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Thinner Induced Multiple Organ Toxicities in Wistar Rats: Ameliorative Role of Vitamin E (Alpha-Tocopherol)

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Abstract: Thinner, a common industrial solvent, is a popular choice for recreational self-intoxication, leading to potential serious health consequences. This study delves into the acute toxicity of thinner, focusing on its impact on various organ systems and the potential ameliorative ability of Vitamin E as an antioxidant. Four experimental groups of Wistar rats (n-12) were established as follow: control, Vitamin E only, thinner only and thinner + Vitamin E. The rats were subjected to a 3week inhalation protocol, and various parameters such as body weight, biochemical markers (aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), total bilirubin (TBIL), blood urea nitrogen (BUN) and creatinine levels), oxidative stress markers (malondialdehyde, nitrites, glutathione, catalase and superoxide dismutase), and histopathological examinations were assessed using standard procedures. The results revealed a significant reduction in body weight in the thinner-exposed group. However, the group exposed to both thinner and Vitamin E exhibited marked increase in body weight. Biochemical parameters (AST, ALT, ALP, TBIL, BUN and creatinine) were significantly elevated ($p<0.05$) in thinner only exposed rats. Conversely, the parameters were markedly reduced in the rats co-administered with thinner and vitamin E. The different tissue oxidative stress marker profiles were significantly altered in the thinner only treated rats. Interestingly, vitamin E supplementation significantly reversed the alterations to the markers. Thinner exposure precipitated severe histomorphological disruptions in the liver, kidneys, lungs and spleen. Remarkably, vitamin E co-administration with thinner markedly ameliorate the structural damage to the organs. This study emphasizes the potential therapeutic role of Vitamin E in mitigating the adverse effects of thinner-induced toxicity, suggesting its importance as an antioxidant strategy in managing solvent abuse-related health complications.

Key words: Thinner, toxicity, vitamin E, antioxidant, histopathology.

Wistar Sıçanlarında Tinerin Neden Olduğu Çoklu Organ Toksisiteleri: E Vitamininin (Alfa-Tokoferol) İyileştirici Rolü

Öz: Yaygın bir endüstriyel çözücü olan tiner, keyfi amaçlı kendi kendine zehirlenme için yaygın bir seçimdir ve potansiyel ciddi sağlık sonuçlarına yol açmaktadır. Bu çalışma, tinerin akut toksisitesini, çeşitli organ sistemleri üzerindeki etkisine ve bir antioksidan olarak E Vitamininin potansiyel iyileştirici yeteneğine odaklanarak incelemektedir. Wistar sıçanlardan oluşan dört deney grubu (n-12) şu şekilde oluşturulmuştur: kontrol, sadece E Vitamini, sadece tiner ve tiner + E Vitamini. Sıçanlar 3 haftalık bir inhalasyon protokolüne tabi tutulmuş ve vücut ağırlığı, biyokimyasal belirteçler (aspartat aminotransferaz (AST), alanin aminotransferaz (ALT), alkalin fosfataz (ALP), total bilirubin (TBIL) gibi çeşitli parametreler ölçülmüştür, kan üre azotu (BUN) ve kreatinin seviyeleri), oksidatif stres belirteçleri (malondialdehit, nitritler, glutatyon, katalaz ve süperoksit dismutaz) ve histopatolojik incelemeler standart prosedürler kullanılarak değerlendirilmiştir. Sonuçlar, tiner maruz kalan grupta vücut ağırlığında önemli bir azalma olduğunu ortaya koymuştur. Bununla birlikte, hem tiner hem de E Vitaminine maruz kalan grupta vücut ağırlığında belirgin bir artış görülmüştür. Biyokimyasal parametreler (AST, ALT, ALP, TBIL, BUN ve kreatinin) sadece tinere maruz kalan sıçanlarda önemli ölçüde yükselmiştir ($p<0,05$). Tersine, tiner ve E vitamini birlikte uygulanan sıçanlarda parametreler belirgin şekilde azalmıştır. Farklı doku oksidatif stres belirteç profilleri sadece tinerle tedavi edilen sıçanlarda önemli ölçüde değişmiştir. İlginç bir şekilde, E vitamini takviyesi belirteçlerdeki değişiklikleri önemli ölçüde tersine çevirmiştir. Tiner maruziyeti karaciğer, böbrekler, akciğerler ve dalakta ciddi histomorfolojik bozulmalara yol açmıştır. Dikkat çekici bir şekilde, E vitamininin tiner ile birlikte uygulanması organlardaki yapısal hasarı belirgin bir şekilde iyileştirmiştir. Bu çalışma, tiner kaynaklı toksisitenin olumsuz etkilerini hafifletmede E Vitamininin potansiyel terapötik rolünü vurgulamakta ve solvent kullanımına bağlı sağlık komplikasyonlarının yönetiminde antioksidan bir strateji olarak önemini ortaya koymaktadır.

Anahtar kelimeler: İnceltici, toksisite, E vitamini, antioksidan, histopatoloji.

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1. Introduction

Intentional abuse of solvent is a popular form of inhalant abuse common among children and adolescent age [1, 2]. This involves sniffing (nasally inhaled), huffing (orally inhaled) or bagging of thinner solvent for recreational self-intoxication [3]. It is a habit found most appealing among adolescents due to easy accessibility, inexpensiveness and legal acceptability in some part of the world [4, 5]. Thinner is an industrial solvent that is composed of volatile solvents such as toluene, acetone, methanol, benzene, hexane and other substances with the toluene constituting the greatest proportion [6]. A study using a gas chromatography has confirmed toluene, as the major components found in commercial thinner samples, making up to 60-70% of the solution [7].

Evidences have shown that prolonged or regular use or over-exposure to thinner can affect the body and induce serious toxicity resulting in organ system dysfunction and sudden death [7]. Since thinner solvent (toluene and benzene) is volatile; hence, it may be sniffed (nasally inhaled), huffed (orally inhaled) or bagged by abusers. Following inhalation, a potential user reported a pleasurable or euphoric effects, which may be intoxicating [6]. This effect which occurs rapidly and disappears fairly quickly create a sober attitude in individual abusers that makes early detection more difficult [8], thus creating the possibility of continuous abuse which may persist into adulthood and subsequently, may lead to medical complications. Consistent inhalation of thinner can serve as a gateway to the abuse of more addictive substances [9].

Toluene, the major component of thinner is a well-known organo-toxins, mostly implicated in oxidative damage of several body organs including the brain, lung, liver, kidney, testes and ovaries [7, 10]. In recent time, most of the research on inhalant abused is primarily focused on central nervous system, however, solvent abuse also exerts effects on other organ systems and cellular processes. Organ defects associated with prolonged or regular use or over-exposure to toluene-based solvent includes bone-marrow depression [11], cardiac dysrhythmias, liver and kidney disorders. Gastrointestinal effects include nausea, vomiting, diarrhea, abdominal cramps and loss of appetite [12]. Some of the systemic pathologies recorded from examination of individuals constantly exposed to toluene include renal tubular acidosis [13], renal calculi, haematuria, hypovitaminosis, hypertension and glomerulonephritis [14]. Myocardial infarctions have been attributed to inhalation of toluene-based solvent, with the production of reactive oxygen species (ROS) and increased oxidative stress [15]. Hepatotoxicity with toluene-based solvents results to ascites, jaundice, hepatomegaly and liver failure [16]. Other clinical symptoms associated with thinner exposure includes acute respiratory failure, cardiac arrhythmias, heart failure, choking, kidney damage, weight loss, hearing loss, skin rashes and sudden death [12, 17]. Leukemia, lymphoma, multiple myeloma and aplastic anaemia are also haematological malignancy associated with chronic inhalant abuse [18].

Although the mechanism underlying thinner toxicity has not been clearly elucidated [19]. Some evidences have implicated oxidative stress as the main etiology of thinner intoxication [7, 10]. Toluene being the principal component of thinner, also induce oxidative stress effect [15]. Some of the proposed mechanisms by which thinner induced cellular damages in biological tissue includes production of toxic end product of oxidative metabolism (toluene and benzene) which generates free radicals [20], cytosolic NADH [21] and cytotoxic ROS [22], which subsequently disrupt cellular redox system. The other mechanisms include upregulation of agents of inflammation (TNF-α, NF-κB) and oxidative stress markers (NO) produced in the organ tissue in response to thinner induced toxicity. Cellular oxidative damages linked with thinner toxicity are peroxidation of macro molecules, reduction of antioxidant enzymes and upregulation of inflammatory mediators [23, 24]. A recent study found increased levels of thiobarbituric acid reactive substances (TBARS), glutathione reductase (GR), glutathione disulfide (GSSG) and superoxide dismutase (SOD) in various rat organs after chronic toluene inhalation [25]. Rats exposed to toluene also exhibited elevated lipid peroxidation products and evidence of oxidative damage to the DNA [26].

Since the induction of oxidative stress has been proposed as the fundamental mechanism of toxic substances [27], potent antioxidants can antagonize toxic effect elicited by free radicals generated by toxic chemicals in the body [7]. Vitamin E (Alpha-tocopherol) is a fat-soluble compound with distinctive antioxidant activities [28, 29]. It stops the production of reactive oxygen species (ROS) formed during oxidative processes and thus delays oxidative damage in the tissue [30]. Vitamin E exists in eight chemical forms: α-, β-, γ-, and δ-tocopherols, along with their corresponding tocotrienols [31]. Among these, α-tocopherol is the most biologically active form, playing a crucial role in neutralizing reactive oxygen species (ROS). It achieves this by donating phenolic hydrogen to unstable lipid radicals (such as peroxyl radicals and singlet oxygen), thereby inhibiting the lipid peroxidation chain reaction. This process results in the formation of a relatively stable tocopheroxyl radical, which does not readily propagate further oxidative damage within the cell [32, 33, 34]. The tocopheroxyl radical can be recycled back to its active form through interactions with other antioxidants, including Vitamin C (ascorbic acid) and glutathione (GSH) [35, 36]. Additionally, Vitamin E enhances cellular antioxidant defense by upregulating superoxide dismutase (SOD) [37], which converts superoxide radicals ($O_2^{\bullet-}$) into hydrogen peroxide (H_2O_2), and by supporting glutathione peroxidase (GPx) in detoxifying hydrogen peroxide and lipid peroxides [38].

The ameliorative effect of vitamin E against chemical toxicity has been explored in several epidemiological and clinical studies [39]. Cellular damage of toluene via the formation of ROS was effectively ameliorated with the use of vitamin E [40]. Therefore, antioxidant therapy may be an important strategy for managing thinner-induced toxicity. Despite the plethora of works on the influence of thinner on central nervous system disruption [7, 41-42], there is still scanty information on the high possibility of thinner abuse impacting other organs. Therefore, this study was designed to extensively evaluate thinner-induced multiple organ damage in Wistar rats and the ameliorative potential of vitamin E.

2. Materials and Methods Chemicals

ABRO # 11 nitrocellulose Thinner (Part. No. #11-4-5L) (was obtained from ABRO Industries, International. (Court South Bend, USA), while all other reagents, of analytical grade, were sourced from the British Drug Houses (Poole, Dorset, UK).

Animal Protocol and Ethical Considerations

A total of 48 juvenile male Wistar rats (100g) were acquired and bred in the Central Animal House at the College of Medicine, University of Ibadan, Ibadan, Nigeria. The rats were housed under standardized lighting conditions with food and tap water available ad libitum. All procedures were conducted under the Institutional Animal Care and Use Committee (IACUC) protocol of the Institute of Health, United States of America. Every effort was made to minimize animal suffering, ensuring the study met ethical standards.

Experimental Design

The study design is as follows:

Group 1 (Control): Rats were unexposed to thinner fumes via inhalation throughout the treatment
Group 2 (Vitamin E only): Rats were orally administered with vitamin E 500mg/kg body weight 72 hourly for 3 weeks after being acclimatized for 3 weeks.
Group 3 (Thinner): Rats were exposed to thinner fumes via inhalation at 3000ppm, 1 hour per day for 3 weeks following a 3-week acclimatization period.
Group 4 (Thinner + Vitamin E): Rats were exposed to thinner fumes via inhalation at 3000ppm, 1 hour per day for 3 weeks and orally administered with 500mg/kg body weight of Vit. E 72 hourly in the same period.

Inhalation Protocol

The inhalant used in this study was paint thinner, commonly sold in cans and typically used to thin oil-based paints and also to clean excess paint from work surfaces or application tools. The experimental animals were divided into four groups (n=12 per group): Control, Vitamin E only, Thinner, and Thinner + Vitamin E. The amount of inhalant used was modified based on a preliminary study by Baydas et al. [43] and Fifel et al. [44]. The conversion factor used was (1 µl≈1.5 ppm). Specifically, 1.5-2.0ml of liquid thinner was applied to a filter paper placed on a glass Petri dish, which was covered with a wire mesh and positioned on the floor of the inhalation chamber, achieving an estimated thinner concentration of 3000 ppm. To examine the effects of acute thinner exposure, Thinner, and Thinner + Vitamin E groups rats were exposed to 3000 ppm thinner inhalation (1 h/day) between 1700 and 1800 h [43], for 21 days in the whole inhalation chamber. The exposure to thinner was performed in whole-body inhalation as described by Fifel et al., [44], while the control and Vitamin E-only groups had no thinner exposure. The potential mitigating effects of Vitamin E following 21 days of thinner exposure were tested in the Thinner + Vitamin E group, with oral administration of 500 mg/kg body weight of Vitamin E every 72 hours for 21 days.

Body Weight Assessment

Each of the animals were weighed at the commencement of the experiments, during the time of experiment, body weight of the animals was measured two times in a week for all groups using a standard electronic weighing balance. The data obtained was documented for further analysis such as relative organ and body weight.

Biochemical Assays.

Blood samples were collected into lithium heparin bottles and centrifuged at 3500 rpm to obtain plasma, which was then stored at -20ºC until analysis. Biochemical values were measured using an automatic chemistry analyzer, Cobas® Integra 400 plus (Roche Diagnostics Ltd., Switzerland). The parameters assayed included serum

creatinine, blood urea nitrogen, alkaline phosphatase (ALP), alanine transaminase (ALT), aspartate transaminase (AST), and total bilirubin (TBIL).

Antioxidant Assay

Following treatment, rats from each group were sacrificed by rapid decapitation on ice. Organs (liver, kidney, lungs, and spleen) were removed using the method described by Folarin et al. [35 45]. The tissues were homogenized in eight volumes of 50 mM phosphate buffer, and the homogenate was centrifuged at 10,000×g for 15 minutes at 4 °C. The supernatant (post-mitochondrial fraction) was collected and stored at −20 °C for biochemical analysis. Oxidative stress in organ homogenates was assessed by measuring malondialdehyde (MDA) levels. Antioxidant enzyme activities were evaluated by measuring superoxide dismutase (SOD), glutathione peroxidase (GPx), reduced glutathione (GSH), and glutathione transferase (GST) using commercially available kits from Northwest Life Sciences Specialties, Washington, USA. The activity of catalase (CAT) was determined using kits from Oxford Biomedical Research, Michigan, USA.

Histopathological Preparations of the Tissues

Immediately after sacrifice, the liver, kidney, lungs, and spleen were excised and fixed in 10% buffered formalin. The tissues underwent conventional processing techniques, including dehydration, clearing, and infiltration, before being embedded in paraffin wax. Sections of 5 μm were cut using a Leica RM 2115 rotary microtome. These sections were stained with hematoxylin and eosin for light microscopy. Photomicrographs were captured at ×100 magnification using a spinning disc laser confocal system (Nikon Eclipse 80i) with Nikon Microphotography system (DS-Fi1, NIS-Elements BR 3.2 software). Histopathological examination was conducted to identify the presence of lesions in the organs.

Statistical Analysis

Data on body weight, relative organ weight, biochemical, and antioxidant analyses were analyzed using one-way analysis of variance (ANOVA), with post hoc comparisons made using the Tukey test. Results are presented as means ± standard error of the mean, and significance was determined at $p < 0.05$.

3. Results Examination of Body Weight

The study involved an examination of the body weights of animals over the course of exposure to the toxicant (thinner). Notably, there was a progressive increased bodyweight in rats exposed to thinner only from week 1 to 4. However, there was a marked decline in bodyweight from week 4 downwards when compared to the control and Vitamin E (Figure 1). Conversely, rats exposed to thinner and Vitamin E displayed a noticeable increase in body weights from week 1 – 4 then followed by a decrease between week 4-5 and a steady progressive increase from week 5 to 6.

Biochemical Examination

The results on biochemical assays are presented in Tables 1 & 2. The liver function marker [aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and total bilirubin (TBIL)] levels were significantly higher in the rats exposed to thinner only compared to the control. However, co-administration of vitamin E with thinner markedly reduced the serum level of AST, ALT, ALP and TBIL when compared to the group exposed to acute thinner only (Table 1). Regarding the renal function markers, rats exposed to acute thinner showed significantly higher creatinine and blood urea nitrogen when compared to the control. Similarly, the level of the renal markers was significantly reduced in rats treated with co-administration of thinner +Vit E compared to thinner only (Table 2).

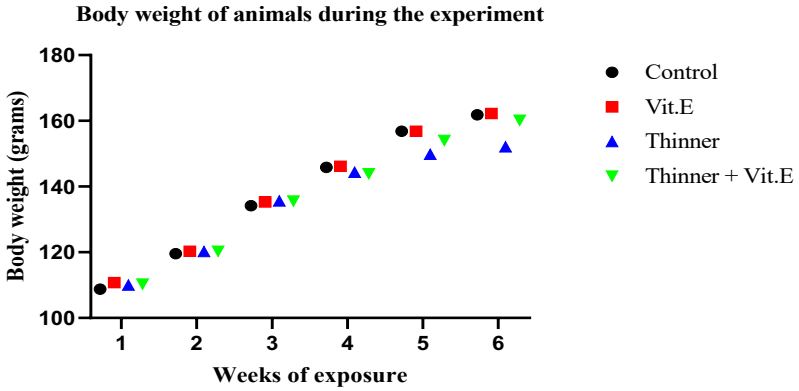

Figure 1. Profile of body weight of rats during the course of the study. There was reduction in body weights during the period of exposure for toxicity group when compared with the control. However, Toxicity+ Vit. E group showed increase in weight during recovery period with treatment with Vit.E when compared with the Toxicity Group.

Table 1. Effect of acute thinner inhalation (500ppm) on liver function markers in male Wistar rats.

Treatments	AST (U/L)	ALT (U/L)	ALP (U/L)	TBIL (µmol/L)
Control	11.2 ± 0.58	12.2 ± 0.37	48.4 ± 1.27	0.48 ± 0.06
Thinner	28.0 ± 0.71 [a]	31.0 ± 0.71 [a]	77.6 ± 1.81 [a]	1.44 ± 0.12 [a]
Thinner + Vit. E	17.2 ± 0.86[d]	23.6 ± 0.51 [d]	52.2 ± 0.86 [d]	0.88 ± 0.06[c]
Vit. E	8.6 ± 0.51	9.6 ± 0.51	42.6 ± 1.08	0.62 ± 0.06

AST: aspartate aminotransferase; ALT: alanine aminotransferase; ALP: alkaline phosphatase; TBIL: total bilirubin; Values with different superscripts are significantly different; *p*-values ([a]p<0.001: Thinner compared with normal control); ([b]p<0.05, [c]p<0.01, and [d]p<0.001: Thinner compared with Thinner +Vit. E); Vit.E vs Control: not significantly different (ns).

Table 2. Serum creatinine and Blood Urea Nitrogen levels after acute thinner inhalation (500ppm) in male Wistar rats

Treatments	CR (µmol/L)	BUN (mg/dL)
Control	0.58 ± 0.004	36.20 ± 1.36
Thinner	2.58 ± 0.007 [a]	101.4.4 ± 1.6 [a]
Thinner + Vit.E	1.4 ± 0.007[d]	62.60 ± 2.79 [d]
Vit.E	0.62 ± 0.06	42.6 ± 0.93

CR: Creatinine; BUN: Blood Urea Nitrogen. Values with different superscripts are significantly different; *p*-values: ([a] p<0.0001: Thinner compared with normal control); ([b]p<0.05, [c]p<0.01, and [d]p<0.001: Thinner+ Vit. E compared with Thinner); [Vit.E vs Control: not significantly different (ns)].

Oxidative Stress Marker Profiles in the Liver, Lungs, Kidneys, Spleens and Testes of Rats Exposed to Thinner

There was a marked increase in hepatic MDA level of thinner only exposed rats compared to the control and Vit. E groups (Figure 2). However, the MDA level was significantly reduced in thinner + Vit E group when compared to thinner only group. With the exception of the non-significant difference (p>0.05) in the hepatic nitrites levels across the different treatment groups, the levels of the hepatic endogenous antioxidant (GSH, SOD, GST and CAT) enzymes were significantly decreased (p<0.05) in the thinner only treated rats when compared to the control. On the other hand, co-administration of thinner and Vit E elicited significantly elevated (p<0.05) level when compared to thinner only (Figure 2).

The trend in the changes in the oxidative stress markers observed in other organs (lungs, kidneys, spleen and testes) seemed to be similar to the profile recorded for liver (Figures 3, 4, 5 and 6). The MDA and nitrite levels in the lung, renal, splenic and testicular tissues were significantly elevated ($p<0.05$) in the thinner only treated rats when compared to the control and Vit E. Interestingly, the MDA and nitrite profiles were significantly reduced ($p<0.05$) in thinner + Vit E group relative to thinner only. With respect to the endogenous enzymes (GSH, SOD, GST and CAT) in the four tissues, the thinner only treated rats displayed markedly reduced levels when compared to the control. However, the endogenous antioxidant levels were remarkably elevated in rats co-administered with thinner and Vit E when compared to thinner only.

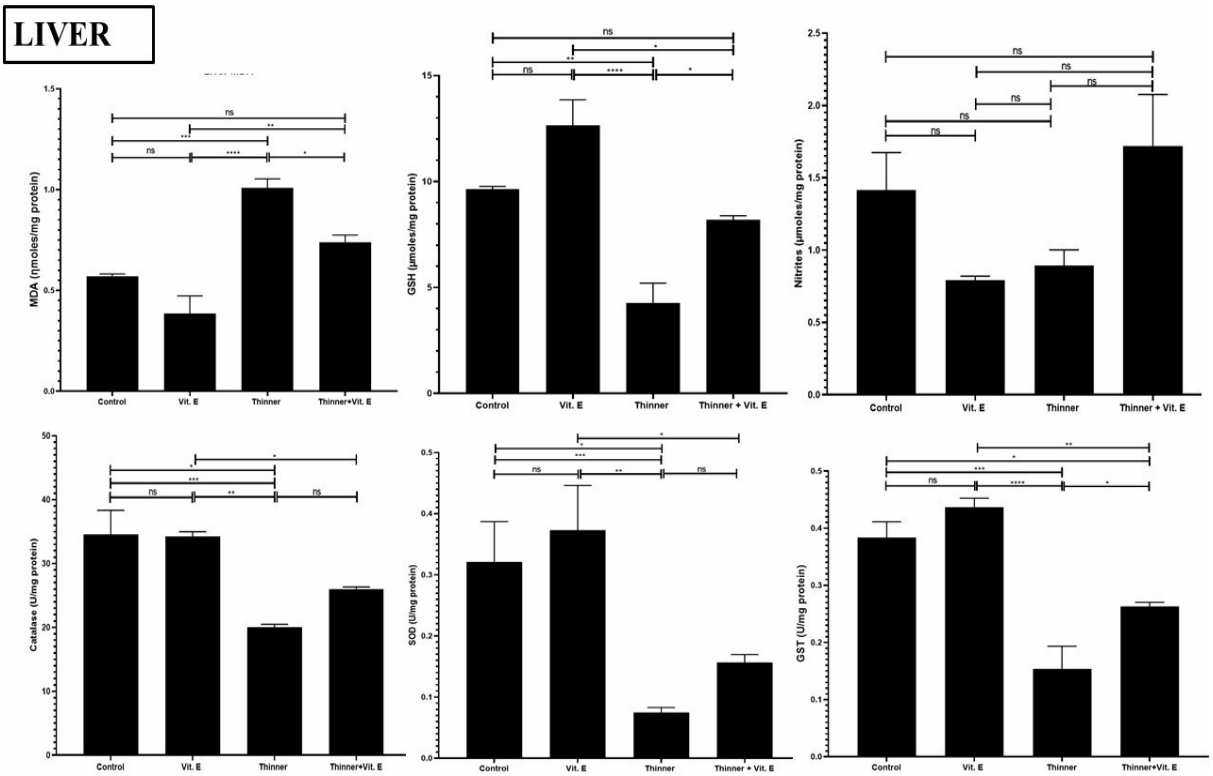

Figure 2. Changes in the oxidative stress markers profile in the liver of rats exposed to acute thinner inhalation (500ppm). Bars with different asterisks are significantly different; *p*-values ($^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$, $^{****}p<0.001$ & ns (not significantly different) were established between the experimental groups.

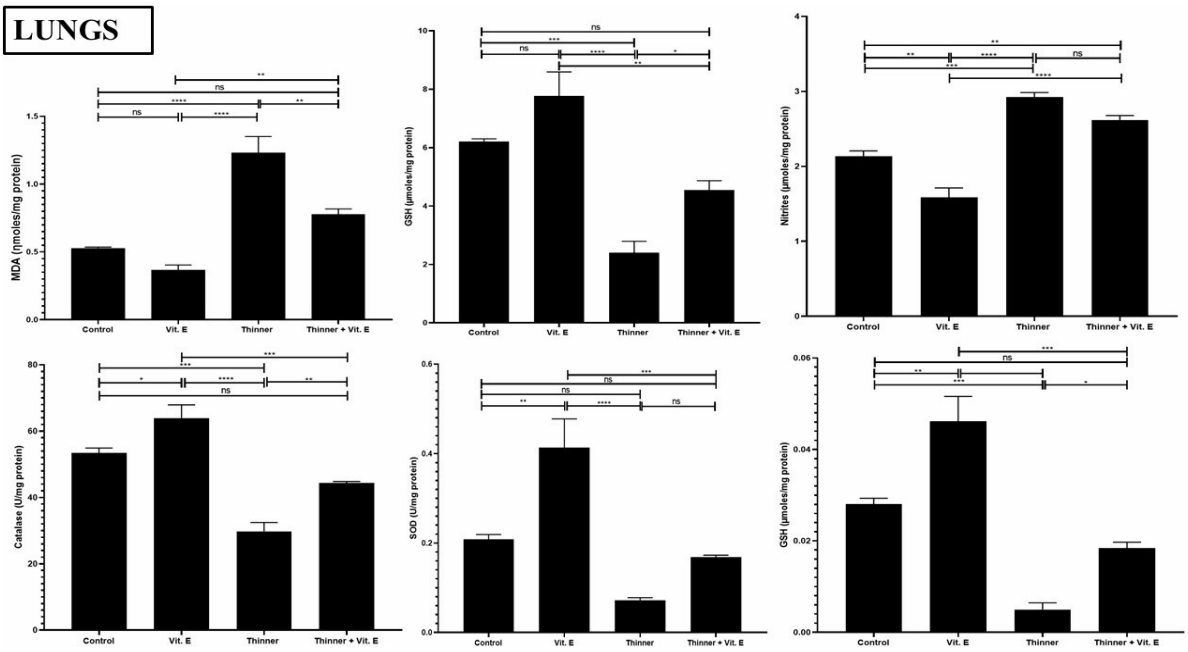

Figure 3. Changes in the oxidative stress markers profile in the lungs of rats exposed to acute thinner inhalation (500ppm). Bars with different asterisks are significantly different; *p*-values ($^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$, $^{****}p<0.001$ & ns (not significantly different) were established between the experimental groups.

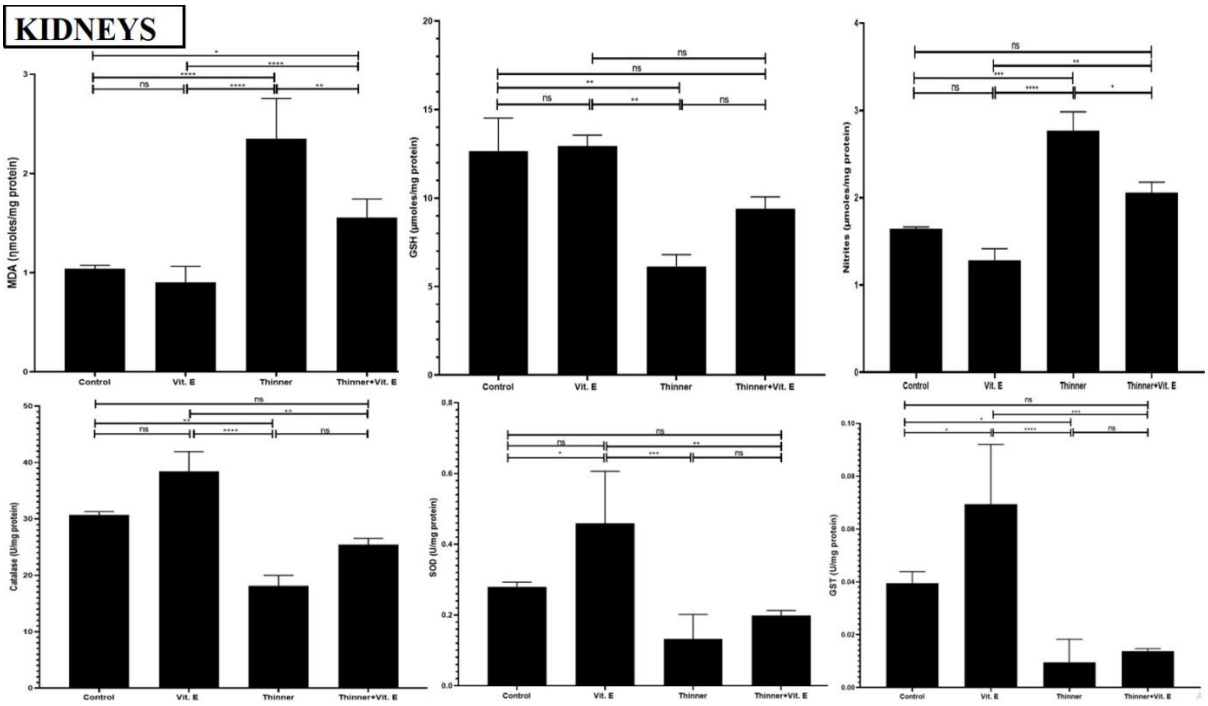

Figure 4. Changes in the oxidative stress markers profile in the kidneys of rats exposed to acute thinner inhalation (500ppm). Bars with different asterisks are significantly different; *p*-values ($^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$, $^{****}p<0.001$ & ns (not significantly different) were established between the experimental groups.

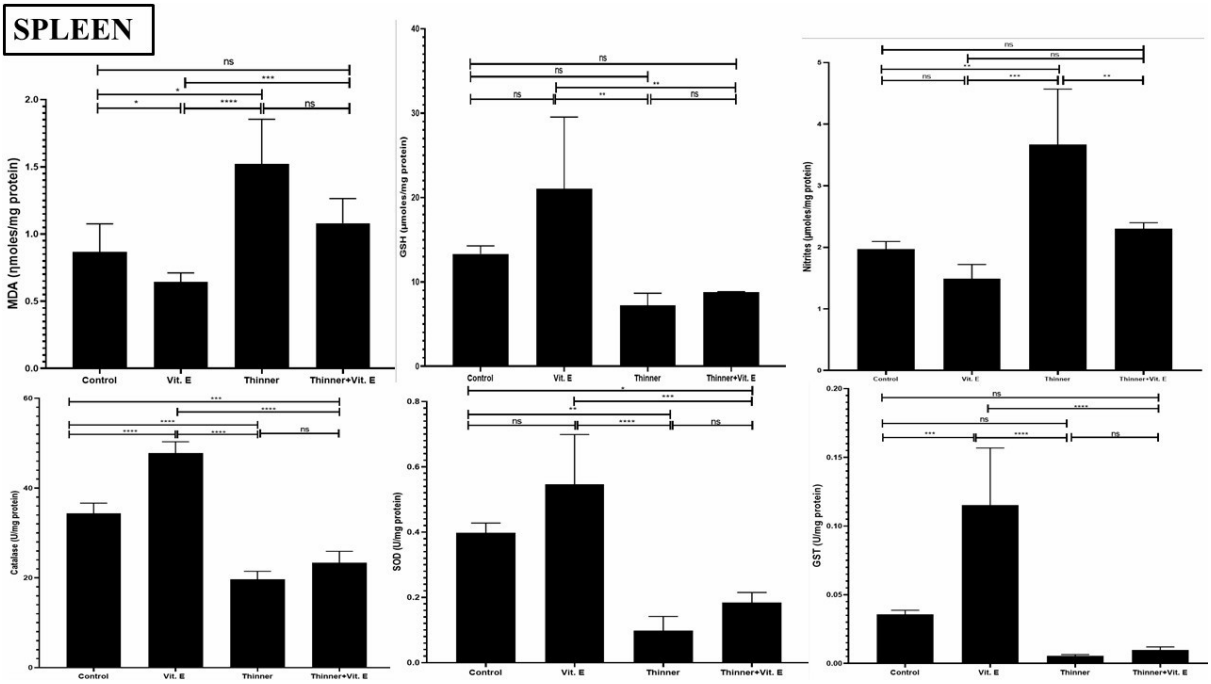

Figure 5. Changes in the oxidative stress markers profile in the spleen of rats exposed to acute thinner inhalation (500ppm). Bars with different asterisks are significantly different; *p*-values ($^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$, $^{****}p<0.001$ & ns (not significantly different) were established between the experimental groups.

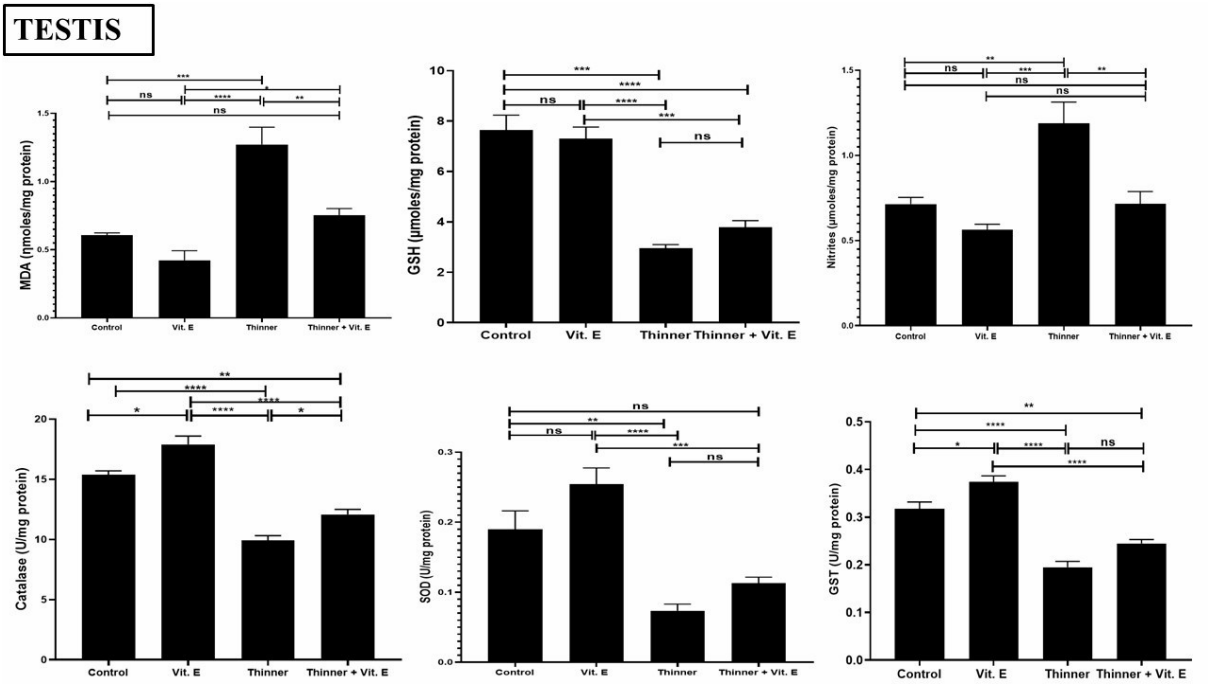

Figure 6. Changes in the oxidative stress markers profile in the testis of rats exposed to acute thinner inhalation (500ppm). Bars with different asterisks are significantly different; *p*-values ($^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$, $^{****}p<0.001$ & ns (not significantly different) were established between the experimental groups.

Histopathological Examination

The histopathological results from this study were presented in Figures 7-11. The hepatic parenchyma was devoid of lesions in rats treated with Vit. E only and the control rats (Figure 7A & B). However, moderate to severe hepatic histo-architectural alterations (hepatocellular degeneration and sinusoidal congestion) were observed in Vit. E + thinner (Figure 7D) and thinner only group (Figure 7C) respectively.

The lung tissue parenchyma was intact in the control, Vit E only and thinner +Vit E treated rats (Figure 8A, B & D). On the contrary, the lung histomorphological integrity was markedly disrupted in the thinner only exposed rats when compared to others (Figure 8C).

With regard to the renal tissue histological integrity, the kidneys of the control, Vit. E only and thinner +Vit E treated rats were devoid of histo-architectural alterations (Figure 9A, B & D). Conversely, there was a marked renal interstitial congestion (arrowhead) (Figure 9C) in the thinner only rat.

The splenic histo-architecture was not altered in the control and Vit E only treated as typified by the presence of intact white and red pulp (Figure 10A & B). However, moderate to severe splenic histomorphological disruptions (congestion of red pulp) were observed in thinner + Vit E (Figure 10D) and thinner only (Figure 10C) respectively.

The testicular seminiferous tubules in the control and Vit. E exposed rats bear normal histo-architectural organization characterized by distinct seminiferous tubular germinal epithelium, considerable luminal contents and intact interstium (Figure 11A & B). However, rats exclusively given thinner and those co-administered thinner and vitamin E (Figure 11 C & D) showed only mild focal germinal epithelial depletion when compared to the control rats.

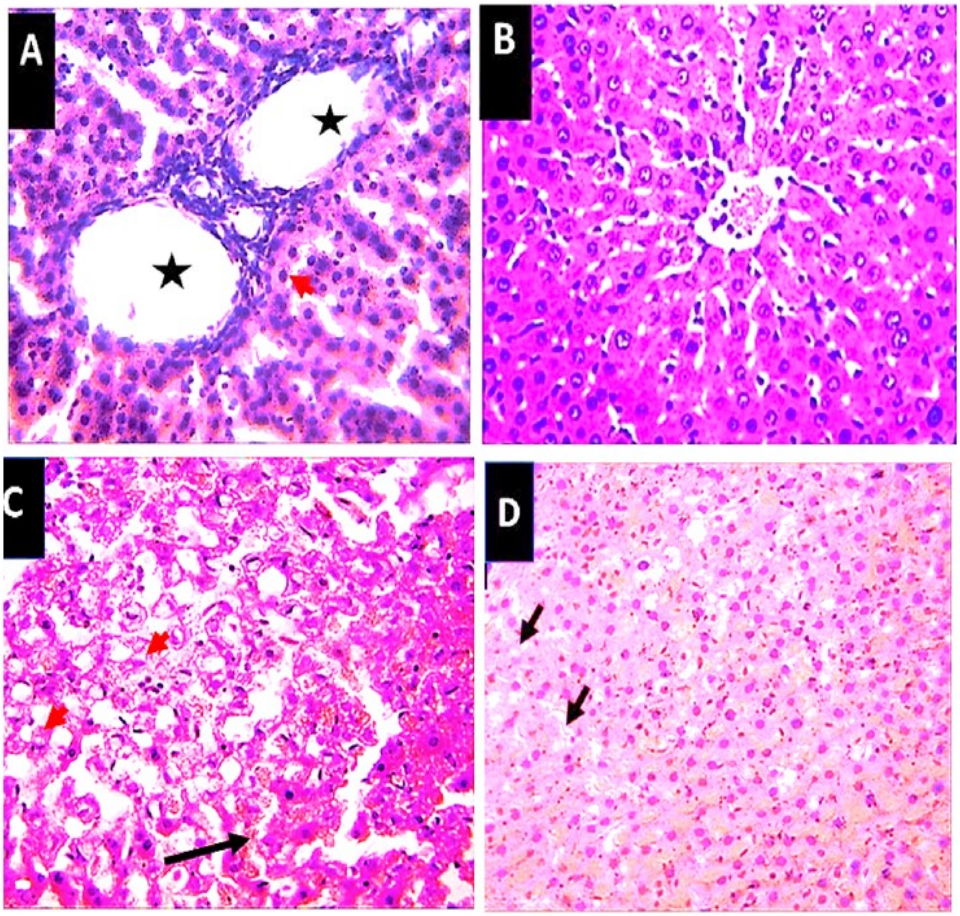

Figure 7. Histopathology of the liver of rats exposed to acute thinner inhalation (500ppm) from 3 weeks of exposure: A (Control) & B (Vit. E only): showed normal liver histoarchitecture as evidenced by intact hepatocytes (red arrowhead) and portal vessels (star). C (Toxicity): showed severe sinusoidal congestion (black arrow) with marked hepatocellular degeneration (red arrowhead). D (Toxicity + Vit. E): showed moderately diffused sinusoidal congestion (black arrow) Magnification: X400; Stain: Haematoxylin-eosin.

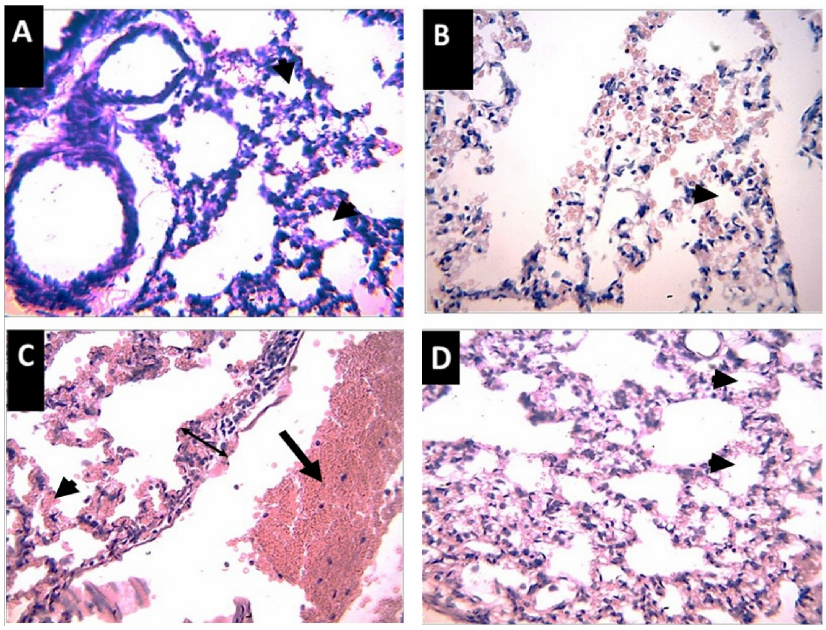

Figure 8. Histopathology of the lungs of rats exposed to acute thinner inhalation (500ppm) from 3 weeks of exposure: A (Control), B (Vit E only) & D (Toxicity + Vit E): showed normal histoarchitecture of the lungs including intact alveoli (arrowhead). C (Toxicity): showed peribronchiolar hemorrhage (black arrow), interstitial congestion with hemorrhagic foci in the alveoli (black arrowhead) and pneumocytes proliferation. Magnification: X400; Stain: Haematoxylin-eosin.

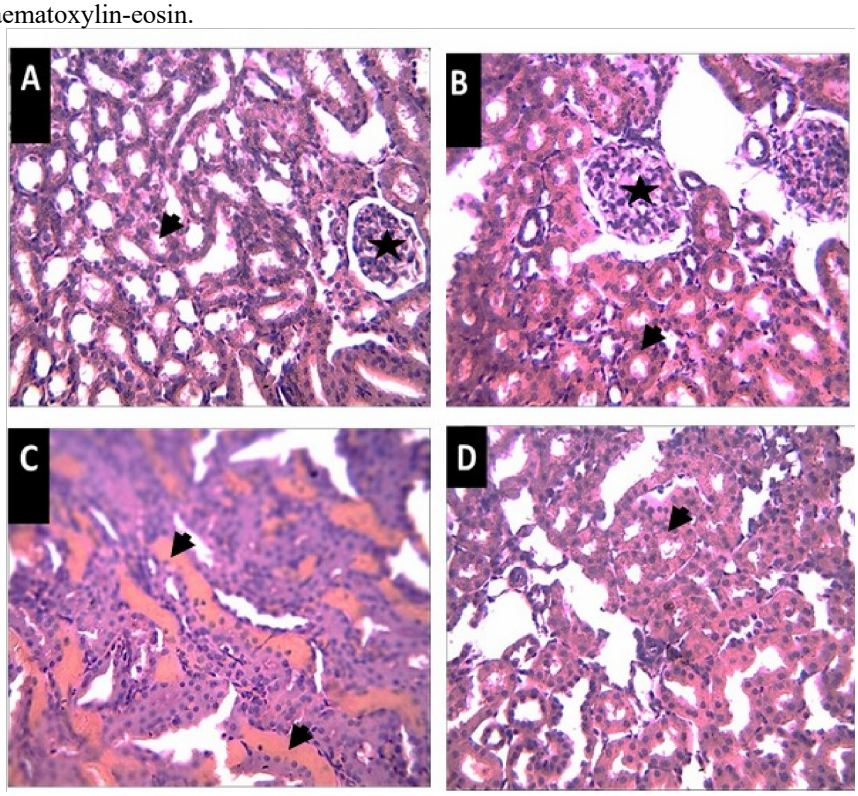

Figure 9. Histopathology of the kidney of rats exposed to acute thinner inhalation (500ppm) from 3 weeks of exposure: A (Control), B (Vit E only) & D (Toxicity + Vit E): showed normal histoarchitecture as evidenced by intact glomerulus (star) and renal tubular epithelium (black arrowhead). C (Toxicity): showed very severe renal interstitial congestion (arrowhead). Magnification: X400; Stain: Haematoxylin-eosin.

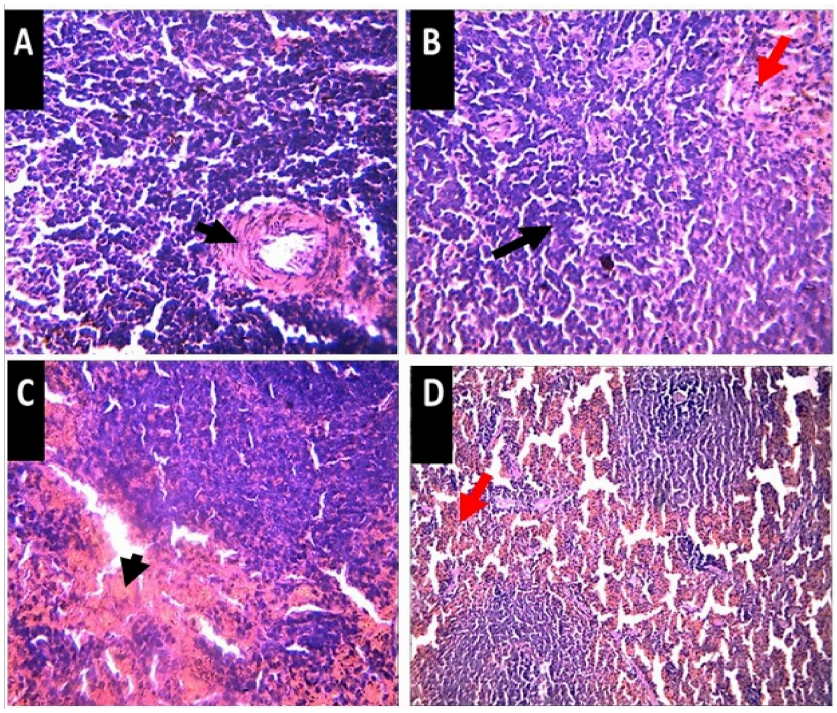

Figure 10: Histopathology of the spleen of rats exposed to thinner inhalation (500ppm) from 3 weeks of exposure: A (Control) & B (Vit E only): splenic parenchyma is devoid of lesion as revealed by intact white pulp (black arrow), red pulp (red arrow) and splenic arteriole (arrowhead) C (Toxicity): severed generalized congestion of red pulp (black arrowhead). D (Toxicity + Vit E): showed moderate congestion of red pulp (red arrow). Magnification: X400; Stain: Haematoxylin-eosin.

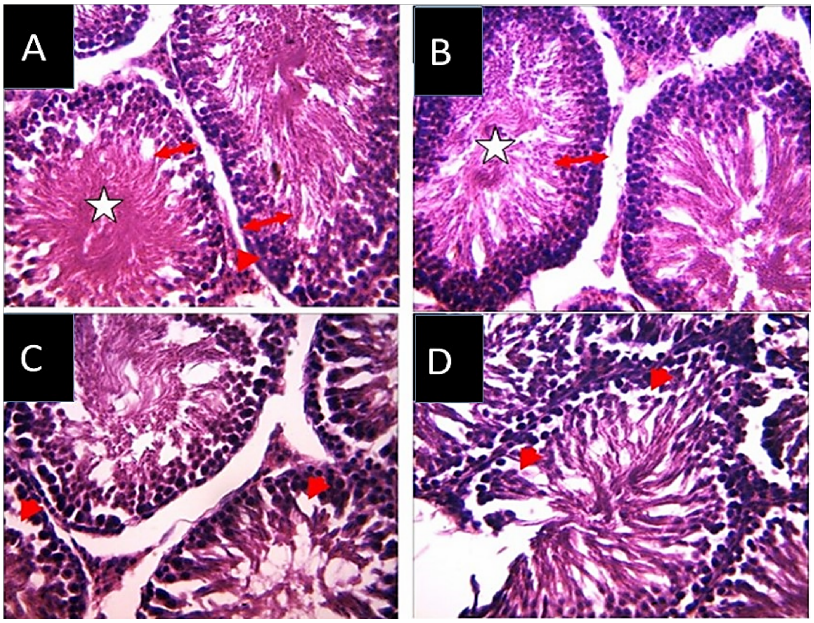

Figure 11. Photomicrographs of the testes of rats exposed to thinner inhalation (500ppm) and exposed to Vitamin E. **A. Control**; **B. Vitamin E only:** There is no visible histo-architectural alterations as evidenced by intact seminiferous germinal epithelium (double arrow-head), substantial luminal contents (star) **C. Thinner only; D. Thinner + Vit E:** With the exception of mild germinal epithelial focal depletion (arrowhead), there was no visible histo-architectural alterations to the testes. (H & E: X 100).

4. Discussion

This study demonstrated that thinner, a toluene rich solvent, intoxication resulted in some multiple organ toxicities as reflected in significant increase in biochemical parameters (AST, ALT, ALP, total bilirubin, BUN and creatinine), deranged antioxidant marker profiles and marked histomorphological alterations. Remarkably, vitamin E co-administration with thinner strikingly ameliorated the perturbed biochemical and antioxidant profiles as well as improved the compromised histo-architectural integrity.

The body weight of laboratory animals is a crucial indicator of distress and can independently signify pain and discomfort [46 – 48]. Therefore, the significant decrease in body weight observed in rats exposed to acute thinner inhalation compared to the control rats seemed to be in tandem with reports of consistent gradual decline in body weights and appetite in rats exposed to acute thinner inhalation as previously documented by MartínezAlfaro et al. [49] and Malloul et al. [50]. The validation for the decrease in the body weight sequel to gum sniffling has been suggested by Brohi et al. [51] and Fialkow et al. [24] to be due to progressive muscle weakness, nerve denervation and loss of functions potentially emanating from increased tissue free radical production leading to lipid and protein degradation. Interestingly, rats co-administered with vitamin E and thinner showed a significant improvement in body weight loss. This finding on improved body weight following acute toluene intoxication and treatment with vitamin E is consistent with report of Muti et al. [40].

Hepatic function tests or markers consist of arrays of blood tests that are usually performed for the assessment of the preclinical studies and understanding of the liver health [52]. Bearing this in mind, the increased levels of hepatic function markers such as AST (aspartate aminotransferase), ALT (alanine aminotransferase), ALP (alkaline phosphatase), and TBIL (total bilirubin) in rats treated solely with thinner indicated significant hepatocellular damage. These findings further underscore the toxic nature of thinner, as previously documented in the literature by Rahimi et al. [53] and Lala et al. [54]. Vitamin E is well-known for its potent antioxidant properties as documented by Rizvi et al. [55]. It functions by neutralizing reactive oxygen species generated during oxidative stress. Therefore, it is reasonable to suggest that the markedly reduced serum levels of hepatic markers observed in rats co-administered with thinner and Vitamin E may stem from the antioxidative actions of Vitamin E. In essence, Vitamin E likely mitigates the hepatocellular damage induced by thinner exposure through its ability to scavenge free radicals and protect cells from oxidative stress. This suggests a potential therapeutic role for Vitamin E in attenuating liver injury associated with toxic exposures such as thinner. The observed liver markers findings in our study concurs with the report of Sekihashi et al [56] and Upperman et al. [57].

The renal function markers, including serum creatinine and blood urea nitrogen (BUN), provide valuable insights into the health status of the kidneys. In the context of rats exclusively treated with thinner, these markers markedly deranged and the elevated levels of serum BUN and urea nitrogen primarily indicate severe renal morpho-physiological alterations that are detrimental to the normal functioning of the kidneys. These findings suggest renal dysfunction, possibly resulting from the toxic effects of thinner on renal tissue. Interestingly, the improved renal function markers observed in rats treated with both thinner and vitamin E lend credence to the potential protective effect of vitamin E against thinner-induced renal damage. The amelioration of renal function markers in the presence of vitamin E suggests that vitamin E may mitigate the adverse effects of thinner on kidney function, possibly through its antioxidant properties. The renal function marker profile recorded in this study agree with report of Latief et al. [58].

Lipid peroxidation is a process wherein free radicals attack lipids containing carbon-carbon double bonds, leading to the formation of reactive lipid peroxides. The remarkable increase in malondialdehyde (MDA) levels across all the visceral organs (liver, lungs, kidneys, and spleen) of rats exposed only to thinner could be attributed to the reactive oxygen generating potential of thinner. This signifies the heightened oxidative stress induced by thinner exposure. On the other hand, the decrease in MDA levels in the visceral organs resulting from the co-administration of Vitamin E with thinner appeared to mitigate the oxidative damage to the organs caused by thinner treatment. This observation further reinforces the antioxidant properties of Vitamin E in combating oxidative stress. In similar manner, the nitrite composition in the tissues is also indicative of increased oxidative stress. Therefore, the elevated nitrite levels in the various organs of rats treated solely with thinner, as measured in this study, further confirms the oxidative damage caused by thinner exposure. The observation of a remarkable reduction in nitrite levels in the various tissues of rats exposed to both thinner and Vitamin E lends support to the notion that Vitamin E effectively mitigates oxidative stress induced by thinner exposure. These findings from our study on nitrite levels in the internal organs corroborate with the work of Sekihashi et al. [56].

Endogenous antioxidants refer to the body's natural defense mechanisms against oxidative stress. These include Glutathione (GSH), Superoxide Dismutase (SOD), Glutathione-S-Transferase (GST), and Catalase (CAT). The observed decline in the levels of endogenous antioxidants (GSH, SOD, GST, CAT) enzymes in the different visceral organs of rats exposed solely to thinner suggests a depletion of the body's antioxidant defense mechanisms in response to oxidative stress. However, Pajaro-Castro et al. [59] reported increased in GST gene

expression after thinner and toluene exposure. Conversely, the significantly elevated levels of endogenous enzymes observed in rats exposed to both thinner and co-administered Vitamin E indicated the protective effect of Vitamin E in preserving the antioxidant defense mechanisms against thinner-induced oxidative stress.

The observed severely damaged hepatic parenchyma in rats exclusively exposed to thinner, characterized by marked hepatocellular degeneration and sinusoidal congestion, confirms the potential toxic nature of thinner which align with previous report by Tas et al. [60]. These histomorphological alterations align with the biochemical and antioxidant derangements recorded for this category of rats in the study. The histological results provide further evidence of the adverse effects of thinner on liver health. However, the co-administration of thinner and Vitamin E noticeably improved the severe histo-architectural disruptions elicited by thinner. This observation aligned with the histological findings reported by Bansal et al. [61] in a similar study that investigated the protective role of N-nitrosodiethylamine induced oxidative stress in rat liver. Thus, it suffices to suggest that Vitamin E may have a protective effect against the hepatic damage induced by thinner exposure, as evidenced by the restoration of liver histology towards normalcy.

The distinct histo-architectural disruptions observed in the lungs of rats exclusively exposed to thinner, such as peri-bronchiolar hemorrhage, interstitial congestion, and pneumocyte proliferation, further substantiate the toxic potential of thinner on lung health which mirrors observations by Fujimaki et al. [24] and Muti et al. [40]. On the other hand, the moderate distortion of lung parenchyma histomorphology noticed in rats treated with Vitamin E in addition to thinner, relative to rats exposed solely to thinner, suggests that Vitamin E supplementation may provide a partial protection against lung damage induced by thinner. In addition, the renal histological alterations, particularly parenchymal interstitial congestion, observed in rats treated exclusively with thinner, further establish the nephrotoxic potential of thinner which correspond with reports of Martinez-Alfaro et al. [7] and Latief et al. [58]. Conversely, the marked renal histomorphological amelioration observed in the form of visibly intact renal histo-architecture in rats treated with both thinner and Vitamin E suggests that coadministration of Vitamin E may protect against renal damage induced by thinner exposure. The findings on ameliorative potential effect of Vitamin E on kidney tissue in this study corroborate the reports of Baltusnikiene et al. [62].

The respective histological variations, ranging from moderate to severe, in splenic histo-morphological integrity of rats treated with thinner + Vitamin E and thinner only, depict the slight mitigating potential of Vitamin E against the inherent toxic effect of thinner on splenic morphology. These lesions observed as red pulp congestion and hemorrhagic foci in the spleen, are consistent with a previous work [63]. The moderate splenic histological disruption observed could be attributed to the antioxidant properties of Vitamin E, which might have helped in counteracting some of the damage caused by thinner exposure. Multi-organ toxicity recorded in this study appeared to further validate the previous documentation on paint thinner fumes inhalation reported by Zaidi et al. [64].

The observed apparently intact testicular histomorphological integrity in both thinner only and thinner + Vit. E treated rats implies that testicular tissues markedly tolerate thinner toxicity relative to other visceral organs. Though, the data on oxidative stress markers in this study contradicts the histological results as the former established the toxic potential of thinner. It is envisaged that if the exposure time is prolonged beyond the 21 days used in study, there is great chances of presentation of thinner induced testicular histological alterations. The testicular results from this study is at variance with the report of precipitation of testicular histological lesions by Kanter [65].

5. Conclusion

The study suggests that exposure to thinner induces oxidative stress and organ damage, particularly in the liver, lungs, kidneys, spleen and testes. Vitamin E supplementation shows promise in mitigating thinner-induced toxicity by reducing oxidative stress, preserving organ function and ameliorating histopathological alterations. These findings underscore the importance of antioxidant therapy in mitigating the adverse effects of toxicant exposure and highlight Vitamin E as a potential therapeutic agent against thinner toxicity. However, further research, including dose-response studies and investigation of underlying mechanisms, is warranted to fully understand the protective effects of Vitamin E and its potential clinical applications in toxicant-induced organ damage.

Ethics

The study was approved by the University of Ibadan Animal Care and Use Research Ethics Committee (UIACUREC), under ethical code number UI-ACUREC/APP/21/133.

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