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# Fansidar drug induces cytotoxicity in some vital tissues in a rat model: combination defensive effect of selenium and zinc capsules

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***Abstract***

**Aim:** Fansidar (FAN) is widely used as an antimalarial drug, but it may cause hepatoxicity, nephrotoxicity, and neurotoxicity. Hence, the study examines the cytoprotection of selenium (Se) and zinc (Zn) tablets against FAN induced toxicity.

**Method:** Group I was given distilled water. Groups II, III, IV, and V received 50 mg/kg FAN by gavage. Group III was co-treated with a 50 mg/kg Se tablet. Group IV was co-treated with a 50 mg/kg Zn tablet. Group V was co-treated with a 50 mg/kg Se tablet + 50 mg/kg Zn tablet. The exposure lasted for 7 days (sub-acute exposure).

**Result:** FAN causes cytotoxicity through significant ($p < 0.05$) alteration of antioxidant molecules and hepatic enzymes. It also significantly ($p < 0.05$) induces renal, hepatocyte, and purkinje cell damage, but no visible lesion on testicular cells. The FAN induced cytotoxicity was significantly ($p < 0.05$) reversed on treatment with both single and combined antioxidant tablets.

**Conclusion:** Our study supports the view that antioxidant micronutrient (Se and Zn) tablets may be a useful modulator in alleviating FAN induced oxidative stress and cytotoxicity in male rats.

## Plain language summary

### Combined selenium and zinc capsules: better therapy against cytotoxicity

Fansidar was approved by United States' Food and Drug Administration as an anti-malarial drug to treat acute and complicated malaria fever among patients in West Africa; however, its usage elicits toxicity to several organs of the body. It was elucidated that the combination of selenium and zinc capsules promotes organ wellness on co-treatment with Fansidar.

***Keywords:*** capsule, cytotoxicity, drug, fansidar, selenium, zinc

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## Introduction

In the last decades, the use of synthetic drugs has greatly increased. For instance, Fansidar (FAN), popularly known as sulfadoxine and pyrimethamine, was approved by United States' Food and Drug Administration as an anti-malarial drug to treat acute and complicated malaria fever among patients in West Africa.[1–3] The mechanism of FAN action is either *via* inhibition of *Plasmodium falciparum* or decreasing its biomass per cycle in the erythrocyte.[4,5] However, studies have reported FAN as a cytotoxicity agent in the liver, kidney, and brain.[6,7] It was also reported that repeated intake of FAN can cause epidermal necrolysis in humans and experimental animals by acting as a mediator of hepatomegaly and nephrotoxicity.[8]

Further studies have also showed that treatment of malaria in pregnant women by sulfadoxine and pyrimethamine caused severe cutaneous adverse reactions, teratogenic abnormality, and alterations in bilirubin production.[9,10] In addition, several studies have reported the toxicity of anti-malarial drugs in mice models; however[11,12] their effects on rat models have been scarcely studied. This forms the choice of rat model in this study.

In addition, drugs obtained from natural products or micronutrients have shown alternative therapeutic agents to annul the side effects of various synthetic drugs.[9,10] For this reason, natural multiple drugs are advocated owing to their efficacy and non-toxic action.[12] Selenium capsules are antioxidants found in trace amount in foods.[13] They help in metabolism and exist as selenocysteine, selenomethionine, and selenite in the body.[13] Selenium triggers the activity of metalloenzymes, glutathione peroxidase, and thioredoxin reductase.[14,15] Recent studies have reported that dietary supplementation of selenium nanoparticles alone could modulate systemic and mucosal immune status and stress resistance of red sea.[16] Similarly, zinc inhibits nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, which catalyzes the assembly of the singlet oxygen radical from oxygen by using NADPH as an electron donor.[17] It acts as a co-factor of superoxide dismutase, which converts the singlet oxygen radical into hydrogen peroxide.[18] Zinc activates the level of metallothionein, the scavenger of hydroxyl radical.[19] Competitively, zinc competes with copper while binding the cell wall to decrease the generation of hydroxyl radicals.[19] Other studies had suggested that the zinc drug alone exhibits pharmacological properties as antioxidant activity, radical scavenging, and cytoprotective properties.[13,20] Collectively, studies had reported the anti-inflammatory, anti-apoptotic, anti-ulcer, and anti-osteoporotic activities of the Se and Zn capsules.[14] Zn and Se protect against metabolic syndrome, genetic damage, and central nervous system (CNS) disorders.[21,22] Considering that enzymatic antioxidants [catalase (CAT) and superoxide dismutase (SOD)] and non-enzymatic antioxidant such as glutathione (GSH) have the potential to inhibit overproduction of reactive oxygen species (ROS) with a corresponding depletion of malondialdehyde (MDA) levels, we speculated that the combined intake of Se and Zn capsule (Se-Zn interaction) will alleviate the FAN-induced oxidative damage better than single administration.

As a result, this study investigates the cytoprotective effect of natural capsules (Se and Zn) against oxidative stress and histopathological changes in sub-acute FAN induced toxicity in rat model.

## Materials and methods

### *Chemicals and reagents*

Epinephrine, reduced GSH, and hydrogen peroxide were purchased from Sigma (St. Louis, MO, USA). All other reagents were of analytical grade

### *Animal protocol*

Forty (40) healthy adult male Wistar rats weighing approximately 200–220 g were obtained from the Department of Biochemistry, Bells' University of Science and Technology Ota, Nigeria. They were acclimatized for 2 weeks and randomly assigned into five groups of eight animals per group ($n = 8$). They were housed in a wooden suspended cage placed in a well-ventilated rat house, provided rat pellets and water *ad libitum*, and subjected to a natural photoperiod of 12-h light and 12-h dark cycle. Group 1 was given distilled water only *via* an oral route. Group 2 was given 50 mg/kg FAN only. FAN is usually taken by patients when treating malaria. Group 3 (FAN + Se) was co-treated with 50 mg/kg FAN and a 50 mg/kg selenium tablet. Group 4 (FAN + Zn) was co-treated with 50 mg/kg FAN and a 50 mg/kg zinc tablet. Group 5 (FAN + Se + Zn) was co-treated with 50 mg/kg FAN and a 50 mg/kg selenium tablet and a 50 mg/kg zinc tablet. FAN dose was selected following the previous method of Michalová *et al.*[23] Se (50 mg/kg) and Zn (50 mg/kg) tablets were chosen because they are pharmaceutically prepared as 50 mg. People do apply these doses directly as prescribed by the physicians. The rats were fed with the same standard food and had free access to drinking water throughout the experiment. The experiment lasted for 1 week (7 days). The Se and Zn drugs are pure curative drugs purchased from a pharmaceutical shop in Sango Ota, Nigeria. All the animals received humane care according to the criteria outlined in the 'Guide for the Care and Use of Laboratory Animals' prepared by the National Academy of Science and published by the

National Institute of Health.[24] Ethic regulations were followed in accordance with national and institutional guidelines for the protection of animal welfare during experiments. The identification approved number of the researcher is-2458

### *Necropsy*

The animals fasted overnight, weighed, and sacrificed by decapitation 24 h after the last treatment and blood samples were collected by cardiac puncture. Livers, kidneys, testes, and brain were removed and cleared of adhering tissues, washed in ice-cold 1.15% potassium chloride and dried with blotting paper. The blood was allowed to clot and centrifuged at low speed (3000*g*) at room temperature for 15 mins. The supernatant (serum) was removed and used for the determination of biochemical parameters. The liver, kidney, testes, and brain tissues were collected and cut into pieces, fixed in Bouin's fluid for 6 h, then transferred to formalin, sectioned, and stained routinely with hematoxylin and eosin for histopathological examination.

### *Enzyme assay*

The livers, kidneys, brain, and testes were homogenized in 50 mM Tris-HCl buffer (pH 7.4) containing 1.15% KCl and the homogenate was centrifuged at 10,000*g* for 15 mins at 4°C. The supernatant was collected for the estimation of CAT activity using hydrogen peroxide as a substrate according to the method of Sinha.[25] Protein concentration was determined by the method of Lowry *et al.*[26] Serum alanine aminotransaminase (ALT) and serum aspartate aminotransaminase (AST) were estimated by the method of Reitman and Frankel.[27]

### *GSH assay*

Reduced GSH was determined at 412 nm using the method described by Jollow *et al.*[28]

### *Histopathological evaluation*

The liver, kidney, and brain were fixed in 10% formalin while testes were fixed in Bouin's solution. Morphological examination of the aforementioned tissues was done using an ocular micrometer scale under the light microscope

### *Data analysis*

The results of the replicates were pooled and expressed as mean and standard error. A one-way analysis of variance (ANOVA) was used to analyze the results and Duncan multiple tests were used for the *post hoc*.[29] Statistical Package for Social Science (SPSS) 17.0 for Windows was used for the analysis and the least significance difference (LSD) was accepted at $p < 0.05$.

## Results

### *Effect of antioxidant micronutrient (Se and Zn) drugs on oxidative stress biomarkers (GSH and catalase)*

Tissue enzymatic and non-enzymatic antioxidants in animals treated with FAN for 7 days is presented in Table 1. *Post hoc* analysis showed that GSH level (liver, kidney, and testes) was significantly ($p < 0.05$) depleted by sub-acute administration of 50 mg/kg/day FAN for 7 days (Table 1) when compared to their corresponding control groups. The level of GSH brain (Table 1) was not significantly reduced when compared with the control, due to its non-brain-targeted drug. However, treatment with single and combined antioxidant micronutrient (50 mg/kg selenium or 50 mg/kg zinc) drugs prevented the GSH decrease in the liver, kidney, and testes (Table 1). In addition, the administration of 50 mg/kg/day FAN to male rats for 7 days significantly ($p < 0.05$) exacerbated the CAT activity in the liver, kidney, and testes when compared with the control group (Table 2). FAN administration (50 mg/kg) inhibited the SOD activity when compared with the control (Table 2). Antioxidant micronutrient (Se and Zn) drugs significantly ($p < 0.05$) prohibited the altered CAT activity in both single and combined administration (Table 2)

### *The effect of antioxidant micronutrient (Se and Zn) drugs on liver enzyme activity in FAN-induced toxicity rats*

Liver enzyme activity reflects the extent of organ toxicity is presented in Table 3. As observed, the activities of serum AST and ALT, which are the key biochemical markers of hepatocellular necrosis and indicators of hepatic injuries significantly ($p < 0.05$) increased in male rats treated with 50 mg/kg/day FAN when compared with the corresponding control (Table 3). The co-treatment

**Table 1.** Effect of Se and Zn drugs on the level of reduced GSH in FAN-induced toxicity of selected vital tissues in male rats.

| Treatment | GSH (mg/ml) | | | |
|---|---|---|---|---|
| **Group** | **Liver** | **Kidney** | **Testes** | **Brain** |
| Control | $45.17 \pm 6.98^{a}$ | $42.97 \pm 2.55^{a,b}$ | $39.38 \pm 4.97b$ | $35.85 \pm 4.61^{a}$ |
| FAN only | $44.81 \pm 8.18^{a}$ | $39.09 \pm 2.28^{a}$ | $33.58 \pm 0.81^{a}$ | $32.39 \pm 2.03^{a}$ |
| FAN + Se | $72.31 \pm 11.35^{c}$ | $45.98 \pm 4.26^{b}$ | $37.59 \pm 4.67^{b}$ | $35.90 \pm 2.57^{a}$ |
| FAN + Zn | $61.30 \pm 10.38^{b}$ | $42.49 \pm 6.76^{a,b}$ | $33.00 \pm 1.75^{a}$ | $33.90 \pm 2.70^{a}$ |
| FAN + Se + Zn | $74.25 \pm 14.21^{c}$ | $44.12 \pm 4.55^{b}$ | $37.68 \pm 3.51^{b}$ | $35.39 \pm 4.04^{a}$ |

Results are presented as mean ± SD; $n = 8$; values with different letters on the same column are statistically different ($p < 0.05$).
FAN, Fansidar; GSH, glutathione; SD, standard deviation; Se, selenium; Zn, zinc.

**Table 2.** Effect of Se and Zn drugs on the activity of CAT in FAN-induced toxicity of selected vital tissues in male rats.

| Treatment | CAT (μmol $H_2O_2$ consumed/min/mg protein) | | | |
|---|---|---|---|---|
| **Group** | **Liver** | **Kidney** | **Testes** | **Brain** |
| Control | $31.42 \pm 2.85^{b}$ | $15.45 \pm 1.49^{a}$ | $7.39 \pm 5.76^{a,b}$ | $7.26 \pm 0.31^{a,b}$ |
| FAN only | $50.50 \pm 4.43^{c}$ | $26.51 \pm 1.57^{a}$ | $9.56 \pm 5.01^{b}$ | $5.90 \pm 0.33^{b}$ |
| FAN + Se | $48.91 \pm 3.12^{b,c}$ | $22.17 \pm 1.16^{a}$ | $5.34 \pm 3.41^{a}$ | $10.64 \pm 0.61^{a}$ |
| FAN + Zn | $17.22 \pm 2.63^{a}$ | $25.64 \pm 13.08^{a}$ | $4.11 \pm 1.53^{a}$ | $11.12 \pm 0.81^{a}$ |
| FAN + Se + Zn | $49.94 \pm 14.21^{b,c}$ | $20.07 \pm 1.46^{b}$ | $8.29 \pm 4.56^{a,b}$ | $9.60 \pm 0.80^{a,b}$ |

Results are presented as mean ± SE; $n = 8$; values with different letters on the same column are statistically different ($p < 0.05$).
CAT, catalase; FAN, Fansidar; $H_2O_2$, hydrogen peroxide; Se, selenium; SE, standard error; Zn, zinc.

of male rats with 50 mg/kg Se and 50 mg/kg Zn antioxidants dropped the hepatic activity of AST and ALT in both single and combined administration (Table 3).

### *The effect of antioxidant micronutrient (Se and Zn) drugs on histopathological changes in liver tissues*

Figure 1 showed that administration of FAN drug (50 mg/kg/day) for 7 days caused degeneration, dissociation, and necrosis of hepatocytes revealing inflammation of the hepatic cells. In contrast, the hepatic lesions on administration with 50 mg/kg selenium and 50 mg/kg zinc drugs were abated (Figure 1). Single treatment with a 50 mg/kg zinc drug showed moderate dissociation of hepatocytes while co-treatment with 50 mg/kg selenium and 50 mg/kg zinc drugs depicted moderate individualization of hepatocytes with no visible lesions to the hepatocytes (Figure 1).

### *Effect of antioxidant micronutrient (Se and Zn) drugs on histopathological changes in renal tissues*

Male rats treated with FAN drug (50 mg/kg) per day for 7 days displayed contracted glomerulus and expanded space with distended Bowman's capsule and random tubular necrosis; in addition,

**Table 3.** Effect of Se and Zn drugs on the activities of ALT and AST in FAN-induced toxicity in male rats.

| Treatment | Bio-indicator | |
|---|---|---|
| **Group** | **ALT (U/l)** | **AST (U/l)** |
| Control | $89.76 \pm 1.99^{a,b}$ | $55.64 \pm 0.98^{b,c}$ |
| FAN only | $94.87 \pm 1.64^{b}$ | $57.12 \pm 0.91^{c}$ |
| FAN + Se | $93.60 \pm 0.99^{b}$ | $51.91 \pm 1.36^{a}$ |
| FAN + Zn | $86.78 \pm 2.07^{a}$ | $53.78 \pm 1.46^{a,b,c}$ |
| FAN + Se + Zn | $86.73 \pm 1.64^{a}$ | $52.39 \pm 1.04^{a,b}$ |

Results are presented as mean ± SE; $n = 8$; values with different letters on the same column are statistically different ($p < 0.05$).
ALT, alanine aminotransferase; AST, aspartate aminotransferase; FAN, Fansidar, Se, selenium; SE, standard error.

it also showed hyaline cast (Figure 2). The focal interstitial inflammation was equally observed on administration with 50 mg/kg FAN-an antimalarial drug. The abnormities exhibited irregular kidney function following necrosis of the nephrons. In contrast, the animals administered with 50 mg/kg Se or 50 mg/kg Zn drug reflected tubular ectasia, hyalinization, and congestion of glomerular capillary (Figure 2).

### *The effect of antioxidant micronutrient (Se and Zn) drugs on histopathological changes in testicular tissues*

As reflected in Figure 3, there was little or no impairment to testicular cells in male rats treated with FAN drug (50 mg/kg) for 7 days. Similarly, the 50 mg/kg Zn drug treated group had no visible lesions; in contrast, the administration of 50 mg/kg Se drug showed mild degeneration of tubular basement membrane (Figure 3). The group treated with both Se and Zn drugs showed no visible lesions to Leydig and Sertoli cells.

### *The effect of antioxidant micronutrient (Se and Zn) drugs on histopathological changes in neuronal cells*

Figure 4 showed that animals treated with the FAN drug (50 mg/kg/day) for 7 days exhibited neuronal disintegration in the nucleus and necrosis of the neurons. The necrosis of Purkinje cells (indication of cognitive misbehavior, memory dysfunctions, Alzheimer's, and Parkinson disease) was also observed on administration with 50 mg/kg/day of FAN (Figure 4). Combined administration of Se and Zn drugs reversed the lesions by showing no severe visible lesions; however, no mild ischemic change and vacuolation of the white matter were recorded in the brain (Figure 4).

## Discussion

Recently, the safety of the use of sulfadoxine, pyrimethamine, and artemisinin as anti-malarial drugs cannot be overemphasized in humans. At therapeutic doses, FAN is metabolized *via* glucuronidation, and acetylation reactions occurring primarily in the liver and form water-soluble metabolites that are excreted in the kidney. Following the metabolic conversion of FAN by the microsomal CYP-450 enzyme, a highly reactive intermediate is formed.[10] The highly reactive intermediate directly reacts with GSH, causing the depletion of cellular GSH. This consequently binds to cellular proteins and initiate lipid peroxidation, leading to renal and hepatic injury.[15] The generation of reactive oxygen species attacks other extrahepatic tissues causing brain damage.[19] Current evidence reports that intracellular GSH depletion plays a crucial role in detoxification of FAN-induced toxicity in the brain, liver, and kidney.[10] Remarkable reduction in the tissue concentrations of glutathione was linked to drug-induced cellular damage including nephrotoxicity, hepatotoxicity, and neurotoxicity.[30] Consistent with these studies[21,22,31]; FAN administration in our study led to the depletion of GSH with corresponding alteration of catalase activity. This occurrence initiated substantial peroxidation of membrane lipids on treatment with FAN in the tissues of rats. The declined antioxidant status confirmed the mechanism of nephrotoxicity, hepatoxicity, neurotoxicity, and cytotoxicity induced by FAN, due to free radical generation which implicated damage to the cells, tissues and organs. Micronutrients, as antioxidant drugs, can scavenge various types of radicals in aqueous and lipid milieu.[32] The co-single and co-combined treatment of Se and Zn drugs and micronutrients, to rats however restored the activity of these endogenous antioxidant molecules in the tested tissues. The recovery of these molecular

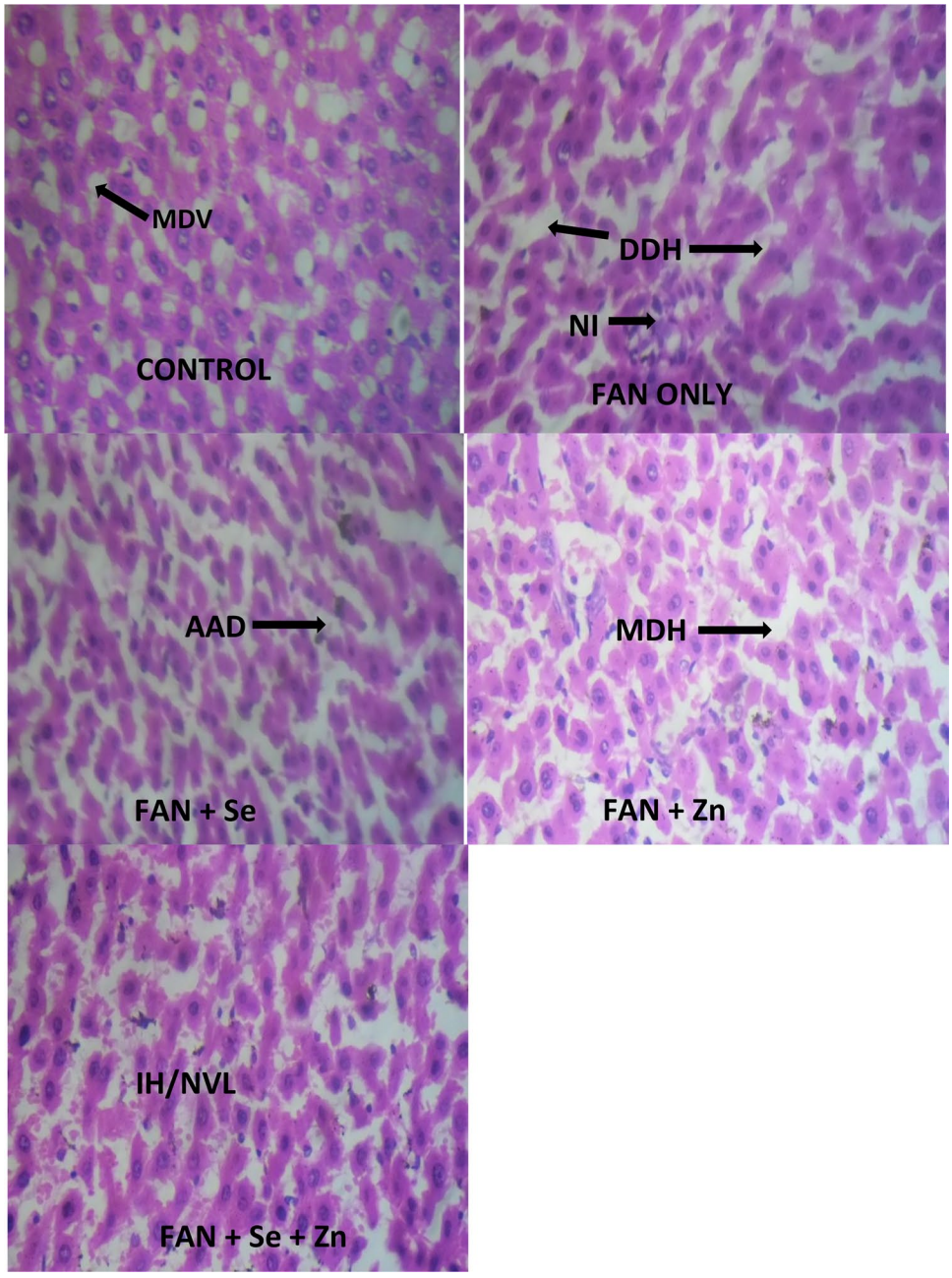


**Figure 1.** Hepatic histopathology changes on rat administered with FAN, FAN + Se, FAN + Zn, and FAN + Se + Zn (original magnification ×400). (Control): there was moderate diffuse vacuolation (MDV) of hepatocytes; (FAN only): rat treated with Fansidar only showed DDH or NI of the hepatic cells (FAN + SE): the group of animals administered with Fansidar plus Se drug depicted AAD of hepatocytes; (FAN + Zn): the rat treated with Fansidar plus Zn revealed MDH; (FAN + Se + Zn) showed IH or NVL to the hepatocytes.
AAD, atrophy and dissociation; DDH, degeneration, dissociation of hepatocytes; FAN, Fansidar; IH, individualization of hepatocytes; MDH, moderate dissociation of hepatocytes; MDV, moderate diffuse vacuolation; NI, necrosis and inflammation; NVL, no visible lesions; Se, selenium; Zn, zinc.

antioxidant proteins amplifies the protection endowed by Se and Zn drugs to rats against cytotoxicity and oxidative stress induced by FAN. Its protection may be associated to their active co-factors in seleno-proteins of GSH reductases or their ability to form complexes with antioxidant

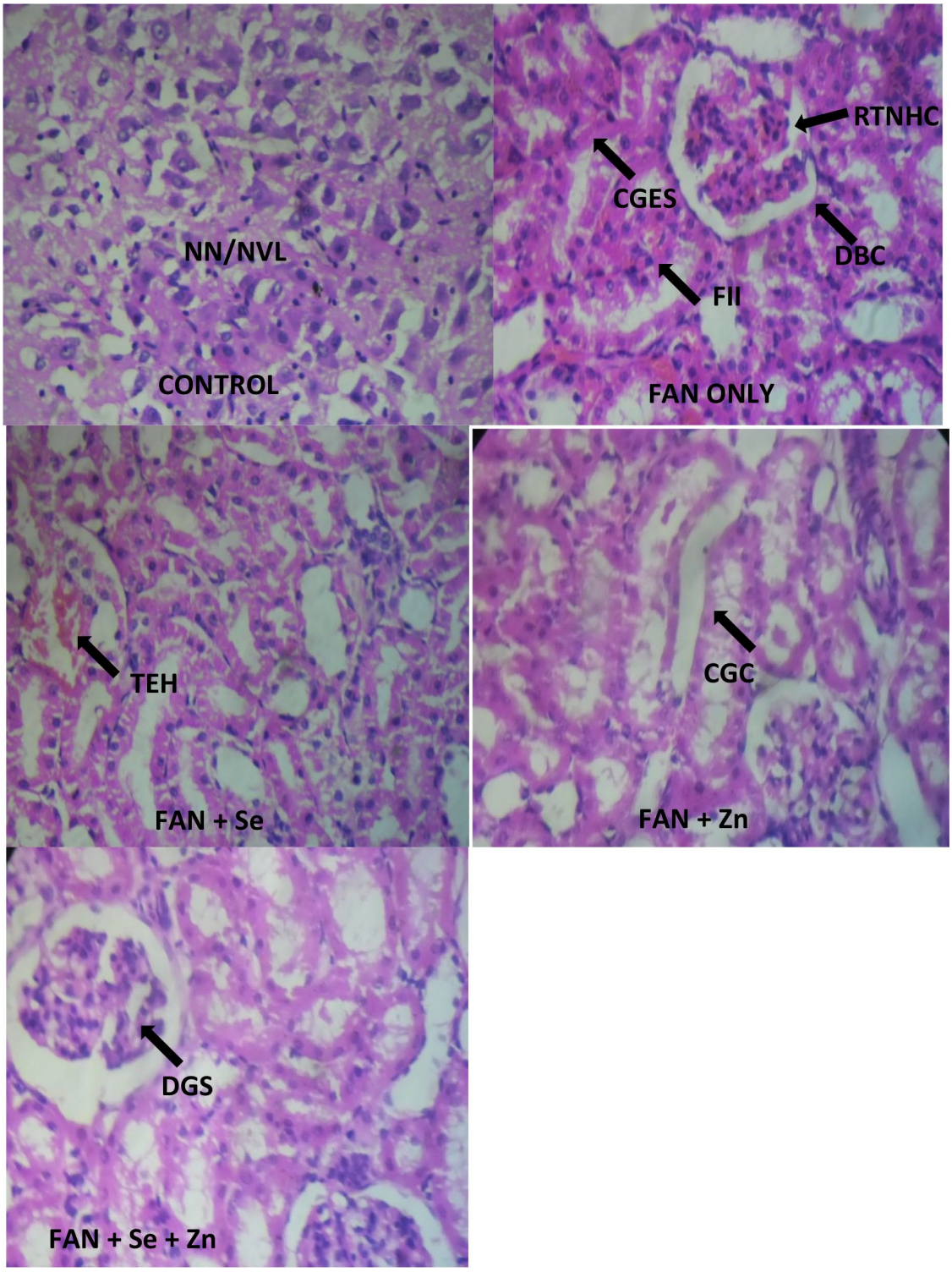


**Figure 2.** Nephritic histopathology changes on rat administered with FAN, FAN + Se, FAN + Zn, and FAN + Se + Zn (original magnification ×400). (Control): this group showed or NVL (FAN only): rat treated with FAN only showed CGES. In addition, there was DBC, and RTNHC. Furthermore, FII (macrophages, lymphocytes) was observed (FAN + Se): group of animals treated with FAN plus Se depicted TEH; (FAN + Zn): the rat administered with FAN plus Zn showed CGC; (FAN + Se + Zn): This group of animals revealed DGS and tubular coagulation.

CGC, glomerular capillary; CGES, contracted glomerulus and expanded space; DBC, distended Bowman's capsule; DGS, distended glomerular space; FAN, Fansidar; FII, focal interstitial inflammation; NN, normal nephron; NVL, no visible lesions; RTNHC, random tubular necrosis with hyaline cast; Se, selenium; TEH, tubular ectasia and hyalinization; Zn, zinc.

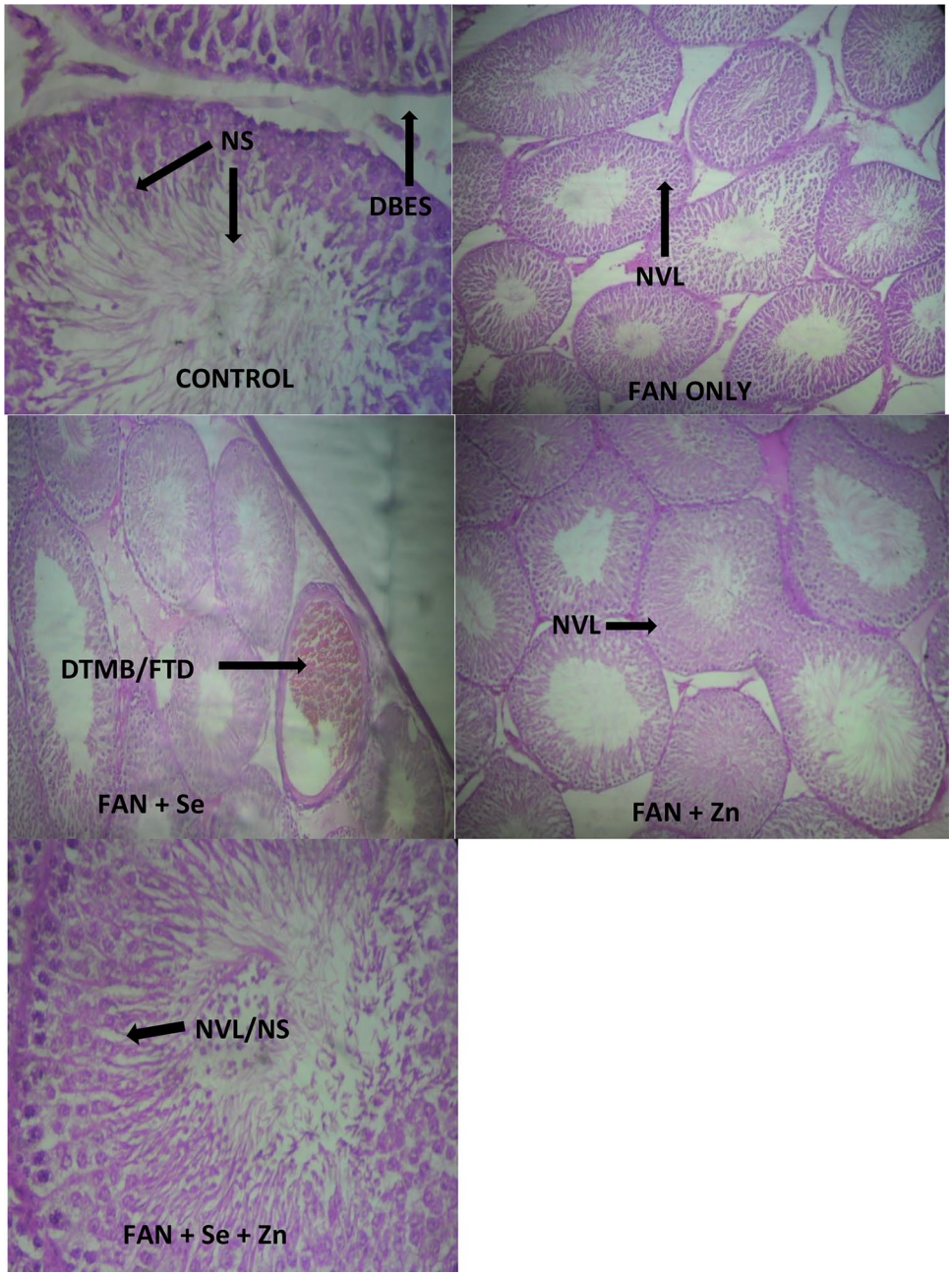


**Figure 3.** Testicular histopathology changes on rat administered with FAN, FAN + Se, FAN + Zn, and FAN + Se + Zn (original magnification ×400). (Control): this group showed NS with mild DBES; (FAN only): rat treated with FAN only showed NVL; (FAN + Se): group of animals treated with FAN plus Se depicted DTBM or FTD; (FAN + Zn): the rat administered with FAN plus Zn showed NVL; (FAN + Se + Zn): this group of animals revealed showed NVL or NS.
DBES, dissociation of basal epithelium and spermatogonia; DTBM, degeneration of tubular basement membrane; FAN, Fansidar; FTD, focal tubular degeneration; NS, normal spermatocytes; NVL, no visible lesions; Se, selenium; Zn, zinc.

enzyme.[14,15] Studies have also showed that complex formation of the antioxidant enzymes with micronutrients promote cell survival; inhibiting cellular inflammations[1,13] in human patients.[33] Early study had shown that selenium and zinc micronutrients are potent antioxidants and offer protection against tissue damage, cell death, apoptosis, cell necrosis,[34] and ATP diminution by

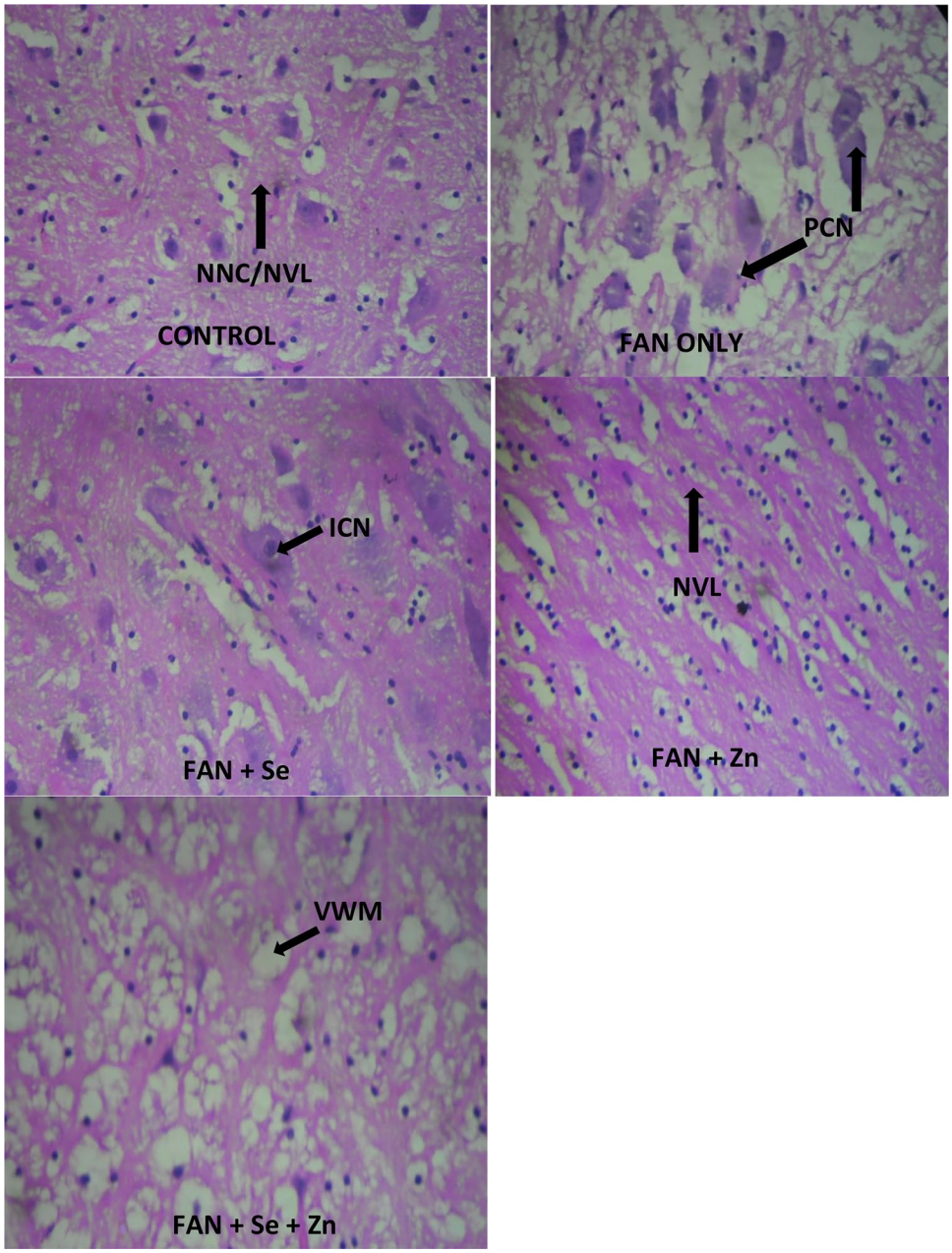


**Figure 4.** Neuronal histopathology changes on rat administered with FAN, FAN + Se, FAN + Zn, and FAN + Se + Zn (original magnification ×400). (Control): this group showed NNC or NVLs; (FAN only): rat treated with FAN only showed neuronal degeneration in the nucleus and necrosis of neurons as well as PCN; (FAN + Se): group of animals treated with FAN plus Se depicted ICN; (FAN + Zn): the rat administered with FAN plus Zn showed NVL; (FAN + Se + Zn): this group of animals revealed VWM.
FAN, Fansidar; ICN, ischemic change of the neurons; NNC, normal neuronal cells; NVL, no visible lesions; PCN, purkinje cell necrosis; Se, selenium, VNM, vacuolation of the white matter; Zn, zinc.

their synergistic and additive interaction.[35] In addition, physiological preponderance of GSH potentiated by micronutrients had been shown to interrupt biological function of the cells as well as bone marrow by abrogation of carcinogenesis and abnormal fetal developmental.[36] In particular, Se prevents the predisposition of the tissues to damage; thereby promoting cellular metabolism.[37] Zn drugs are likely to help the mitochondrial complexes leading to promotion of the electron

transport and abundance of ATP accompanied by less production of ROS that supports the antioxidant system. As a result, we supposed that both single and combined supplementation of Se and Zn are involved against FAN-mediated oxidative damage in rat tissues. A therapeutic combination of Se and Zn would offer multiple sources of H-atom donors and in turn boost the ROS scavenging capacity. Based on that premise, our current information discovered that co-administration of Se and Zn capsules could elicit better protective effect than their individual therapy against FAN toxicity, particularly in liver tissue.

Se and Zn are minerals derived from natural sources. They have strong free radical scavenging activity due to their chemical structure. Se is covalently bound to carbon in organic molecules as selenomethionine (SeMet), selenocysteine (SeCys) and Se-methylselenocysteine (SeMSC).[32] These forms can each be metabolized to selenide ($H_2Se$), which serves as a compulsory intermediate in selenoprotein synthesis.[32] SeMet can substitute for methionine (Met) in protein synthesis.[32] Selenide can also be metabolized by methylation and sugar-derivation to produce a variety of excreted products. These include selenosugars, which are the major urinary metabolites in humans, and methylselenol ($CH_3SeH$), which is regarded as the major anti-hepatic carcinogenic Se metabolite.[32] However, in the current study, Se administration reduced the liver function enzymes such as AST and ALT which may be due to formation of methylselenol ($CH_3SeH$) in the liver. In addition, high AST and ALT were reduced by Zn intake due to its ability to activates the level of metallothionein in the liver thereby preventing the hepatic necrosis of the liver cells.[38] This action reveals the hepatoprotective potential of Zn capsule[39] against FAN-induced hepatoxicity. Our previous study reported Zn capsules as natural antioxidants, anti-mediators of cellular inflammation, anti-liver cancer, and inhibitor of hepatocyte necrosis.[14] Other similar studies had also reported that Zn could prevent the accumulation of $H_2O_2$ in the hepatocytes to delay tubular ectasia, hyalinization, and inflammation.[39,40]

Histopathological examination in the present study showed that FAN caused degeneration, dissociation, and necrosis of hepatocytes revealing inflammation of the hepatic cells. This action confirms FAN as a hepatotoxin. Recent similar study reported that repeated intake of synthetic drugs could trigger injury of the hepatic cells.[14] Combined therapy of micronutrient capsules prevented the hepatic lesions. But the single administration sustains the liver viability and integrity with mild symptoms of atrophy and dissociation of hepatocytes. It is interesting to report that combined therapy of Se and Zn capsules showed a better efficacy than single administration. The failure of Se and Zn capsule to adequately obviate the toxic effect of FAN suggests that the liver is highly susceptible to synthetic drug intoxication[41] or possibly, follows pass effect metabolism. In addition, the liver is the direct site of drug metabolism.[42,43]

Furthermore, the intake of FAN exhibited contracted glomerulus and expanded space with distended Bowman's capsule including random tubular necrosis with hyaline cast. In addition, focal interstitial inflammation and nephritic necrosis following distorted glomeruli[44] was observed in FAN administered animals. The damage to renal tubular cells establishes nephrotoxicity in rats.[45] Combined as well as single therapy of Se and Zn capsule could not inhibit FAN-induced nephrotoxicity. This may be linked to the fact that nephron ultrafiltration system had been impaired beyond repair or there was uncontrollable excretion of Se and Zn micronutrients in the urine. Study had supported this finding as kidney can easily eliminate micronutrients faster in the body due to their mall sizes or trace amount availability.[13] For reproductive cells, FAN administration did not elicit significant lesions to the Leydig and Sertoli cells. This suggests that FAN drug is not an endocrine disruptor in rat model because it cannot cross the thick testicular blood barrier (TBB).[46] This action also confirms FAN as semi-lipophilicity agent.

In the study, FAN administration causes damage to Purkinje cells and neurons. Report had indicated that purkinje cell necrosis triggers cognitive misbehavior, dementia known as Alzheimer's disease. It can also lead to attention deficit/hyperactivity disorder (ADHD), communication defect and Parkinson disease.[47] This further confirms FAN as neurotoxin in rat model. The management with micronutrient capsules (Se and Zn) inhibited FAN induced neurotoxin by reversing the disintegration and necrosis of the Purkinje cells. The ripple effect or lesions after micronutrient treatment may be due to the quick oxidation of polyunsaturated fatty acids in the brain. Study

had reported that brain had low levels of antioxidants and defense proteins but utilizes high oxygen.[48–50]

## Conclusion

Micronutrient capsule (Se and Zn) promotes renal, hepatic, testicular and neuronal antioxidant molecules in rat model by inhibiting the activity of AST and ALT in the liver of rat induced by FAN. They also reversed inflammation of the hepatocytes; impairments of the nephrons, necrosis of the Purkinje cells with no significant effect in testicular cells. Therefore, we suggest that micronutrient capsule particularly Se and Zn drugs may have therapeutic potential for patients insulted with FAN toxicity.

### *Limitations of the study*

The study is expected to make use of mice model for malarial finding. However, our future finding will be based on the effect of these antioxidant drugs on malarial parasite using the malarial mice model. In addition, the finding is projected to elucidate the specific biochemical and molecular mechanisms associated with malarial toxicity.

## Conflict of interest statement

The authors declare that there is no conflict of interest.

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