-way ANOVA alongside Dunnett's post-test was adopted using GraphPad Prism version 4.00 for Windows, GraphPad Software, (San Diego California USA, www.graphpad.com).

RESULTS AND DISCUSSION

Biological systems have been challenged directly or indirectly by myriads of pollutants arising from pesticides of organophosphate origin. Residues from malathion (one of the extensively utilized OP insecticides) after utilization, are biosynthetically incorporated into water bodies and food chains contaminating these biosystems as well as destabilizing their micro flora [38]. In recent times, contamination of water and food has become a worrisome trend in Nigeria, as both terrestrial and aquatic health are in jeopardy as a result of biotransformation of some of these xenobiotics into more hydrophobic active molecules and oxidative stress emanating from these pollutants.

Myriads of case-controlled research have associated insecticides such as malathion to parenteral exposure culminating into diverse metabolic disorders. This current investigation entails the oral administration of malathion at dose 1/50 of LD_{50} i.e. 27 mg/kg body weight/day dissolved in 0.5ml CMC for 60 days. Each exposure occasion, generated behavioral responses like hypersensitivity which ensued 5-10 minutes of malathion dose delivery. Aside hypersensitivity, other physically observed signs are decreased feeding and elevated H_2O intake, mild eye discharge, red nose with lethargy. However, no animal mortality was recorded. This connotes that the dose introduced in this study was tolerable for the subjects and above lethal dose. Related study demonstrates that oral LD_{50} for malathion in male rats as 1350mg/kg [39].

Body weight changes and liver weight were examined in other to evaluate toxicity impact. It was deciphered that liver and body weight changes were significantly lowered ($p<0.05$) upon malathion treatment alone when compared with the control group and selenium coadministered group (Table 1). Previous studies recounts that the brain and liver tissues are target tissues for OP toxicity [39, 40]. Weight reduction is a physical manifestation of oxidative stress generated by prolong malathion toxicity thus enhancing an imbalance of the antioxidant/ ROS ratio [41]. Decrease in food and fluid consumption might as well be a contributory effect on the drastic reduction in weight as indicated with malathion administration in the tissues being measured. The toxicity generated as consequences of malathion treatment appears to outweigh the effects of selenium and vitamin E treatment alone in groups II and III respectively. This was expressed by its non-significant ($p>0.05$) changes in weight when compared with the toxicant group alone (Group II). The co-administration of selenium and α-tocopherol reflected positively in the physical signs as stated above for malathion administration alone. Result from group V was statistically significant ($p<0.05$) when compared with the toxicant treatment alone by restoring body and liver relative weights to near control values. The body weight was alleviated to a near normal as rats in this group showed no eye discharge with bulging of eyes, no red nose, nor lethargy but showed appreciable food and water intake.

In the assessment of malathion toxicity on liver damage, the determination of liver biomarker enzymes levels in serum such as ALT and AST was adopted. As it relates membrane disruption

or necrosis and as makers of hepatic damage, these enzymes are discharged into circulation and therefore measured in the serum. Results from table 2 depicted significant increase ($p<0.05$) in serum ALT, AST and ALP upon malathion treatment alone when compared with the control. The outrageously high level of serum ALT, AST, and ALP as observed, can be ascribed to the dysfunction and damage of toxicants on hepatic histostructure [42]. Studies have deduced and elucidated that malathion potentially cause necrosis or damage of hepatic membrane via ROS generation hence releasing liver associated enzymes into the circulatory system [43, 44].

Selenium and vitamin E coadministration decreased the serum levels of ALT, AST and ALP to near normal values probably by stabilization of membrane integrity. Revelations from histological microscopy tend to buttress biochemical findings (Plate 3). Selenium which is an integra component of glutathione peroxidase, facilitated peroxyl radical reduction by permitting more α-tocopherol [45-47]. As observed with other malathion treated groups (groups III and IV) with respect to the liver enzymes, no significant ($p>0.05$) difference was indicated with respect to selenium and α-tocopherol alone treatment.

Alterations in biochemical parameters were detected in serum total cholesterol, triacylglycerol, HDL and LDL cholesterol concentration as depicted in Table 3 upon the toxicant introduction. With the exception of HDL cholesterol (which was decreased significantly ($p<0.05$), malathion caused a marked increase in serum TAG, LDL, and total cholesterol which was significantly ($p<0.05$) different from the control (Table 3). The assessment of lipid profile in the serum is predicated on the fact that malathion exhibit rapid asymmetrical transmembrane uptake by the liver making it a potential target for malathion toxicity [48]. Since malathion is lipophilic its incorporation into the lipid bilayer of the cell makes it possible to affect cell architecture and its composition [49]. Furthermore, the liver is the main site of lipoprotein synthesis, any damage to hepatic tissue might disrupt or destabilize lipid profile. The aftermaths of selenium administration alone or coadministered with α-tocopherol from this study did not ameliorate malathion induced lipid profile alteration, as the value obtained when compared with the toxicant group alone was not statistically significant ($p>0.05$). Foregoing studies have indicated alteration in lipid profile, which might consequentially arise from TAG accumulation, bile acid inhibition

from cholesterol synthesis which is synthesized in the liver or derived from plasma lipids, leading to increase in cholesterol levels [50]. It seems presumably that malathion toxicity might have overwhelmed effect of selenium and α-tocopherol treatment alone as values obtained for TAG, HDL, LDL and T-cholesterol were statistically not significant (0>0.05). Upon co-treatment of selenium with α-tocopherol, an ameliorative trend was statistically visible. This corroborates and aligns with studies indicating a change is hyperlipidemia upon selenium administration [51]. Total cholesterol, TAG, and LDL were dropped to near normal while HDL was maintained at normal level which might imply bile acid normalization.

No striking alteration was also captured in serum electrolytes and represented in Table 4. Although Blood urea nitrogen (BUN) was abnormally higher in malathion treated groups, the administration of selenium and vitamin alone did not impact significantly upon malathion induced toxicity. Furthermore, the short experimental time frame might possibly be a factor culminating to the statistically unnoticeable changes in the serum electrolyte.

Table 1: Effect of selenium and vitamin E cotreatment on malathion induced toxicity on body weight

Groups and design of treatment	Initial body weight	Final weight (g)	Change body weight	Liver weight (g)	Relative Liver weight
Control (I)	139.19±4.60	153.21±5.79	9.15±1.62	1.60±0.029	1.88±0.23
Malathion alone (II)	137.63±7.74	141.33±9.14	2.61±2.86[a]	1.56±0.013[a]	2.96±0.13[a]
Malathion + Selenium (III)	138.25±6.88	142.97±5.31	3.37±2.00[a]	1.51±0.017	2.77 ±0.22
Malathion + Vitamin E (IV)	137.15 ±5.87	145.50 ±5.22	5.85 ±4.69 [ab]	1.54 ±0.026	1.99 ±0.12
Malathion + Selenium + Vitamin E (V)	138.88 ±6.14	150.71 ±9.57	7.85 ±3.28[b]	1.61 ±0.077	1.93 ±0.06

Values are represented as Mean ± SD (n =5). Values with superscript ([a])depict malathion treated group p<0.05 from control group, Superscript ([b]) indicates administered group p<0.05 when compared with toxicant group alone. ([ab]) superscript indicates treated group statistically different as compared with both the control and toxicant groups.

Table 2: Effect of selenium and vitamin E coadministration on malathion induced toxicity on liver biomarkers in serum

Groups and design oftreatment	ALT (IUL^{1})	AST (IUL^{1})	ALP (IUL^{1})
Control (I)	62.40±3.60	115.21±5.70	156.53±5.62
Malathion alone (II)	144.33±2.74[a]	239.33±6.14[a]	321.20±7.86[a]
Malathion + Selenium (III)	85.25±2.88[ab]	173.07±4.41[ab]	264.00±7.00[ab]
Malathion + Vitamin E (IV)	60.15 ±7.80[b]	187.50 ±3.22[ab]	209.15±9.63[ab]
Malathion + Selenium + Vitamin E (V)	61.66 ±5.11[b]	139.11 ±2.55[b]	136.16 ±6.20[b]

Values are represented as Mean ± SD (n =5). Values with superscript ([a])depicts malathion treated group $p<0.05$ from control group, Superscript ([b]) indicates administered group $p<0.05$ when compared with toxicant group alone. ([ab]) superscript indicates treated group statistically different as compared with both the control and toxicant groups.

Table 3: The effect of selenium and vitamin E cotreatment on malathion induced toxicity on serum lipid profile

Groups and design of treatment	Total cholesterol (mdL^{1})	Triglyceride ($mgdL^{1}$)	HDL cholesterol ($mgdL^{1}$)	LDL cholesterol ($mgdL^{1}$)
Control (I)	92.40±5.50	61.21±5.79	56.53±3.62	13.33±0.29
Malathion alone (II)	144.13±3.74[a]	69.33±6.14[a]	37.73±5.86[a]	19.92±0.13
Malathion + Selenium (III)	145.28±3.87[a]	66.00±5.51[a]	40.00±5.03[a]	17.92±0.17
Malathion + Vitamin E (IV)	145.45 ±7.87[a]	67.50 ±5.82[a]	39.55 ±7.10[a]	18.64 ±0.26
Malathion + Selenium + Vitamin E (V)	101.09 ±5.14[a]	63.11 ±6.52[a]	52.16 ±5.28[a]	17.08 ±0.10

Values are represented as Mean ± SD (n =5). Values with superscript ([a])depicts malathion treated group $p<0.05$ from control group, Superscript ([b]) indicates administered group $p<0.05$ when compared with toxicant group alone. ([ab]) superscript indicates treated group statistically different as compared with both the control and toxicant groups.

Table 4: The effect of selenium and vitamin E cotreatment on malathion induced toxicity on serum electrolyte

Groups and design of treatment	Na^+(mmolL^1)	K^+(mmolL^1)	Cl(mmolL^1)	HCO_3 (mmolL^1)	BUN (mgdL^1)
Control (I)	137.00±4.43	5.14±0.71	103.03±2.59	18.33±2.29	59.88±4.77
Malathion alone (II)	134.50±2.38[a]	5.35±0.17	101.00±1.26	19.13±1.21	78.34±8.63[a]
Malathion + Selenium (III)	135.15±4.99[a]	4.00±0.11	103.07±1.09	18.22±1.11	76.01±5.48[a]
Malathion + Vitamin E (IV)	131.10 ±1.11[a]	4.90 ±0.22	103.15 ±2.19	20.64 ±1.26	66.40±7.55[ab]
Malathion + Selenium + Vitamin E (V)	133.09 ±3.11[a]	4.14 ±0.55	105.16 ±2.22	19.25 ±2.20	68.00±3.39[ab]

Values are represented as Mean ± SD (n =5). Values with superscript ([a])depicts malathion treated group $p<0.05$ from control group, Superscript ([b]) indicates administered group $p<0.05$ when compared with toxicant group alone. ([ab]) superscript indicates treated group statistically different as compared with both the control and toxicant groups.

Histological Assessment

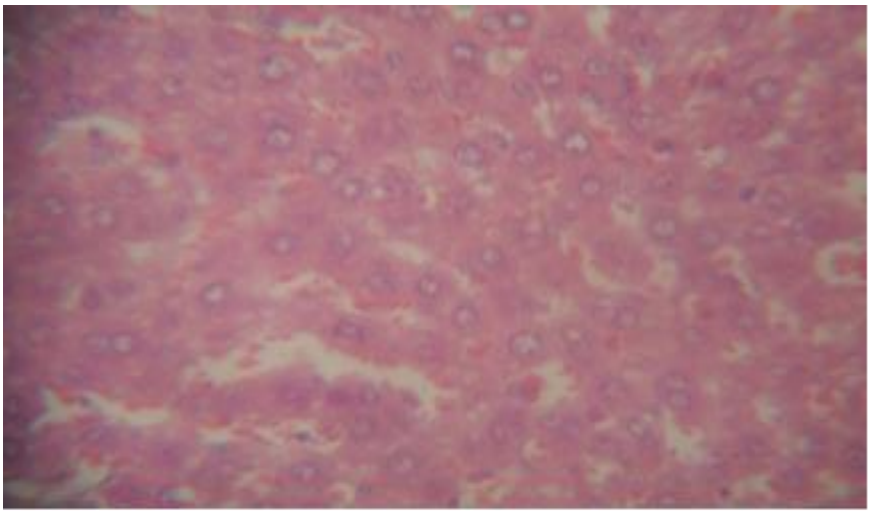

Plate 1 (Control group): Distinct liver cells with centeral vein in centre hepatic tubule. Nucleus appearance is coarse with observable distinct polyhedral arrangement of cells. (H and E) ×10 magnification.

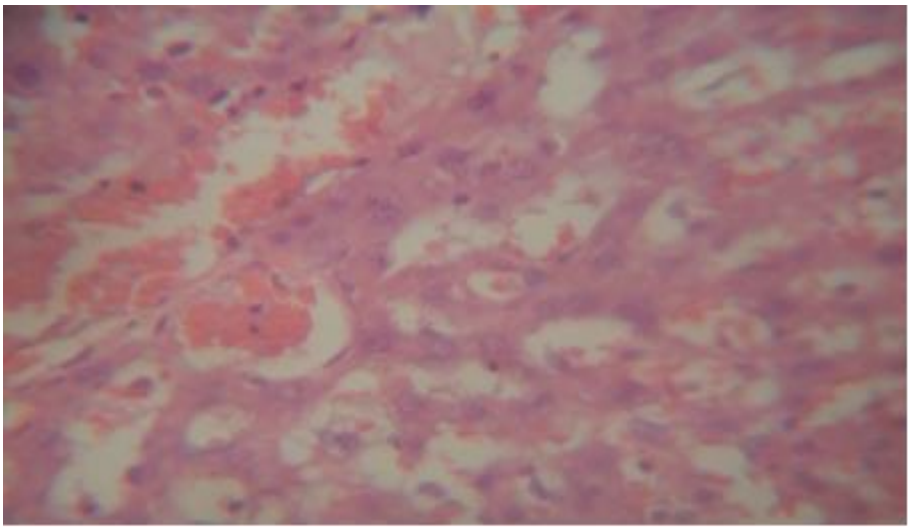

Plate 2 (Malathion alone): Hepatocyte depicts an eosinophilic background, separated vascular channels sinusoid withbrown lipofuscin granules. Deranged arrangement when compared to control slide. The nucleus appears large and tri-tetra nucleated. (H and E) ×10 magnification.

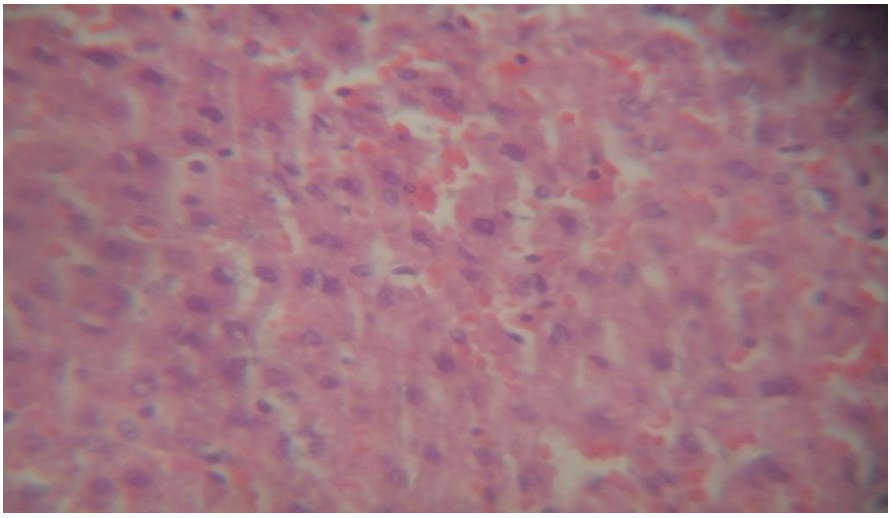

Plate 3 (Malathion + Selenium + Vitamin E): Eosinophilic appearance with mild granulation. At higher magnification, some nucleus appears vacuolated and slightly coarse with mild ballading of hepatocytes (H and E) ×10 magnification.

CONCLUSION

In this current work, we demonstrated the effectiveness of combined treatment of selenium and Vitamin E at a particular dose as against selenium only and Vitamin E alone over hepatic injury by malathion. By this application of malathion induced hepatotoxicity, we demonstrated that coadministration of selenium at a dose of 0.1 mg Se/kg b. wt. and vitamin E at a dose of 100 mg α-tocopherol/kg b.wt, significantly decreased ($p<0.05$) ALT, AST and ALP concentration. Empirical evidence derived from biochemical assay and histological examination of this research, suggest that selenium alone may not be potent or efficacious enough in curbing malathion toxicity at the dose employed in this study. In other words, the potency of selenium alone is dose dependent. However, selenium in combination with Vitamin E might provide more invigorating, attenuating and protective principles on hepatocytes against oxidative stress and subacute toxicity of malathion in rats.

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