Evaluation of the Antioxidant Activities of Zinc, Selenium and Vitamin B12 on Isoniazid and Rifampicin induced liver damage in mice

Abstract

Aim: This study aimed at evaluating the antioxidant activities of Zinc, Selenium and Vitamin B_{12} on liver toxicity induced by Isoniazid-Rifampicin (INH-RIF).
Study Design: Original Research
Place and Duration of Study: National Institute for Pharmaceutical Research and Development (NIPRD), Department of Pharmacology and Toxicology Idu, Abuja, Nigeria. Between August-September 2023.
Methods: 25 Swiss mice (25-35g) were divided into 5 groups, GP1 received distill water, GP 2-5 INH-RIF 150mg/kg for the first two days; while groups 3 received Selenium 1mg/kg, group 4 received VitaminB_{12} 0.01mg/kg, group 5 received Zinc 15mg/kg respectively from day 3 till day 14 orally. Sera samples were obtained, and organ (liver) for weighing and histopathology on day 15.
Results: This study indicated that Selenium (1mg/kg) showed reduction in the biochemical parameter ALT only; histo-anatomy of organ showed inflammation. Vitamin B_{12} indicated reduction in ALT and ALP and no lesion seen in the organ, Zinc showed significant decrease in the level of ALP and ALT, and the histo-picture showed no lesion compared to INH-RIF group. Weights of the liver showed significant reduction in Selenium and Vitamin B_{12} respectively compared to the toxic group.
Conclusion: It was concluded that Selenium 1mg/kg, Vitamin B_{12} and Zinc can mitigate toxic effects of Isoniazid-Rifampicin on the liver but Zinc indicated more ameliorative potentials. It was recommended that Zinc and /or Vitamin B_{12} can be co-administered with these anti-tuberculars to mitigated the toxicity that may emanate from their administration.

Key words: ameliorative, antioxidants, histo-anatomy, biochemicals

INTRODUCTION

“The liver plays a crucial role in various physiological processes in the body, such as the production of bile, vitamin K, and angiotensin hormone” [1]. Additionally, it is responsible for metabolizing substances, which exposes it to harmful xenobiotics and toxins that can potentially harm or impair its function [2].

“Isoniazid, or isonicotinylhydrazide (INH) and Rifampicin (RFP) are frequently prescribed medicine for treating both active and latent forms of tuberculosis caused by *Mycobacterium tuberculosis* infection and considered a first-line therapy option” [3,4]. “A major concern during treatment with INH is hepatotoxicity leading to even liver failure” [5]. “Isonicotinylhydrazide-induced liver toxicity is related to the production of reactive metabolites including hydrazine and acetyl hydrazine through N-acetyltransferase and amidohydrolase produced by the hepatic metabolism of this drug. Toxic and reactive metabolites of INH covalently bind to the liver macromolecules and induce oxidative damages” [6]. “It has been shown that the oxidative damage caused by INH is attributed to the formation of highly active oxygen species and alteration in various protective mechanisms, including enzymatic and non-enzymatic antioxidants” [7]. “Rifampicin-induced liver injury is mainly related to cholestasis, endoplasmic reticulum stress, and liver lipid accumulation. Rifampicin can increase INH-induced hepatotoxicity by regulating the expression of drug-metabolizing enzymes and transporters” [8]. “Regarding the role of oxidative stress in the hepatotoxicity of INH and RFP, dietary supplementation with antioxidants is proposed to prevent and treat liver toxicity” [8].

“Zinc, an essential trace element, has been reported to play various pivotal roles in the human body” [9]. “Notably, in the liver, it is needed for the activation of many enzymes, such as ornithine transcarbamylase (OTC) and glutamate dehydrogenase (GDH), which are utilized in the urea cycle and the glutamine synthetase cycle respectively. Superoxide dismutase (SOD), which requires zinc for its activation, has strong antioxidant activity” [10]. “Zinc deficiency may cause SOD inactivity, followed by increased reactive oxygen species (ROS)” [11].

“Vitamin B_{12} is stored primarily in the liver; this vitamin is essential for one-carbon metabolism and cell division” [12]. “It acts as a cofactor for two enzymatic reactions, namely, methionine synthesis from homocysteine and succinyl-CoA synthesis from methylmalonyl-CoA, in mammalian systems” [13].

“The generation of ROS in a limited dose is one of the processes induced by the immune system to destroy microbial pathogens and viruses. However, the over-production of ROS can also cause damage to the host cells that need to be protected by Selenium (Se) at various stages in the immune system” [14]. “Foods are major natural source of Se, and its levels generally depend on soil Se levels” [15]. “Since its discovery as an important component of antioxidant enzymes, such as glutathione peroxidase (GPx), thioredoxin reductase (TrxR) and iodothyronine deiodinases (IDD), there has been an increased interest

in the study of other Se-containing proteins (selenoproteins) or enzymes (selenoenzymes)" [16, 39-41].

This study therefore evaluated the antioxidant potentials of Zinc (Zn), Selenium (Se) and Vitamin B12 (Vit B12) in liver damage from Isoniazid and Rifampicin and determine which is preferable in case of liver damage during management of tuberculosis with these drugs.

Material and Methods

Experimental Animals

Swiss mice were obtained from the Animal Facility center of the Department of Pharmacology and Toxicology, National Institute for Pharmaceutical Research and Development (NIPRD). They were kept under standard environmental conditions (temperature 24 ± 2 °C) with free access to water and diet. They were kept for two weeks to acclimatized before the studies.

Induction of toxicity in animals

The animals were grouped into 5 groups of 5 animals each for the study. All administration was done orally.

Group 1 received distilled water daily as control, Group 2 negative control received (INH-RIF) (150mg/kg) orally for the first two days, Group 3 received INH-RIF (150mg/kg) for the first two days before Se (1mg/kg) on day 3 till day 14, Group 4 received INH-RIF (150mg/kg) for the first two days before $VitB_{12}$ (0.01mg/kg) on day 3 till day 14, Group 5 received INH-RIF (150mg/kg) for the first two days before Zn (15mg/kg) on day 3 till day 14.

Biochemical Analysis

After the experiments, animals were anesthetized with chloroform and blood samples collected from the heart into plain bottles for the determination of biochemical parameters such as Alkaline phosphatase (ALP), Aspartate transaminase (AST), Alanine transaminase (ALT), Well-labeled plain bottles were used to collect 5 mL of blood sample, which was allowed to clot for 4 hours before centrifuging using Uniscope Laboratory Centrifuge (Model SM 112, Surgifriend Medicals, England) at 2000 revolution per minute for 20minutes to separate the sera from clotted blood cells. Each serum was carefully separated in the plain bottles that were well label accordingly at room temperature of 23-26^{o}C. The activities of AST, ALP and ALT were estimated using Randox test kit (UK).

Gross and histopathology

The organ was identified (liver) and weighed, then sectioned and rinsed in normal saline. The tissues were fixed in 10% formal saline, dehydrated with 100% ethanol solution and embedded in paraffin, sectioned at 5µm, stained using H&E method, cleared in xylene and mounted in a mountant [17]; using the magnification of 400x.

Data analysis

Data obtained were expressed as mean± standard error of mean (SEM). One way analysis of variance (ANOVA) was used to compare the means between groups. It was followed by Dunnet test using GraphPad Prism software. $P<0.05$ was considered statistically significant.

Result

Biochemical parameters for mice treated with Selenium (1mg/kg)

Selenium (1mg/kg) showed a significant reduction (P<0.05) in ALT, and the reduction in ALP was not significant; no significant difference in Total protein (T.Pro), and AST compared to the toxic group INH-RIF. When compared Se with the normal group, There was no significant difference in AST, ALT, and T.Pro but the difference in ALP only as shown in Fig1.

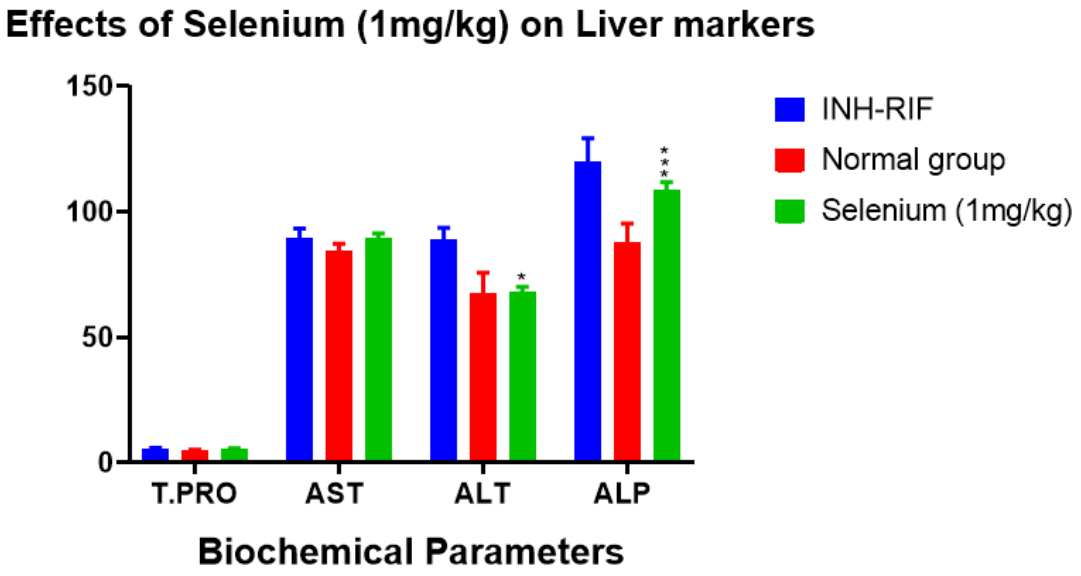

Fig 1. Effects of Se (1mg/kg) on liver biomarkers of mice with liver injury induced by Isonazid-Rifampicin, INH-RIF. AST: Aspartate Aminotransferase, ALT: Alanine aminotransferase, ALP: Alkaline Phosphatase, Normal control: normal untreated group.

Biochemical parameters for mice treated with Vitamin B_{12} (0.01mg/kg)

Vitamin B_{12} (0.01mg/kg) showed significant reduction P<0.05 (0.0026 and 0.0001) in ALT and ALP respectively but no significant difference in Total protein and AST when compared with the toxic INH-RIF group as described in Fig 2.

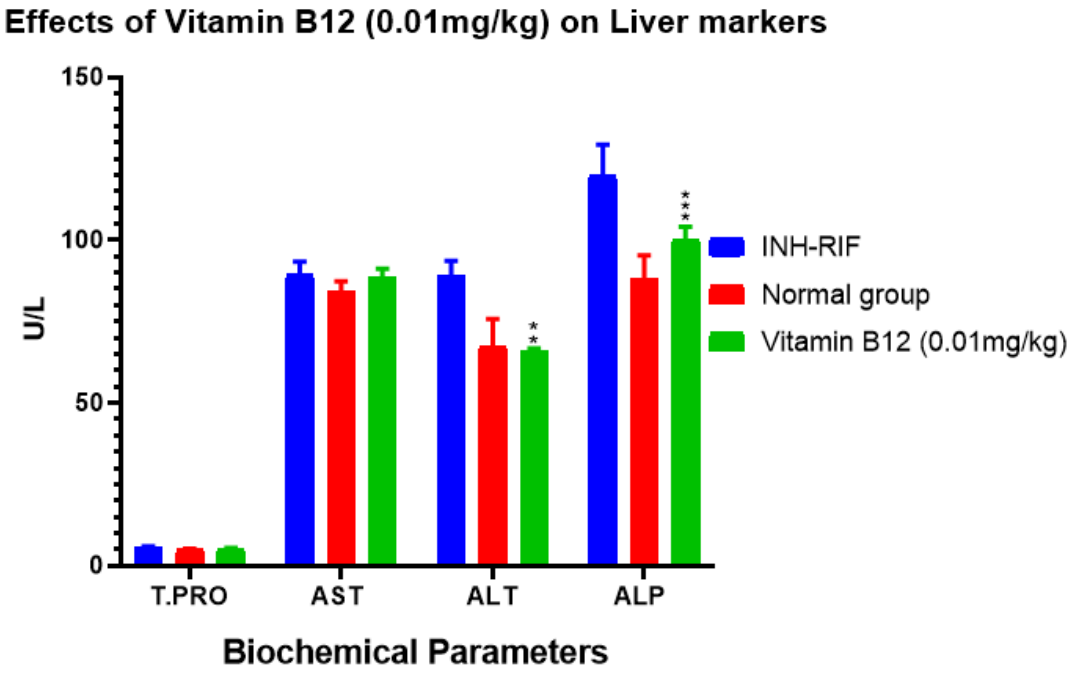

Fig 2. Effects of Vit B_{12} on liver biomarkers of mice with liver injury induced by Isonazid-Rifampicin, INH-RIF. AST: Aspartate Aminotransferase, ALT: Alanine aminotransferase, ALP: Alkaline Phosphatase, Normal control:normal untreated group.

Biochemical parameters of serum of mice treated with Zinc (15mg/kg)

Zinc (15mg/kg) showed significant reduction (P= 0.0007 and 0.0001) in the level of ALT and ALP respectively, but no significant reduction in AST and T.Pro when compared to the toxic group INH-RIF as described in fig3.

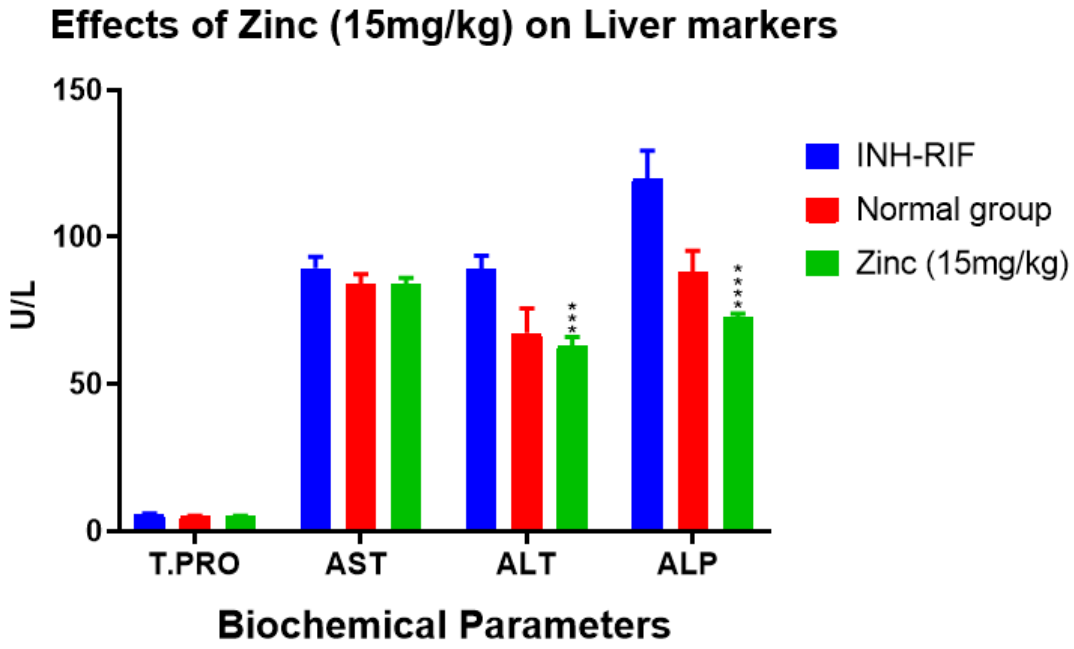

Fig 3. Effects of Zinc (15mg/kg) on liver biomarkers of mice with liver injury induced by Isonazid-Rifampicin, INH-RIF, AST: Aspartate Aminotransferase, ALT: Alanine aminotransferase, ALP: Alkaline Phosphatase, Normal control:normal untreated group.

Effects of Selenium, Vitamin B_{12} and Zinc on the weight of the liver

Selenium, Vit B_{12} and Zinc showed no significant reduction in the weight of the liver compared to INH-RIF group as shown in fig 4.

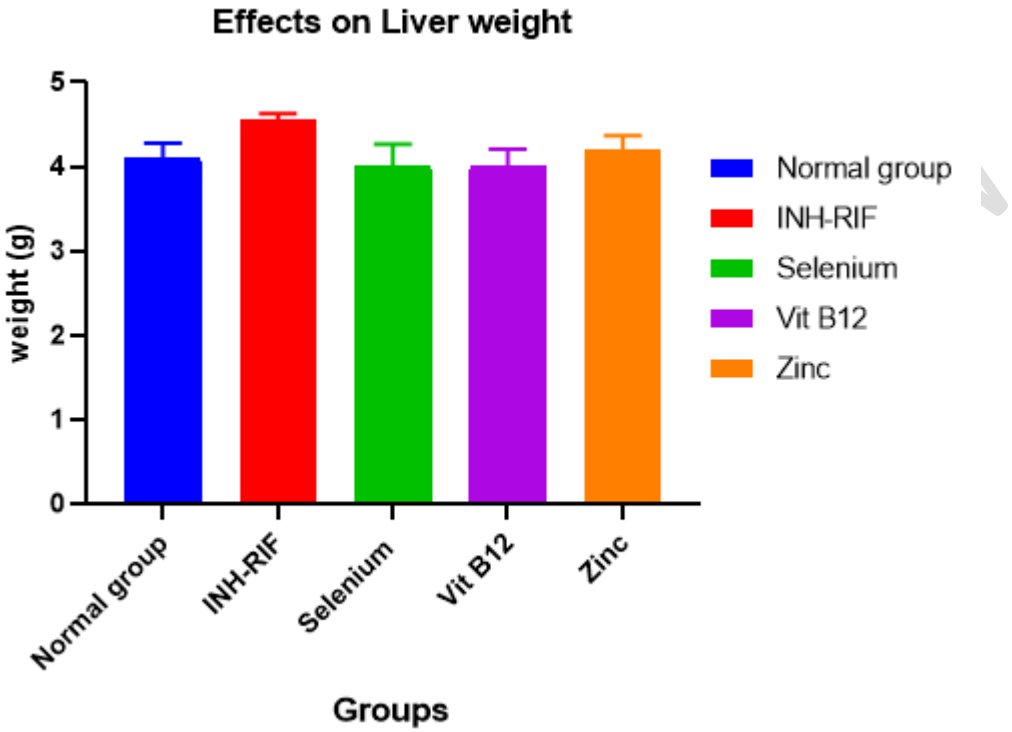

Fig 4. Effect of Isoniazid-Rifampicin on the weight of the liver compared to that of the other antioxidants.

Histopathology

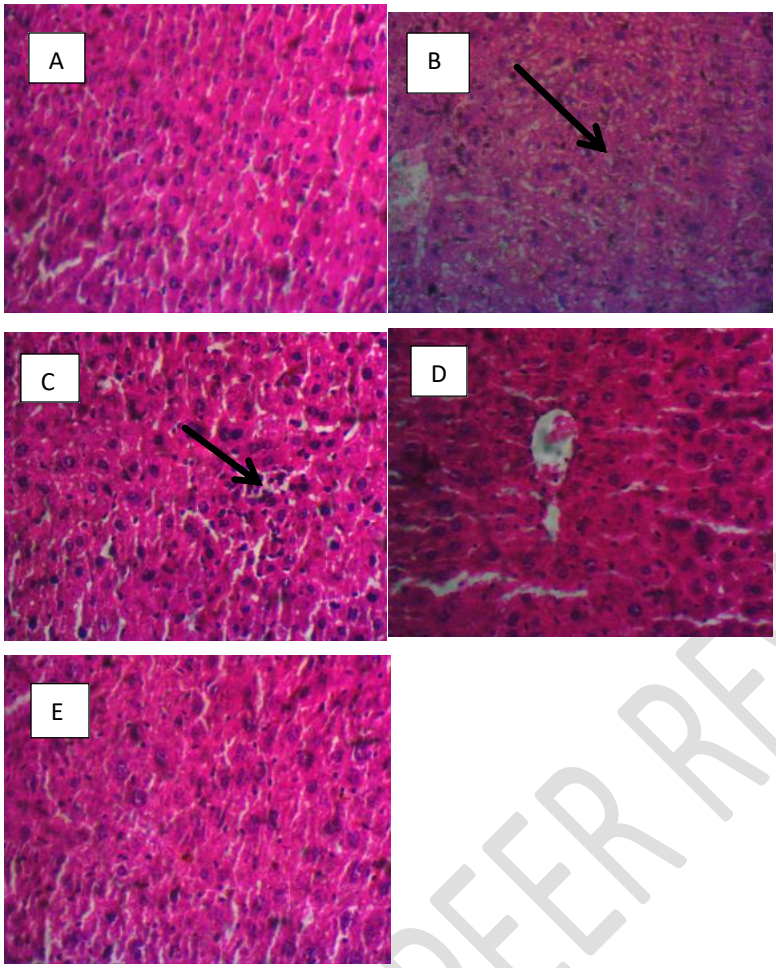

Fig. 5

(A) Unchallenged:No visible lesion seen; (B) INH-RIF:There is random hepatocellular vacoular degeneration (arrow); (C) Se:There is moderate centrilobular inflammation (arrow); (D) Vit B_{12} :No visible lesion seen; (E) Zinc: No visible lesion seen. (H&E stained and x400)

DISCUSSION

There are drugs that induced liver injury either at overdose or at therapeutic dose, these drug-induced-liver-injury (DILI) is the main concern in drug production and prescription; many drug have been banned due to DILI or toxicity [18]. Isoniazid and Rifampicin in this study induced liver injury though the combination is used in the management of tuberculosis, there was increase in the biochemical parameters ALT, AST and ALP as shown in fig 1-3 and increase in weight indicating inflammation as shown in fig 4, this correspond with the previous work which showed increased in ALT, AST, and ALP in isoniazid and rifampicin toxicity but was attenuated by Dendrobine [19], and confirming that Isoniazid and Rifampicin can induced liver toxicity [20].

This study shows that selenium (1mg/kg) reduced significantly the level of ALT only but no significant reduction in other parameters (Fig 1) compared to the INH-RIF group; with decrease in the weight of the liver (Fig 4) and lesions seen in the histopathology. This correspond with the work done by Okwulu [21], which showed that Selenium was not able to

significantly reduced the oxidative stress induced by Acetaminophen on the liver because the tissue histo-picture showed hyperplastic bile duct with mild fibrous hyperplasia. Research has shown that combination of Selenium with plant extracts has efficacy due to synergism, compared to when it was used alone [22]. Its combination with *Harungana madagascariensis* has been reported to have more ameliorative potential in Acetaminophen-induced hepatotoxicity compared to when it was used alone [23]. Its combination with *Pyracantha fortueans* reversed the elevated liver biochemical parameters induced by Carbon-tetrachloride (CCL_4) [24]. The combination of Selenium nano particles with *Moringa oleifera* inhibit hepatocellular carcinoma in rats [25]. Selenium combination with *Hertia cheirifolia* ameliorates the effects of CCL_4 on the liver of rats [22], its co-administration with *Tribulus terrestris* rejuvinated liver injury induced by acetaminophen in rats [26]; its combination with *Aerva Monsoniae* also block cadmium-induced liver toxicity in rats [27] Although selenium is essential for functioning of the immune system in both humans and animals, it exercises synergistic antioxidant activity along with vitamin E to protects the membrane lipids, proteins, and other essential intracellular biomolecules from reactive oxygen attacks [13]. Selenium is a part of glutathione peroxidase that protects the organisms from oxidative stress by catalysing the degradation of H_2O_2, hydroperoxides phospholipids, and other free radicals [28] but its combination with other antioxidants or medicinal plant extracts has more efficient ameliorative potentials.

Vitamin B_{12} showed significant reduction in the elevated biochemical parameters (Fig 2), decrease in liver weight and no lesion in the tissues, this shows that it can combat the free radicals release as a result of oxidative stress induced by INH-RIF in the liver. Previous study showed that hypovitaminosis of Vitamin B_{12}, aging and oxidative stress can cause Alzheimer disease but large dose of Vitamin B_{12} can manage the condition due to its antioxidant effects [3]. This findings also corroborates with previous study which showed that it exhibit hepatoprotective effects in acetaminophen-induced toxicity in rats [12] and its combination with vitamin C and E has been demonstrated to ameliorates the effects of acetaminophen on the liver [29]. It has also been shown to have protective effects on the hepatocytes of Wistar rats intoxicated with daizinon [30]. It is assumed that vitamin B_{12} plays a role in cell division and serves as a modulator of human immunity [31]; stimulates development of T lymphocytes [32], restores an abnormally increased CD4/ CD8 ratio, and retains normal lymphocyte subgroup counts [13]; thus demonstrated anti-inflammatory and free radical scavenging activities shown in this study.

Zinc is an essential component of other enzymes in the body, such as carbonic anhydrase, alkaline phosphatase, alcohol dehydrogenase [33]. It is essential for antioxidant function, as a component of superoxide dismutase [34; 35]. There was significant reduction in ALT and ALP with no lesions in the liver tissue indicating that zinc demonstrated antioxidant activities. This corresponding with the work of Aioub *et al* 2022, which demonstrated the ameliorating effects of zinc nanoparticles in abamectin-induced hepato-renal injury in rat model. It has also been demonstrated to have both preventive and management effects in non-alcoholic fatty liver

disease [36]; and its deficiency has been shown to exacerbate Bisphenol A-induced liver and kidney injury [37], thus zinc has shown to be essential in the healing process of the liver. Zinc produced significant reduction in ALP and ALT compared to Se and Vit B_{12}.

Conclusion

This study showed that VitB_{12} and Zn reversed the toxic effects of the anti-tuberculars and aid recovery better than Selenium; though Zn exhibits better ameliorative potential. Therefore VitB_{12} and Zn are recommended for patients taking INH-RIF for co-administration or hepatotoxicity induced by INH-RIF.

Ethical Approval

Ethical approval was obtained from Animal Care and Ethics committee, NIPRD (05:03:05-44). All the procedures were carried out according to National Institute of Health guide for the care and use of laboratory animals (NIH, 2008).

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