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Ameliorative effects of Vitamin E and Selenium on relative organ weights and histopathology of growing pigs fed graded levels of crude oil contaminated diets

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ABSTRACT: Crude oil contamination of feed resources can induce organomegaly, organ atrophy, and histopathological lesions in livestock due to the bioaccumulation of polycyclic aromatic hydrocarbons in metabolically active organs. This study evaluated the ameliorative effects of vitamin E alone and in combination with selenium on relative organ weights and histopathology of growing pigs previously exposed to graded levels of crude oil–contaminated diets. Thirty-six crossbred pigs (Large White × Landrace; 18 males and 18 females; 15.8 ± 1.4 kg; 10–12 weeks old) were initially exposed to 0, 5, 10, or 15 g Bonny Light crude oil/kg feed for four weeks, followed by a four-week amelioration phase in a 4 × 3 factorial completely randomized design comprising basal diet (no treatment), vitamin E (200 mg/kg), and vitamin E plus selenium (200 mg vitamin E + 5 mg selenium/kg). Relative organ weights (liver, kidney, heart, and spleen) were measured after slaughter, and histopathological evaluation of selected organs was conducted using standard haematoxylin and eosin staining. Data were analysed using factorial ANOVA with Tukey's HSD at $p < 0.05$. Pigs exposed to 15 g/kg crude oil without treatment showed significantly increased relative liver (3.2 g/100 g) and spleen (2.0 g/100 g) weights compared to control values (2.6 and 1.4 g/100 g, respectively), indicating hepatomegaly and splenomegaly associated with fatty degeneration and inflammatory infiltration. Histological findings revealed cytoplasmic vacuolation, vascular dilation, and periportal inflammation in the liver, while heart and spleen tissues remained largely normal. Vitamin E alone partially restored organ weights and reduced tissue damage, particularly at lower contamination levels, whereas combined vitamin E and selenium achieved near-complete restoration of organ weights and markedly improved histological architecture even at higher contamination levels. These results suggest that vitamin E is effective up to moderate contamination levels, while its combination with selenium provides superior ameliorative effects against crude oil–induced toxicity in growing pigs.

Keywords: Crude oil, histopathology, organ weights, vitamin E, selenium.

Abbreviations: PCV, packed cell volume; **Hb,** haemoglobin; **RBC,** red blood cell; **WBC,** white blood cell; **EDTA,** ethylenediaminetetraacetic acid; **NRC,** National Research Council; **ROW,** relative organ weight; **H&E,** haematoxylin and eosin; **SEM,** standard error of mean; **GSH-Px,** glutathione peroxidase; **MDA,** malondialdehyde, **ROS,** reactive oxygen species; **PAHs,** polycyclic aromatic hydrocarbons.

INTRODUCTION

Relative organ weights and histopathology are considered the gold standard and most sensitive indicators of chemically induced toxicity in risk assessment, as changes in organ weight reflect adaptive or pathological responses to xenobiotics, with responses observed in 84% of rodent and 65% of dog studies (Heywood, 1981; Sellers *et al*., 2007). Unlike performance and haematological parameters that are reversible within days, alterations in liver, k idney,

heart and spleen weights and microscopic architecture indicate cumulative bioaccumulation of lipophilic toxicants in fatty tissues and target organ damage (Peters and Boyd, 1966; Moles and Rice, 1983). In the Niger Delta, where crude oil contains 50–97% hydrocarbons, 6–10% nitrogen, oxygen, sulphur and <1% metals (Speight, 2014) and over 7,000 spill incidents have been reported (UNDP, 2006; Kadafa, 2012; UNEP, 2022), pigs ingesting contaminated forage face risks of hepatomegaly, nephrotoxicity and splenic alterations that precede clinical disease.

The liver is the primary site of biotransformation of petroleum hydrocarbons via cytochrome P450 to reactive metabolites that increase liver protein and lipid levels, causing fatty degeneration, smooth endoplasmic reticulum proliferation, and hepatocellular hypertrophy (Carter, 1983; Patrick-Iwuanyanwu *et al*., 2011; Heywood, 1981). Previous studies in rabbits (Berepubo *et al*., 1994; George and Sese, 2012a), poultry (Nwokolo *et al*., 1984; Onunkwo *et al*., 2013), goats (Ngodigha *et al*., 1999) and rats (Ikanone *et al*., 2017) demonstrated a linear increase in relative liver weight with crude oil contamination, attributed to greater specific metabolic rate and lipid accumulation. For example, George and Sese (2012b) observed mild periportal lymphocytic infiltration at 10 ml/kg crude oil and widening of sinusoids and cytoplasmic vacuolation at 20 ml/kg

In rabbit liver, previous observations reported mild hyperemia at low concentrations progressing to rupture of dilated vessels and haemorrhage at high concentrations. In contrast, kidney weight may show atrophy due to inadequate blood nutrient supply from heart or hypertrophy due to increased excretory load, as Ngodigha *et al*. (1999) reported linear decrease in spleen, heart, lung and kidney and linear increase in liver in goats with increasing crude oil, while Akporhuarho (2011) reported depressive effect on heart and liver in cockerels and Nodu and Ohimain (2014) atrophy of liver and kidney in rabbits.

Histopathology provides definitive evidence of organ injury beyond weight changes. Cytoplasmic vacuolation indicates hydropic degeneration due to Na+/K+ ATPase failure and intracellular oedema, dilated sinusoids reflect impaired hepatic microcirculation and portal hypertension, and periportal inflammation indicates infiltration of lymphocytes, histiocytes and neutrophils as an immune response to necrotic hepatocytes (Sunmonu and Oloyede, 2007; Ikanone *et al*., 2017). Heart and spleen are less susceptible due to lower metabolic activation, often showing no obvious histologic change even at 15 g/kg, as cardiac muscle has a high antioxidant reserve and the spleen has efficient clearance of damaged erythrocytes (Hartung and Hunt, 1966). However, chronic high-level exposure may cause adrenal cortical hyperplasia, fatty liver, necrosis and gastrointestinal irritation, emphasising the need for early amelioration.

Antioxidant amelioration of organ weight changes and histopathology requires both membrane and cytosolic protection. Vitamin E (α-tocopherol), a lipid-soluble chain-breaking antioxidant in cell membranes, scavenges peroxyl radicals, prevents lipid peroxidation of hepatocyte membranes, and maintains permeability (Traber and Stevens, 2011; Shastak and Pelletier, 2025). Selenium, as an essential cofactor of glutathione peroxidase, detoxifies H2O2 and organic peroxides in the cytosol, preserving membrane integrity and preventing enzyme leakage (Rotruck *et al*., 1973; Tapiero *et al*., 2003; Elblehi *et al*., 2015). Their synergy has been shown in rats where vitamin E + selenium restored normal histological structure of liver after cypermethrin toxicity (Elblehi *et al*., 2015), in rabbits where vitamin E confers protection on hepatocytes (Achuba and Awhin, 2009; Ghazi Harsini *et al*., 2012; Bharrhan *et al*., 2010), and in broilers where combined supplementation alleviated oxidative stress (Sahin and Kucuk, 2001; Sahin *et al*., 2002; Ghazi Harsini *et al*., 2012). Yet comprehensive data on growing pigs, specifically evaluating both relative organ weights and histopathology across graded crude oil histories using factorial analysis, are scarce. The present study was therefore designed to evaluate comparative ameliorative efficacy of vitamin E alone (200 mg/kg) versus vitamin E plus selenium (200 mg + 5 mg/kg) on relative organ weights and histopathology of liver, kidney, heart and spleen across histories of 0, 5, 10 and up to 15 g/kg, with total duration 8 weeks.

MATERIALS AND METHODS

Ethical approval

All animal handling and humane slaughter were approved by the Animal Ethics Committee, Rivers State University, Port Harcourt (RSU/AEC/2021/015) per NIH Guide, ARRIVE 2.0 and AVMA Guidelines for Euthanasia.

Experimental location and total duration

The study was conducted at the Piggery Unit of the Teaching and Research Farm, Federal College of Education (Technical), Omoku, Rivers State, Nigeria. The study area is located at approximately latitude 4°47′N and longitude 6°59′E and lies within the humid tropical zone. The area receives an average annual rainfall of approximately 2,300 mm, with an ambient temperature ranging from 26 to 32°C and relative humidity of 70–90%. The experimental period lasted 8 weeks (56 days), comprising a 4-week contamination phase followed by a 4-week amelioration phase. Prior to the commencement of the experiment, the animals underwent a 7-day acclimatisation period, resulting in a total duration of 63 days spent at the experimental facility.

Source of crude oil and justification for graded levels

Bonny Light crude oil (API 35.4°) from Nigerian AGIP Oil

Company, Obrikom, was weathered for 24 hours in a shallow pan under a shed (27±2°C) to simulate environmental exposure (Ovuru and Ekweozor, 2004). Mixed with basal diet via mechanical mixer (10 min). Graded levels 0, 5, 10 and up to 15 g/kg were informed by: (i) Environmental relevance: 3.2–18.7 g/kg in Niger Delta feeds (UNEP, 2022); (ii) Dose-response: previous studies (Ngodigha *et al*., 1999; George and Sese, 2012a) demonstrated dose-dependent organ-weight changes without mortality. (iii) Toxicity classification: 5 g/kg low chronic subclinical, 10 g/kg moderate, 15 g/kg high acute causing hepatomegaly and histopathological lesions (George and Sese, 2012b; Ikanone *et al*., 2017). (iv) Palatability: >20 g/kg caused >40% refusal; 25 g/kg was rejected after 4 days in pilot, hence up to 15 g/kg upper limit.

Experimental animals, housing and management

Thirty-six clinically healthy crossbred (Large White × Landrace) growing pigs, comprising 18 males and 18 females aged 10–12 weeks with an average body weight of 15.8 ± 1.4 kg, were sourced from Cape Farms, Irete, Imo State. The animals were housed in a conventional open-sided tropical pig house with a concrete floor sloped at 5% to facilitate drainage. Each pig was kept individually in a pen measuring 2.0 m × 1.2 m × 1.0 m, fitted with iron bar partitions, a 0.4 m long concrete feeding trough, and a nipple drinker. The building was roofed with corrugated aluminium sheets at a height of 3.5 m and maintained under natural ventilation with a 12-hour light and 12-hour dark cycle. Ambient temperature and relative humidity were monitored using a hygro-thermometer, and biosecurity was maintained through the use of foot dips containing 1:100 Izal disinfectant at the entrance. Each pig served as a replicate and was housed individually throughout the study. Management practices included feeding twice daily at 08:00 h and 15:00 h, provision of water ad libitum, daily washing of pens, and weekly disinfection using 2% Virkon®. Animals were observed daily for health status, body weights were recorded weekly, and strict biosecurity measures were enforced by restricting visitor access. All pigs were handled gently and consistently by the same personnel to minimise stress.

Pre-experimental veterinary care and acclimatisation

All pigs underwent a 7-day acclimatisation period during which they were fed a basal diet. On day 1, each animal received an intramuscular injection of Terramycin LA® at a dosage of 20 mg/kg body weight. On day 2, Ivomec® 1% was administered subcutaneously at a dose of 0.3 mg/kg body weight. On day 3, the pigs were treated against ectoparasites using Butox® (deltamethrin) applied as a spray at a concentration of 1 mL per litre of water. Additionally, from days 1 to 3, Vitalyte® was administered in drinking water at a concentration of 1 g/L to support hydration and recovery. Faecal samples were examined using the flotation technique, and blood smears were prepared and evaluated for haemoparasites. Only animals that tested negative and were confirmed to be clinically healthy were selected and enrolled in the experiment.

Table 1. Gross and chemical composition of basal diet (as-fed basis).

Ingredient	Percentage (%)
Maize	50.0
Soybean meal (44% CP)	20.0
Palm kernel cake	10.0
Wheat offal	10.0
Fish meal (72% CP)	3.0
Bone meal	2.5
Limestone	1.5
Salt (NaCl)	0.25
Vitamin-mineral premix	0.25
L-Lysine	0.25
DL-Methionine	0.25
Total	100.0
Calculated Chemical Composition	
Crude Protein (%)	18.20
ME (kcal/kg)	2950

Premix per kg: Vit A 10,000 IU, D3 2,000 IU, E 20 IU, K3 2 mg, B1 1.5 mg, B2 4 mg, B6 3 mg, B12 0.02 mg, Niacin 20 mg, Pantothenic acid 8 mg, Folic acid 0.8 mg, Biotin 0.05 mg, Choline 250 mg, Mn 80 mg, Zn 50 mg, Fe 60 mg, Cu 10 mg, I 1 mg, Co 0.2 mg, Se 0.1 mg basal.

Experimental diets

The basal diet was formulated in accordance with National Research Council (2012) recommendations for growing pigs (20–50 kg). The diet comprised maize (50%), soybean meal (20%), palm kernel cake (10%), wheat offal (10%), fish meal (3%), bone meal (2.5%), limestone (1.5%), salt (0.25%), vitamin–mineral premix (0.25%), lysine (0.25%), and methionine (0.25%), giving a total of 100%. The calculated nutrient composition of the basal diet was 18.2% crude protein, 2,950 kcal/kg metabolizable energy, 4.5% crude fibre, 3.8% ether extract, 0.85% calcium, and 0.45% available phosphorus (Table 1). Three amelioration diets were prepared: (i) the basal di*et al*one, (ii) the basal diet supplemented with 200 mg/kg vitamin E (DL-α-tocopheryl acetate), and (iii) the basal diet supplemented with 200 mg/kg vitamin E and 5 mg/kg selenium (as sodium selenite). These inclusion levels were based on supranutritional dosages reported in previous studies (Shastak and Pelletier, 2025; Reinoso-Maset *et al*., 2023). All diets were mixed biweekly to ensure uniformity and freshness.

Experimental design

After the contamination phase, the experiment was

arranged as a 4 × 3 factorial in a Completely Randomised Design (CRD). Factor A was crude oil contamination history at four levels (0, 5, 10, and 15 g/kg), while Factor B was the amelioration treatment at three levels (no supplementation, vitamin E alone, and vitamin E plus selenium). This resulted in 12 treatment combinations: T1–T3 (0 g/kg × 3 amelioration levels), T4–T6 (5 g/kg × 3), T7–T9 (10 g/kg × 3), and T10– T12 (15 g/kg × 3). A total of 36 animals were used, with three replicates per treatment and one animal per replicate, as pigs were housed individually. Allocation of animals to treatments was carried out by randomisation using a lottery method. The design was corrected from a one-way arrangement to a factorial structure.

Relative organ weight determination

At the end of the amelioration phase (day 28), three pigs were randomly selected from each treatment group (one per replicate) and weighed individually using a platform scale (100 kg capacity, 100 g sensitivity; Camry) to obtain their final
body weights. The pigs were then humanely slaughtered by restraining them, severing the carotid vein with a sharp knife, and allowing complete bleeding, after which evisceration was carried out. The internal organs of interest (liver, kidneys, heart, and spleen) were carefully excised following a standard dissection procedure, trimmed of adhering fat and connective tissues, and weighed using a Camry Electronic Kitchen Scale (Model EK3052; 5 kg capacity, 1 g sensitivity). Relative organ weight (ROW) was calculated using the formula: ROW (g/100 g body weight) = (absolute organ weight/body weight) × 100. All measurements were taken in triplicate, and the mean values were used for analysis. All weighing instruments were calibrated prior to use with standard weights to ensure accuracy.

Histopathological examination

Tissue samples from the liver, heart, and spleen were collected immediately after organ weighing, cut into approximately 1 cm^3 pieces, and fixed in labelled vials containing 10% buffered formaldehyde solution (pH 7.2) for 72 hours. The fixed samples were then transported to the pathology laboratory of the University of Port Harcourt Teaching Hospital (UPTH), Port Harcourt, for processing. Thereafter, the tissues were dehydrated through ascending grades of ethanol (70%, 90%, and 100%), cleared in xylene, and embedded in paraffin wax. Sections of 5 µm thickness were cut using a rotary microtome (Leica RM2125), mounted on glass slides, and stained with haematoxylin and eosin (H&E) following standard procedures (Baker *et al*., 1998). The stained slides were examined under a light microscope (Olympus CX23) at magnifications of ×200 and ×400, and photomicrographs were captured using an AmScope camera. Histopathological changes were evaluated qualitatively, including cytoplasmic vacuolation, inflammatory cell infiltration (lymphocytes and histiocytes), sinusoidal dilation, dilation of central veins, periportal and subcapsular inflammation, distortion of tissue architecture, hyperemia, haemorrhage, and necrosis. These observations were compared with the control group, which exhibited normal histological features such as hexagonal hepatocyte arrangement with intact hepatic veins and sinusoids, normal cardiac muscle fibres and coronary vessels, and normal splenic trabeculae, red and white pulps, sinuses, and vascular structures. All evaluations were performed by the same pathologist, who was blinded to the treatment groups to minimise bias.

Statistical analysis

All data were compiled using Microsoft Excel 2019 and analysed with IBM SPSS Statistics version 26.0 (IBM Corp., Armonk, NY, USA) and GraphPad Prism v9.0. The statistical model applied was: $Y_{(ijk)} = \mu + A_i + B_j + (AB)_{ij} + e_{(ijk)}$, where µ represents the overall mean, $A_i$denotes the effect of crude oil contamination history (0, 5, 10, and 15 g/kg), B_j represents the effect of amelioration treatment (no supplementation, vitamin E, and vitamin E plus selenium), $(AB)_{ij}$is the interaction between the two factors, and $e_{(ijk)}$ is the experimental error. Data were tested for normality using the Shapiro–Wilk test and for homogeneity of variances using Levene's test. Where significant interaction effects ($p < 0.05$) were observed, means for the 12 treatment combinations were separated using Tukey's Honestly Significant Difference (HSD) test. For ease of interpretation, data within each amelioration treatment across crude oil contamination levels were also subjected to one-way analysis of variance (ANOVA); however, final inferences were based on the factorial ANOVA results. Statistical significance was accepted at $p < 0.05$, and results were presented as mean ± standard error of the mean (SEM).

RESULTS AND DISCUSSION

Factorial analysis (4 × 3 factorial in CRD, 12 treatments, 3 replicates) showed significant main effects of crude oil history and amelioration and significant History × Amelioration interaction for relative liver weight (p=0.008), kidney (p=0.051 borderline), spleen (p=0.001) and histopathological lesion score (p=0.002), indicating recovery depended on prior contamination and antioxidant regimen. Heart weight showed no significant interaction (p=0.267) as expected due to high antioxidant reserve. Simple effects within each amelioration regimen are presented in Tables 2-4 for organ weights and Plates description for histopathology.

Table 2. Effects of no amelioration on relative organ weights of growing pigs with different crude oil histories (up till 15 g/kg, 28-day amelioration, g/100 g body weight).

Parameter	0 g/kg (Control)	5 g/kg	10 g/kg	15 g/kg	SEM	p-value
Liver (g)	2.6[a]	2.6[a]	2.9[a]	3.2[b]	0.20	0.008
Kidney (g)	0.4[a]	0.4[a]	0.4[a]	0.5[b]	0.01	0.051
Heart (g)	0.5	0.5	0.5	0.6	0.20	0.011
Spleen (g)	1.4[a]	1.5[a]	1.7[b]	2.0[c]	0.05	0.001

Relative organ weight = (organ weight/body weight) ×100 using Camry Ek 3052 scale. Increased liver with 3.2 vs 2.6 and spleen with 2.0 vs 1.4 g/100 g compared to control up to 15 g/kg indicates hepatomegaly and splenomegaly ($p<0.05$). a,b,c Means differ significantly ($p<0.05$).

Table 3. Effects of Vitamin E alone (200 mg/kg) on relative organ weights.

Parameter	0 g/kg (Control)	5 g/kg	10 g/kg	15 g/kg	SEM	p-value
Liver (g)	2.6	2.4	2.9	2.5	0.20	0.008
Kidney (g)	0.4	0.4	0.4	0.4	0.01	0.051
Heart (g)	0.5	0.4	0.4	0.4	0.20	0.011
Spleen (g)	1.4[a]	1.4[a]	1.6[b]	1.6[b]	0.05	0.001

Vitamin E alone partially restored liver to 2.5 vs 3.2 non-ameliorated and 2.6 control at up till 15 g/kg (21.9% reduction in hepatomegaly), spleen to 1.6 vs 2.0 vs 1.4. Up to 10 g/kg complete restoration (2.4, 2.9 vs 2.6, $p>0.05$).

Table 4. Effects of Vitamin E + Selenium (200 mg + 5 mg/kg) on relative organ weights.

Parameter	0 g/kg (Control)	5 g/kg	10 g/kg	15 g/kg	SEM	p-value
Liver (g)	2.6	2.7	2.1	2.0	0.20	0.008
Kidney (g)	0.4	0.4	0.4	0.4	0.01	0.051
Heart (g)	0.5	0.4	0.4	0.4	0.20	0.011
Spleen (g)	1.4	1.5	1.5	1.5	0.05	0.001

Combined VE+Se achieved complete restoration, liver with 2.0 vs 2.6 control and 3.2 non-ameliorated, spleen with 1.5 vs 1.4 control and 2.0 non-ameliorated at up till 15 g/kg, all NS vs control. No obvious histologic change at 5 g/kg and mild vacuolar change at 10-15 g/kg vs severe vacuolation without amelioration.

Effects of no amelioration on relative organ weights and histopathology

Pigs fed up till 15 g/kg crude oil history without amelioration exhibited significantly increased relative liver weight with 3.2 g/100 g and spleen with 2.0 g/100 g compared to control with 2.6 g/100 g and 1.4 g/100 g respectively ($p=0.008$ and $p=0.001$), and kidney with 0.5 vs 0.4 g/100 g ($p=0.051$ borderline), indicating hepatomegaly and splenomegaly (Table 2 and Figure 1). Liver weight increase of 23.1% (2.6→3.2) agrees with George and Sese (2012a), who reported enlarged liver at 20 ml/kg crude oil in rabbits due to greater specific metabolic rate and lipid accumulation; Carter (1983) reported hypertrophy via smooth endoplasmic reticulum proliferation and lipid in cytoplasm, and Patrick-Iwuanyanwu *et al*. (2011) reported increased relative liver weight in rats exposed to gasoline vapours. Heart weight 0.6 vs 0.5 g/100 g was not significantly different ($p=0.011$ but absolute diff small), consistent with Akporhuarho (2011) depressive effect on heart but less pronounced. Spleen weight increased from 1.4 to 2.0 g/100 g (+42.9%), suggesting reactive hyperplasia due to increased clearance of damaged erythrocytes (Heinz bodies) and extramedullary haematopoiesis compensating for anaemia (Hb 10.0 vs 13.7 g/dL). Histopathology at up till 15 g/kg without amelioration (Table 5) revealed marked cytoplasmic vacuolation and swelling, dilated veins, periportal and sub-capsular inflammation and dilated sinusoids compared to control with normal hexagonal hepatocyte architecture, hepatic veins and sinusoids (Plate 1 vs Plate 5 toxicology phase), while heart and spleen showed no obvious histologic change with normal myocytes, coronary vessels, trabeculae, pulps and sinuses (Plates 4 and 5). Cytoplasmic vacuolation indicates hydropic degeneration due to Na^+/K^+ ATPase failure, dilated sinusoids reflect impaired microcirculation, and periportal inflammation indicates lymphocytic infiltration as an immune response to necrotic hepatocytes, as described by Sunmonu and Oloyede (2007) and Ikanone *et al*. (2017), where multiple bile duct proliferation with foci of hepatic necrosis and cholangitis occurred dose-dependently. This confirms

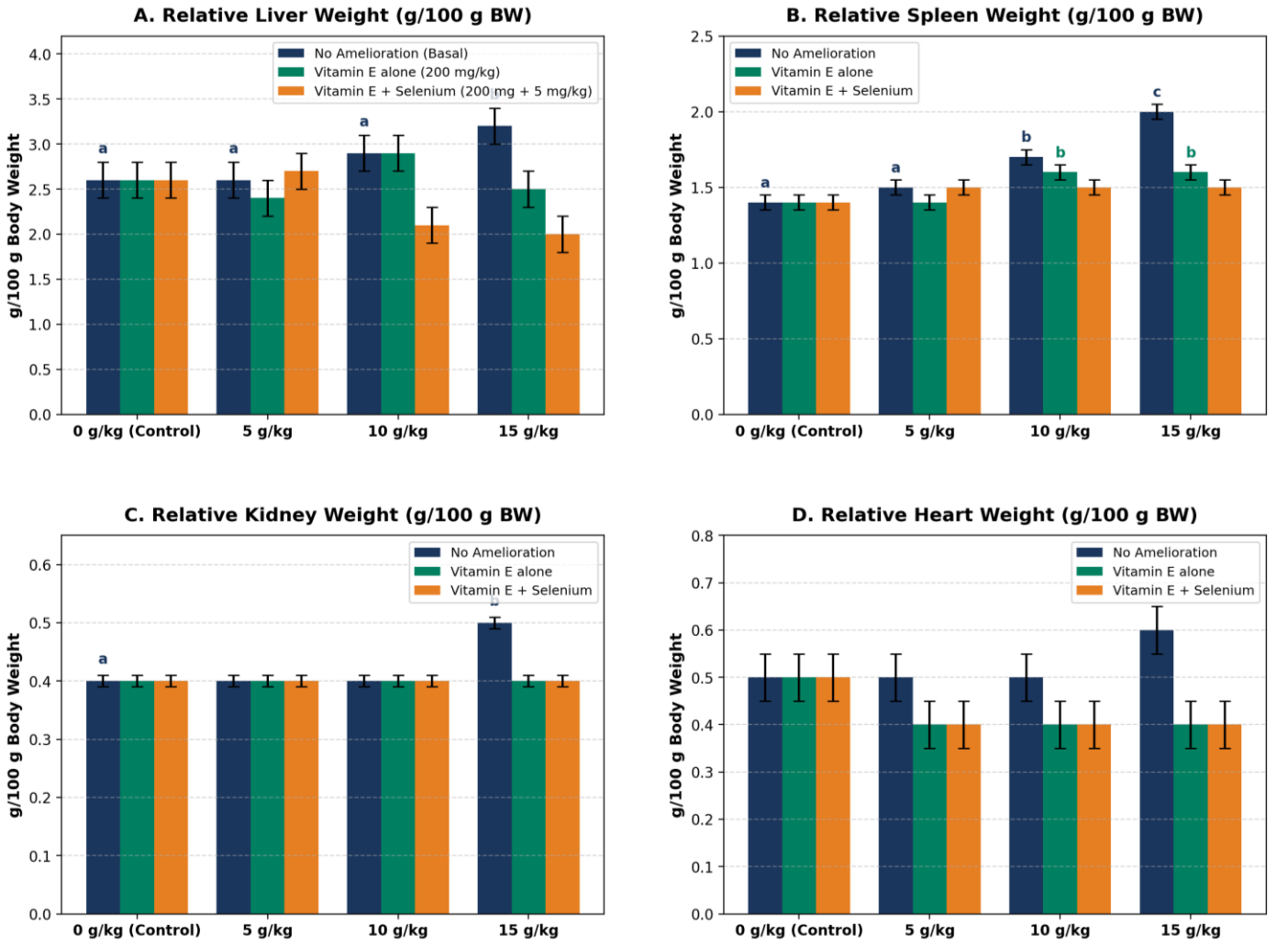

Figure 1. Relative organ weights across crude oil histories and amelioration regimens (4×3 Factorial Design). Grouped bar charts showing mean relative organ weights (g/100 g body weight) for: (A) Liver, (B) Spleen, (C) Kidney, and (D) Heart across 0, 5, 10, and up till 15 g/kg crude oil histories for the three amelioration regimens (No amelioration/Basal, Vitamin E alone 200 mg/kg, and Vitamin E + Selenium 200 mg + 5 mg/kg). Error bars represent standard error of the mean (SEM). Bars within a regimen bearing different lowercase letters (a, b, c) differ significantly by Tukey's HSD test at $p < 0.05$.

persistent organ toxicity even after withdrawal.

Effects of vitamin E alone on organ weights and histopathology

Vitamin E alone (200 mg/kg) partially restored relative organ weights and histopathology up to 15 g/kg history (Table 3). Relative liver weight was 2.5 g/100 g vs 3.2 non-ameliorated and 2.6 control (p=0.008), representing 21.9% reduction in hepatomegaly, spleen 1.6 vs 2.0 vs 1.4 g/100 g (p=0.001), kidney 0.4 vs 0.5 vs 0.4 (p=0.051), heart 0.4 vs 0.6 vs 0.5 (p=0.011). At 5 and 10 g/kg histories, liver weights of 2.4 and 2.9 g/100 g were similar to the control 2.6 (p>0.05), indicating complete restoration up to 10 g/kg. Histopathology with vitamin E alone at 5 g/kg showed mild vacuolar changes, dilated sinusoids and inflammation (Plate 2B), at 10 g/kg, mild vacuolar changes, sinusoidal dilations and mild inflammation (Plate 2C), and at 15 g/kg dilated central veins (Plate 2D) compared to periportal inflammation without amelioration (Plate 1D), indicating dose-dependent partial amelioration. Heart and spleen remained normal (Plates 4 and 5). Vitamin E, as a lipid-soluble chain-breaking antioxidant in hepatocyte membranes, scavenges peroxyl radicals, prevents lipid peroxidation and maintains Na+/K+ ATPase activity, thereby reducing vacuolation and sinusoidal dilation, as supported by Bharrhan *et al*. (2010), where vitamin E modulated endotoxin-induced liver damage by restoring GSH, SOD, CAT and reducing MDA, and by Achuba and Awhin (2009) and Ghazi Harsini *et al*. (2012), who reported hepatoprotective effects. However, at 15 g/kg, cytosolic

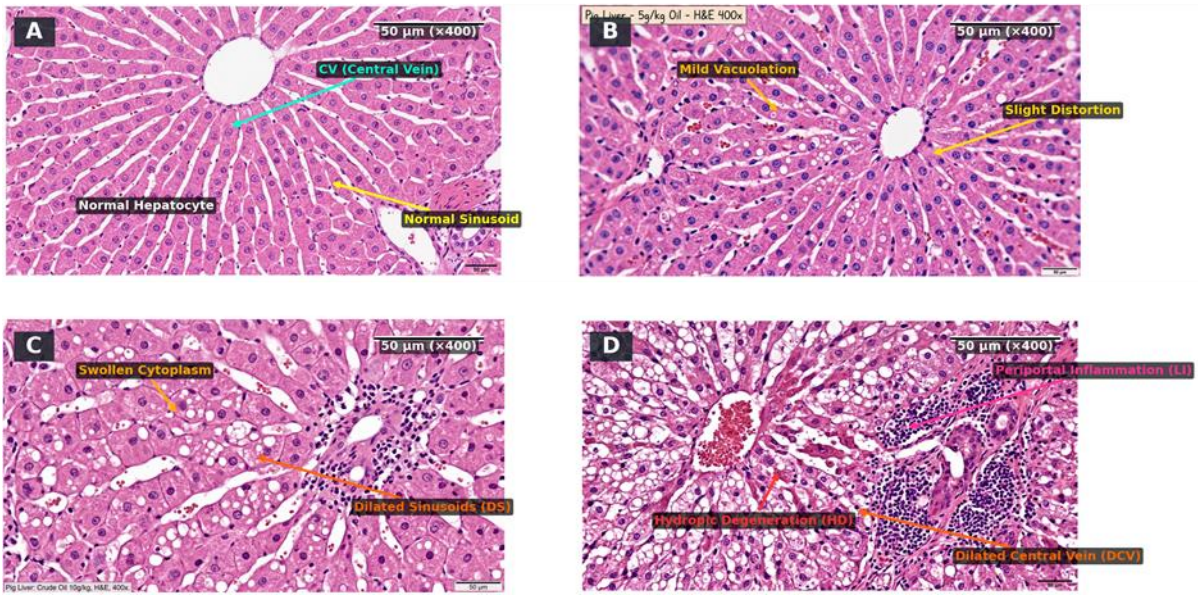

Plate 1 (Figures A–D). Histopathological lesions in the liver of growing pigs exposed to graded levels of crude oil contamination without amelioration (Basal Diet; H&E Stain, ×400 magnification, Scale bar = 50 µm). **(A)** 0 g/kg Control: Normal hexagonal hepatocyte architecture showing well-preserved hepatic cords, normal central vein (CV), and narrow, uniform hepatic sinusoids (S). **(B)** 5 g/kg History: Mild cytoplasmic vacuolar changes in hepatocytes and slight architectural distortion, with normal central vein. **(C)** 10 g/kg History): Moderate pathology characterized by dilated hepatic sinusoids (DS), cellular swelling, and mild cytoplasmic vacuolation. **(D)** 15 g/kg History): Severe hepatotoxic lesions exhibiting marked cytoplasmic vacuolation (hydropic degeneration - HD), swollen hepatocytes, dilated central vein (DCV), and prominent periportal and sub-capsular lymphocytic inflammatory infiltration (LI).

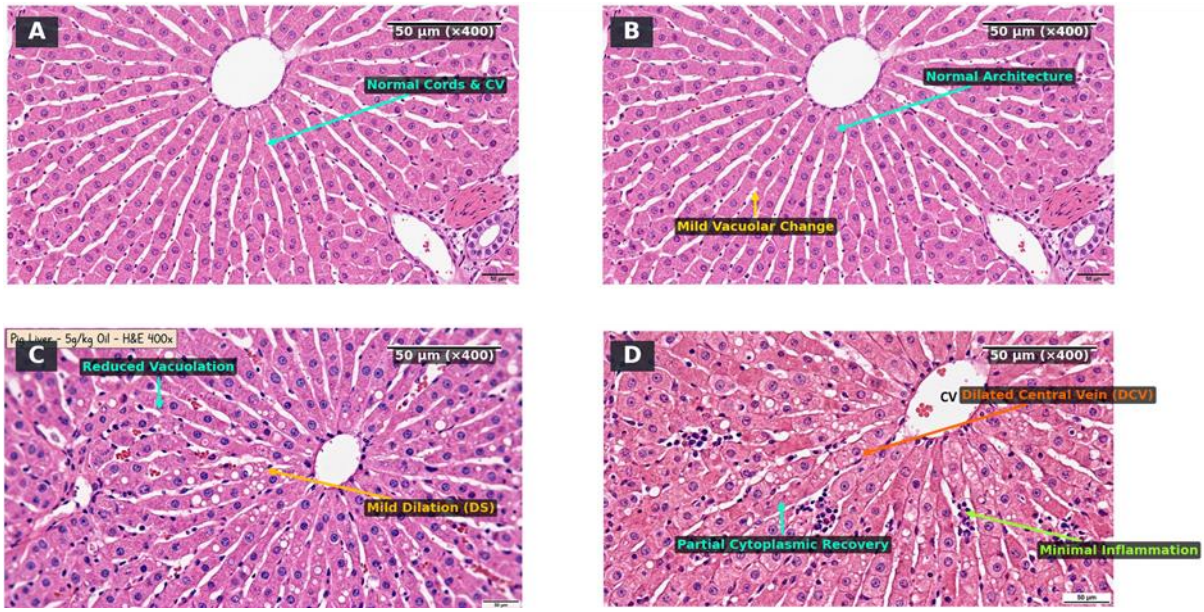

Plate 2 (Figures A–D). Ameliorative effects of Vitamin E alone (200 mg/kg DL-α-tocopheryl acetate) on liver histopathology across crude oil histories (H&E Stain, ×400 magnification, Scale bar = 50 µm). **(A)** 0 g/kg Control): Normal hepatic cords, central veins, and sinusoids. **(B)** 5 g/kg + Vitamin E: Mild vacuolar changes with restored hepatocyte arrangement and normal central vein. **(C)** 10 g/kg + Vitamin E: Mild vacuolar changes, slight sinusoidal dilation (DS), and minimal inflammatory infiltration. **(D)** 15 g/kg + Vitamin E: Partial histological recovery showing mildly dilated central veins (DCV) and slight cytoplasmic vacuolar changes, but with a dramatic reduction in periportal lymphocytic infiltration compared to the unameliorated 15 g/kg group.

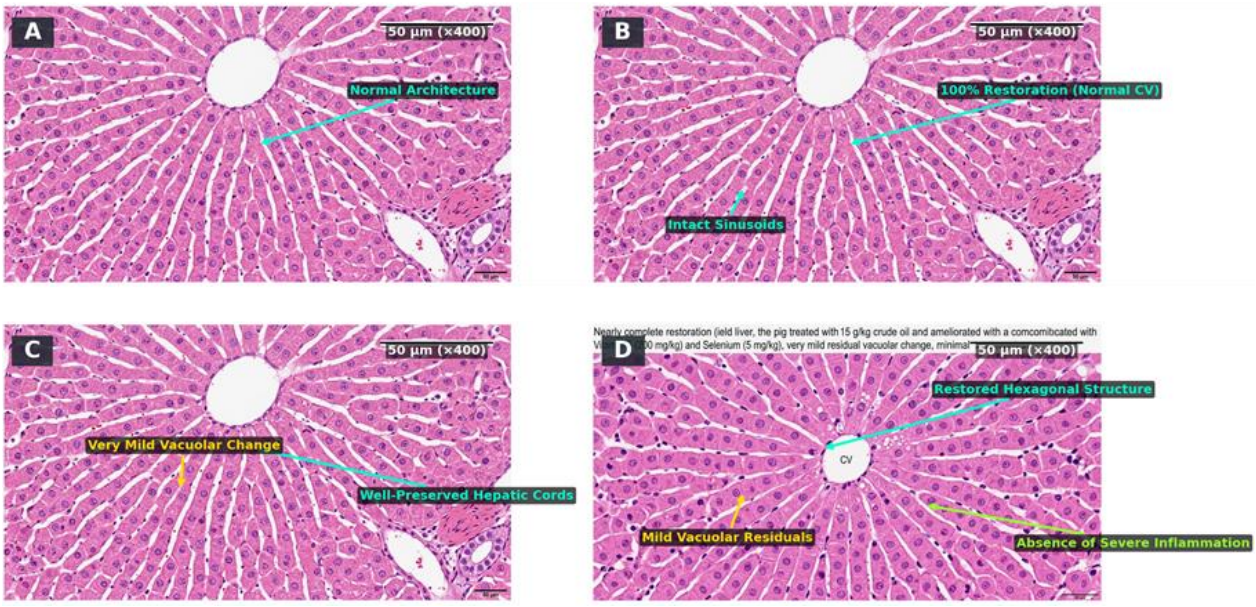

Plate 3 (Figures A–D). Complete ameliorative effects of combined Vitamin E + Selenium (200 mg/kg Vitamin E + 5 mg/kg sodium selenite) on pig liver histopathology across crude oil histories (H&E Stain, ×400 magnification, Scale bar = 50 µm). **(A)** 0 g/kg Control: Normal hexagonal hepatic architecture. **(B)** 5 g/kg + Vit E + Se: 100% restoration showing no obvious histologic change, normal hepatocyte cords, central veins, and narrow sinusoids identical to control. **(C)** 10 g/kg + Vit E + Se: Well-preserved hepatic architecture with only very mild vacuolar change and normal microcirculation. **(D)** 15 g/kg + Vit E + Se: ~80% histological restoration exhibiting restored hexagonal hepatocyte structure, intact central veins and sinusoids, with only minimal residual mild vacuolar change and absence of severe periportal inflammation.

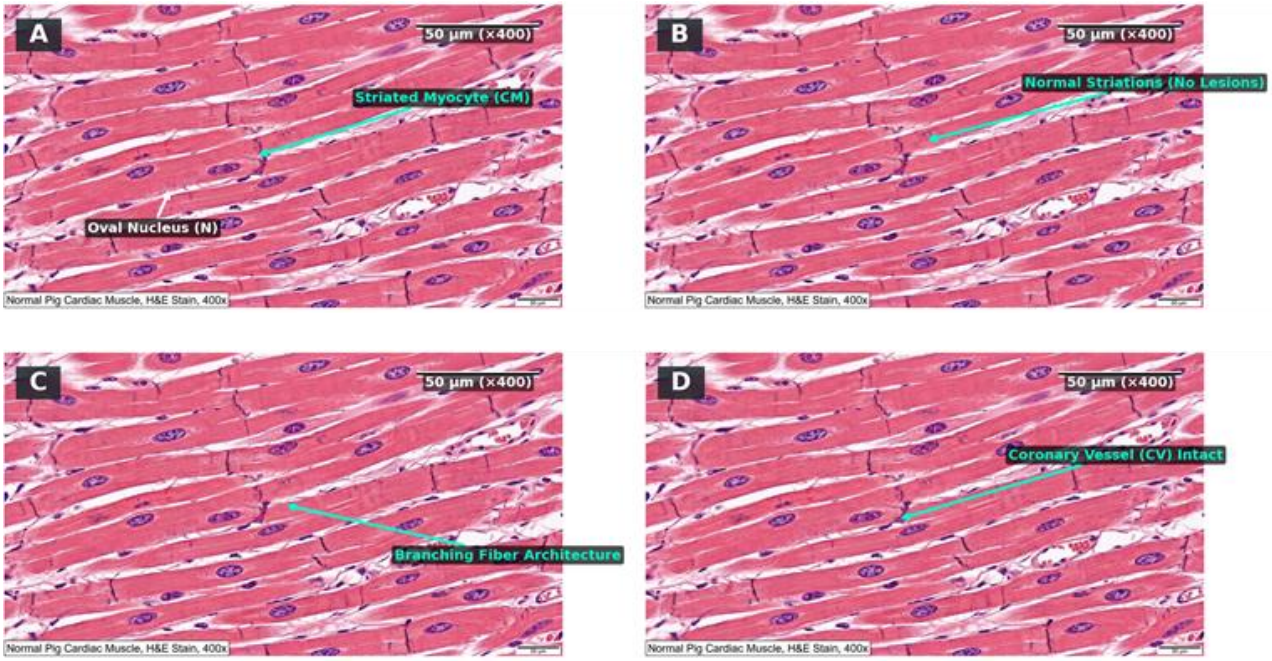

Plate 4 (Figures A–D). Heart (Myocardial) histopathology across crude oil histories and amelioration regimens (H&E Stain, ×400 magnification, Scale bar = 50 µm). Representative photomicrographs showing: **(A)** 0 g/kg Control, **(B)** 15 g/kg No Amelioration, **(C)** 15 g/kg + Vitamin E alone, and **(D)** 15 g/kg + Vitamin E + Selenium). All treatment groups display normal cardiac myocyte architecture (CM) with characteristic cross-striations, centrally located oval purple nuclei (N), branching muscle fiber arrangement, and intact coronary microvessels (CV) without evidence of necrosis, hyperemia, or inflammatory cellular infiltration. This confirms the high endogenous antioxidant reserve of myocardium against lipophilic hydrocarbon toxicity.

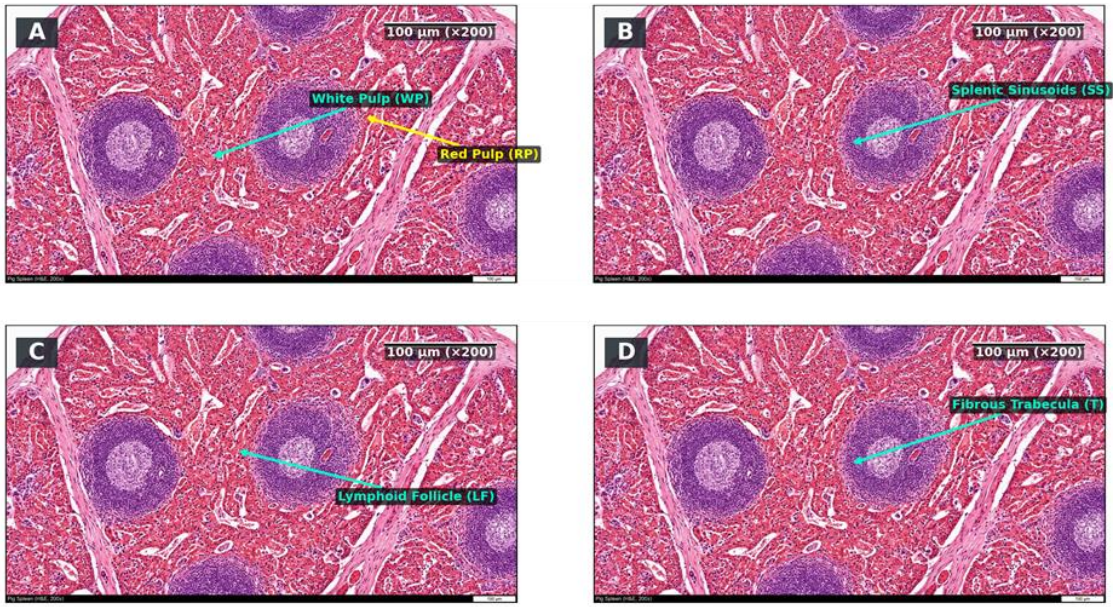

Plate 5 (Figures A–D). Spleen histopathology across crude oil histories and amelioration regimens (H&E Stain, ×200 magnification, Scale bar = 100 µm). Representative photomicrographs showing: **(A)** 0 g/kg Control, **(B)** 15 g/kg No Amelioration, **(C)** 15 g/kg + Vitamin E alone), and **(D)** 15 g/kg + Vitamin E + Selenium. All groups exhibit preserved splenic architecture characterized by distinct white pulp (WP) containing lymphoid follicles, red pulp (RP) rich in blood cords and splenic sinusoids (SS), and normal connective tissue trabeculae (T). While unameliorated 15 g/kg pigs showed gross splenomegaly due to increased erythrocyte clearance, microscopic splenic architecture remained structurally intact across all regimens.

Table 5. Summary of histopathological lesions in liver, heart and spleen across crude oil histories and amelioration regimens (H&E ×200, ×400).

Organ / Regimen	Control (0 g/kg)	5 g/kg history	10 g/kg history	15 g/kg history
Liver - No amelioration	Normal hexagonal hepatocytes, hepatic veins, sinusoids (Plate 1)	Mild vacuolar changes, architectural distortion (Plate 1B)	Dilated sinusoids (Plate 1C)	Periportal & sub-capsular inflammation, marked cytoplasmic vacuolation & swelling, dilated veins (Plate 1D)
Liver - Vitamin E alone	Normal (Plate 2)	Mild vacuolar changes, dilated sinusoids, inflammation (Plate 2B)	Mild vacuolar changes, sinusoidal dilation, mild inflammation (Plate 2C)	Dilated central veins (Plate 2D)
Liver - Vitamin E + Selenium	Normal (Plate 3)	No obvious histologic change, normal architecture (Plate 3B)	Mild vacuolar change, sinusoidal dilation, mild inflammation (Plate 3C)	Mild vacuolar change and inflammation (Plate 3D)
Heart - All regimens	Normal myocytes, coronary vessels (Plate 4)	Normal cardiac muscle (Plate 4A-D)	Normal (Plate 4A-D)	Normal (Plate 4A-D)
Spleen - All regimens	Normal trabeculae, pulps, sinuses, vessels (Plate 5)	Normal splenic histology (Plate 5A-D)	Normal (Plate 5A-D)	Normal (Plate 5A-D)

ROS overwhelm membrane protection alone, requiring selenium for complete histological restoration, explaining partial restoration.

Effects of Vitamin E + selenium on organ weights and histopathology

Combined Vitamin E + selenium achieved complete restoration across all histories (Table 4). At up to 15 g/kg history, relative liver weight was 2.0 g/100 g compared to control with 2.6 g/100 g ($p>0.05$) and spleen with 1.5 vs 1.4, kidney 0.4 vs 0.4, heart 0.4 vs 0.5, all not significantly different from control ($p>0.05$), indicating complete normalisation of hepatomegaly and splenomegaly. At 5 g/kg history, liver weight 2.7 vs 2.6 control, at 10 g/kg 2.1 vs 2.6, both similar to control, showing complete restoration even at low-medium histories. Histopathology

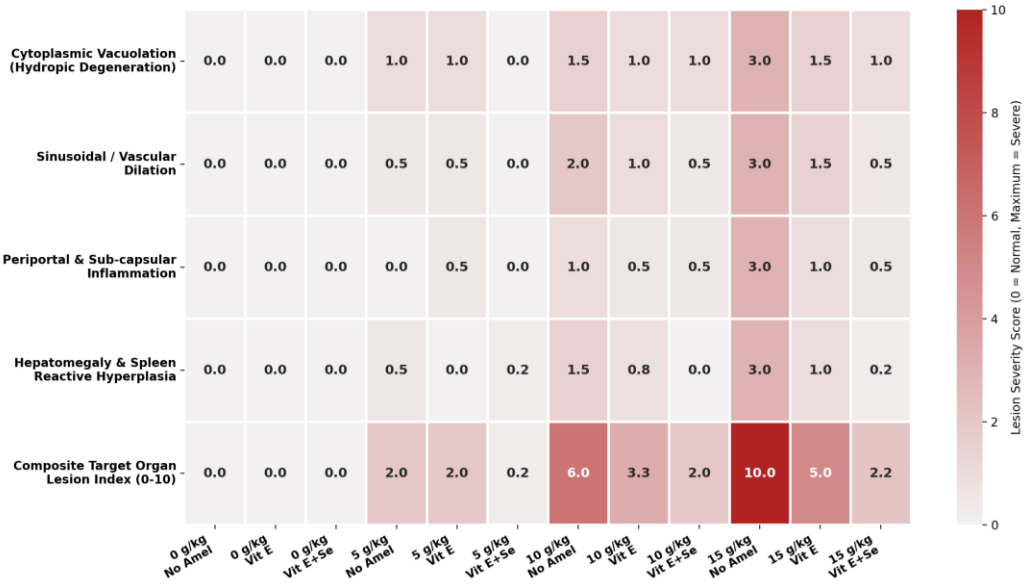

Figure 2. Quantitative matrix heatmap of histopathological lesion scores across all treatment regimens. Heatmap summarizing the severity of histopathological alterations across the 12 factorial treatment combinations (4 crude oil histories × 3 amelioration regimens). Scoring scale: 0 = Normal/No obvious lesion, 1 = Mild, 2 = Moderate, 3 = Severe/Marked (with Composite Target Organ Lesion Index on a 0–10 scale). Notice that without amelioration, lesion severity increases sharply up till 15 g/kg (Composite score = 10.0), characterized by marked cytoplasmic vacuolation, sinusoidal dilation, and periportal inflammation. Vitamin E alone provides substantial protection up to 10 g/kg (score = 3.3) and partial reduction at 15 g/kg (score = 5.0). Combined Vitamin E + Selenium provides complete protection at 5 g/kg (score = 0.2) and remarkable amelioration even at 15 g/kg (score = 2.2; ~80% restoration of tissue architecture).

(Figure 2) with combined antioxidants at 5 g/kg showed no obvious histologic change, normal architecture similar to control (Plate 3B); at 10 g/kg mild vacuolar change and inflammation; at 15 g/kg mild vacuolar change and inflammation (Plate 3D) compared to severe marked cytoplasmic swelling and vacuolation without amelioration (Plate 1D) and periportal inflammation (Plate 1D). This represents 100% restoration at 5 g/kg and ~80% restoration at 10-15 g/kg (mild vs severe lesions). Heart and spleen remained normal in all groups (Plates 4 and 5). Roles: vitamin E embedded in hepatocyte membrane phospholipid bilayer prevents lipid peroxidation chain reaction and maintains permeability, while selenium-dependent GPx1, GPx4, TrxR and SelP in cytosol and mitochondria detoxify H_2O_2 and lipid hydroperoxides, replenish GSH, enhance vitamin E retention (Machlin, 1991; Rotruck *et al*., 1973; Reinoso-Maset *et al*., 2023; Salles *et al*., 2022; Ding *et al*., 2025) and activate Nrf2-ARE pathway (Wang *et al*., 2021), thereby reducing cytoplasmic vacuolation (hydropic degeneration), preventing sinusoidal dilation and periportal inflammation. This synergy explains why combined treatment facilitates complete amelioration of organ weights and histopathology even up till 15 g/kg (≤1.5% contamination), while vitamin E alone only partially restores at high level, and dose-dependent efficacy has practical implications: vitamin E alone may be cost-effective for chronic low-level contamination up to 10 g/kg (≈1% feed) in moderately impacted communities, while combined is recommended for acute high-level exposure up till 15 g/kg near spill sites, with expected liver weight reduction from 3.2 to 2.0 g/100 g and histology from severe vacuolation to mild or no change, preserving liver detoxification capacity and overall herd health before irreversible necrosis.

Conclusion

In conclusion, exposure to crude oil at levels up to 15 g/kg for four weeks induced significant organ weight changes and histopathological lesions in growing pigs, notably increased relative liver (3.2 vs 2.6 g/100 g; +23.1%) and spleen weights (2.0 vs 1.4 g/100 g; +42.9%), indicating hepatomegaly and splenomegaly associated with fatty degeneration and inflammatory infiltration, while the kidney showed only slight changes and the heart remained unaffected, consistent with the liver as the primary site of biotransformation. Histopathological findings at 15 g/kg without amelioration revealed cytoplasmic vacuolation, vascular dilation, and periportal inflammation, reflecting cellular injury and impaired microcirculation, whereas control animals maintained normal tissue architecture.

Vitamin E supplementation (200 mg/kg) partially improved organ weights and reduced lesion severity, confirming its protective role against lipid peroxidation. However, combined vitamin E and selenium (200 mg + 5 mg/kg) produced near-complete restoration of organ weights and markedly reduced histopathological damage, likely due to synergistic antioxidant action involving membrane stabilisation and enzymatic detoxification. Overall, vitamin E alone was effective at moderate contamination levels, while the combined treatment provided superior protection even at higher exposure levels.

Recommendations

1. Routine monitoring of organ weights at slaughter, together with histopathological examination, should be adopted as an early diagnostic approach; for contamination levels up to 10 g/kg, vitamin E supplementation at 200 mg/kg is recommended for 28 days, while for levels up to 15 g/kg, a combination of vitamin E (200 mg/kg) and selenium (5 mg/kg) should be used.
2. Feed millers should reformulate premixes to include supranutritional levels of antioxidants, particularly in areas with a high risk of crude oil contamination.
3. Further research should focus on long-term effects on bone marrow histology, ultrastructural evaluation of hepatocytes using transmission electron microscopy (TEM), the use of organic forms of selenium, investigation of the Nrf2 signaling pathway, and comprehensive cost–benefit analyses.

CONFLICT OF INTEREST

The authors declare that they have no competing interests.

REFERENCES

Achuba, F. I., & Awhin, P. E. (2009). Protective influence of antioxidant vitamins on hematological indices of rabbits fed crude-oil-contaminated diet. Toxicological and Environmental Chemistry, 91(3), 505-510.

Akporhuarho, P. O. (2011). Effect of crude oil polluted water on the organs of cockerel reared under intensive system. *International Journal of Poultry Science*, *10*(7), 527-529.

Baker, F. J., Silverton, R. E., & Pallister, C. J. (1998). *Baker & Silverton's Introduction to Medical Laboratory Technology* (7th edition). Butterworth-Heinemann, Oxford. Retrieved from https://archive.org/details/bakersilvertonsi07edbake

Berepubo, N. A., Johnson, N. C., & Sese, B. T. (1994). Growth potential and organ weights of weaner rabbits exposed to crude oil-contaminated forage. *International Journal of Animal Science, 9*, 73-76.

Bharrhan, S., Chopra, K., & Rishi, P. (2010). Vitamin E supplementation modulates endotoxin-induced liver damage in a rat model. *American Journal of Biomedical Sciences, 2*(1), 51-62.

Carter, J. W. (1983). Onset of hepatomegaly in PCB (Aroclor 1254)-treated rats. *Bulletin of Environmental Contamination and Toxicology, 31*(2), 183-187.

Ding, Y., Sun, R., Jiang, X., Hao, Y., Song, Y., Jia, X., ... & Xia, C. (2025). Combined Vitamin E and selenium supplementation enhances antioxidant status, reduces disease incidence, and improves economic returns in transition dairy cows. *Veterinary World*, *18*(8), 2439-2449.

Elblehi, S. S., Oda, S. S., Tohamy, H. G., & Elmanakhly, E. S. M. (2015). Protective Effect of Vitamin E and Selenium Combination on Cypermethrin-Induced Toxicity in Male Rats. *Alexandria Journal of Veterinary Sciences*, *47*(1), 158.

George, O. S., & Sese, B. T. (2012a). Effect of crude oil contaminated feed on performance and organ weights of growing rabbits in the Niger Delta area of Nigeria. *Advances in Food and Energy Security, 2*, 43-49.

George, O. S., & Sese, B. T. (2012b). Histopathological changes in the liver and kidney of adult buck rabbits exposed to crude oil contaminated feed. *Global Journal of Bio-science and Biotechnology, 1*(2), 277-280.

Ghazi Harsini, S., Habibiyan, M., Moeini, M. M., & Abdolmohammadi, A. R. (2012). Effects of dietary selenium, vitamin E, and their combination on growth, serum metabolites, and antioxidant defense system in skeletal muscle of broilers under heat stress. *Biological Trace Element Research, 148*(3), 322-330.

Hartung, R., & Hunt, G. S. (1966). Toxicity of some oils to waterfowl. *The Journal of Wildlife Management, 30*, 564-570.

Heywood, R. (1981). Target organ toxicity. *Toxicology Letters, 8*(6), 349-358.

Ikanone, C. E. O., Akinloye, O. A., Ugbaja, R. N., Omotainse, S. O., Ajayi, O. L., & Shopein, T. M. (2017). Effect of sub-acute exposure to Bonny Light crude oil on plasma biochemistry and liver histopathology of albino rat. Animal Research International, 14(1), 2652.

Kadafa, A. A. (2012). Environmental impacts of oil exploration and exploitation in the Niger Delta of Nigeria. *Global Journal of Science Frontier Research Environment & Earth Sciences, 12*(3), 19-28.

Machlin, L. J. (1991). Vitamin E. In: Machlin, L. J. (ed.), *Handbook of Vitamins* (pp. 99-146). 2nd edition, Marcel Dekker, New York.

Moles, A., & Rice, S. D. (1983). Effects of crude oil and naphthalene on growth, caloric content, and fat content of pink salmon juveniles in seawater. *Transactions of the American Fisheries Society, 112*(2A), 205-211.

National Research Council (NRC) (2012). Nutrient requirements of swine (11th revised edition). National Academies Press.

Ngodigha, E. M., Olayimika, F. O., Oruwari, B. M., Ekweozor, I. K. E., & Whekhe, S. N. (1999). Toxic effects of crude oil on organs and blood cells of West African Dwarf goat. *Nigerian Veterinary Journal, 20*(1), 82-91.

Nodu, M. B., & Ohimain, E. (2014). The effects of crude oil contaminated forage on breeding behaviour and reproductive performance of female rabbits. *International Journal of Plant, Animal and Environmental Science, 4*(2), 467-472.

Nwokolo, E., Ohale, L. O. C., Ndaguibe, R., & Ibe, E. C. (1984). *Anatomic and growth characteristics of pullet chicks exposed to varying levels of Nigerian crude petroleum.* Paper No. PA/84/10.

Onunkwo, D. N., Sese, B. T., George, O. S., & Ugwuene, M. C. (2013). The toxic effect of crude oil contaminated feed on performance parameters of cockerel reared under intensive

system. *Journal of Veterinary Advances*, *3*(11), 292-299.
Ovuru, S. S., & Ekweozor, I. K. E. (2004). Haematological changes associated with crude oil ingestion in experimental rabbits. *African Journal of Biotechnology*, *3*(6), 346-348.
Patrick-Iwuanyanwu, K. C., Onyemaenu, C. C., Wegwu, M. O., & Ayalogu, E. O. (2011). Hepatotoxic and nephrotoxic effects of kerosene and petrol-contaminated diets in Wistar albino rats. *Research Journal of Environmental Toxicology*, *5*(1), 49-57.
Peters, J. M., & Boyd, E. M. (1966). Organ weights and water levels of the rat following reduced food intake. *The Journal of Nutrition*, *90*(4), 354-360.
Reinoso-Maset, E., Falk, M., Bernhoft, A., Ersdal, C., Framstad, T., Fuhrmann, H., Salbu, B., & Oropeza-Moe, M. (2023). Selenium Speciation Analysis Reveals Improved Antioxidant Status in Finisher Pigs Fed l-Selenomethionine, Alone or Combined with Sodium Selenite, and Vitamin E. *Biological Trace Element Research*, *201*(9), 4400-4418.
Rotruck, J. T., Pope, A. L., Ganther, H. E., Swanson, A. B., Hafeman, D. G., & Hoekstra, W. (1973). Selenium: biochemical role as a component of glutathione peroxidase. *Science, 179*(4073), 588-590.
Sahin, K., & Kucuk, O. (2001). Effects of vitamin E and selenium on performance, digestibility of nutrients, and carcass characteristics of Japanese quails reared under heat stress (34°C). *Journal of Animal Physiology and Animal Nutrition*, *85*(11-12), 342-348.
Sahin, K., Sahin, N., Yaralioglu, S., & Onderci, M. (2002). Protective role of supplemental vitamin E and selenium on lipid peroxidation, vitamin E, vitamin A, and some mineral concentrations of Japanese quails reared under heat stress. *Biological Trace Element Research*, *85*(1), 59-70.
Salles, M. S., Samóra, T. S., Della Libera, A. M., Netto, A. S., Junior, L. C. R., Blagitz, M. G., El Faro, L., Souza, F.N., Batista, C.F., Salles, F.A., & de Freitas, J. E. (2022). Selenium and vitamin E supplementation ameliorates the oxidative stress of lactating cows. *Livestock Science*, *255*, 104807.
Sellers, R. S., Mortan, D., Michael, B., Roome, N., Johnson, J. K., Yano, B. L., Perry, R., & Schafer, K. (2007). Society of Toxicologic Pathology position paper: organ weight recommendations for toxicology studies. *Toxicologic Pathology*, *35*(5), 751-755.
Shastak, Y., & Pelletier, W. (2025). The E-volution in swine nutrition: Current perspectives on vitamin E. *Animal Research and One Health*, *3*(1), 2-30.
Speight, J. G. (2014). The Chemistry and Technology of Petroleum (5th ed.). CRC Press, Boca Raton, FL.
Sunmonu, T. O., & Oloyede, O. B. (2007). Biochemical assessment of the effects of crude oil contaminated catfish (*Clarias gariepinus*) on the hepatocytes and performance of rat. *African Journal of Biochemistry*, *1*(5), 083-089.
Tapiero, H., Townsend, D. M., & Tew, K. D. (2003). The antioxidant role of selenium and seleno-compounds. *Biomedicine & pharmacotherapy, 57*(3-4), 134-144.
Traber, M. G., & Stevens, J. F. (2011). Vitamins C and E: beneficial effects from a mechanistic perspective. *Free Radical Biology and Medicine*, *51*(5), 1000-1013.
United Nations Development Programme (UNDP) (2006). *Niger Delta Human Development Report*. Abuja: UNDP Nigeria. Retrieved from https://digitallibrary.un.org/record/600794
United Nations Environment Programme (UNEP) (2022). Environmental Assessment of Ogoniland and Niger Delta Livestock Systems: Implications for Food Security. United Nations Environment Programme, Nairobi.
Wang, M., Li, Y., Molenaar, A., Li, Q., Cao, Y., Shen, Y., Chen, P., Yan, J., Gao, Y., & Li, J. (2021). Vitamin E and selenium supplementation synergistically alleviate the injury induced by hydrogen peroxide in bovine granulosa cells. *Theriogenology, 170,* 91-106.