

# Original Article: Selenium Safeguards the Liver Against 5-Fluorouracil Induced Toxicity



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## ABSTRACT

**Background:** The hepatotoxic effect of 5-fluorouracil (5-FU) can deprive cancer patients of its maximum therapeutic benefits. Selenium (Se) is a trace element with potential benefits in some animal models of diseases.

**Objectives:** This study assessed the ability of Se to nullify the hepatotoxic effect of 5-FU in albino rats.

**Methods:** In this study, 40 adult male albino rats were grouped into A to D (each 5 rats). Rats in group A (control) were treated intraperitoneally (IP) with normal saline (0.2 mL) daily for 5 days. Rats in groups B1 to B3 were treated IP with Se (0.125, 0.25, and 0.50 mg/kg) daily for 5 days, respectively. Rats in group C were treated IP with 5-FU (20 mg/kg) daily for 5 days. Rats in groups D1 to D3 were treated IP with Se with 0.125, 0.25, and 0.50 mg/kg before treatment with 5-FU (20 mg/kg) daily for 5 days, respectively. After treatment, the rats were euthanized, and their blood samples were collected and evaluated for serum liver function. Liver samples were evaluated for biochemical and histological parameters.

**Results:** Liver and serum aminotransferases, gamma-glutamyl transferase, lactate dehydrogenase, alkaline phosphatase, total bilirubin, and conjugated bilirubin levels were significantly ( $P < 0.001$ ) high in 5-FU-treated rats in comparison to the control group. Liver glutathione peroxidase, superoxide dismutase (SOD), catalase, and glutathione levels were significantly ( $P < 0.001$ ) low whereas the malondialdehyde level was significantly ( $P < 0.001$ ) high in 5-FU-treated rats compared with the control group. Moreover, hepatocyte necrosis was observed in 5-FU-treated rats.

**Conclusion:** Nonetheless, 5-FU-induced hepatotoxicity was significantly nullified in rats supplemented with Se (0.125 mg/kg,  $P < 0.05$ ; 0.25 mg/kg,  $P < 0.01$ , and 0.5 mg/kg,  $P < 0.001$ ) in a dose-dependent fashion in comparison to 5-FU-treated rats. Thus, Se may have a clinical benefit in 5-FU-induced hepatotoxicity

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## 1. Introduction

**L**iver toxicity is a serious complication of using anticancer agents. In clinical practice, liver toxicity associated with anticancer drugs can manifest in diverse pathological forms specific to the causative agent. This phenomenon can culminate in treatment modification such as dose reduction, complete drug withdrawal, or postponement of therapy schedule which may jeopardize the therapeutic outcome and consequently, patients' survival. Severe cases of liver toxicity associated with anticancer agents can cause hepatic failure and death [1]. The mechanisms for the induction of hepatotoxicity by anticancer drugs are not clear, but hepatocyte injury may arise either directly as a result of the interference of drugs with intracellular function and membrane integrity or indirectly as a result of immune-mediated membrane damage [2].

The clinical use of 5-fluorouracil (5-FU) in cancer chemotherapy has reduced mortality associated with various forms of cancer such as breast, gastric, colorectal, neck, and head cancers [3]. The anticancer activity of 5-FU has been attributed to its inhibitory effect on DNA and RNA syntheses in cancer cells and in rapidly dividing cells in humans [4]. A notable curative outcome has been achieved in the fight against cancer with 5FU; however, its maximum therapeutic benefit could be jeopardized due to the frequent occurrence of hepatotoxicity in clinical practice. Hepatotoxic features, including alteration in hepatocyte structure and mild or severe elevations in serum hepatic markers above the normal clinical benchmark, have been associated with 5-FU [4]. Additionally, studies involving animal models have suggested Oxidative Stress (OS), inflammation, and cell apoptosis as possible mechanisms of 5-FU-induced toxicities [5].

Selenium (Se), an essential mineral is a necessary trace element in the human body and is indispensable for maintaining normal metabolic function. It is incorporated in selenoproteins involved in the synthesis of diverse selenoenzymes such as Glutathione Peroxidase (GPx), thioredoxin reductases, and iodothyronine deiodinases which play essential biological functions. Se performs multi-physiological functions, including antioxidant activity, maintenance of immune-endocrine function, metabolic cycling, and cellular homeostasis [6, 7]. The regulatory activity of Se on the immune system may involve mechanisms such as increased T-lymphocyte proliferation, upregulation of Natural Killer (NK) cell activity, increased interferon  $\gamma$  production, and increased antibody-

producing B-cell function [8]. Furthermore, because of the involvement of Se in numerous biological functions, its deficiency can lower the overall health status as a result of increased vulnerability to infectious diseases and impairing reproductive function [9]. In addition to its effects on physiological functions, Se supplementation is beneficial in animal models of some diseases such as hyperlipidemia, diabetes, arteriosclerosis, and hyperphenylalaninemia [8]. Also, Se supplementations protect against a wide range of detrimental chemical agents in animal models [10-12]. However, there is no study on the protective effect of Se against hepatotoxicity induced by 5-FU in rat models. Thus, the current study evaluated the ability of Se to safeguard against hepatotoxicity induced by 5-FU in albino rats.

## 2. Materials and Methods

### Animal handling

The Research Ethics Committee of Department of Pharmacology and Toxicology, Faculty of Pharmacy, Niger Delta University, Nigeria approved (No. 029/2019) the study. This study was performed according to the guidelines of the Canadian Council on Animal Care. Forty adult male albino rats (220-250 g) were used, wherein 5 rats per cage were reared under a standard temperature of  $25\pm 2^\circ\text{C}$  and 12/12 h light/dark cycle. The rats were fed with rat chow and allowed to acclimatize for 2 weeks.

### Animal treatment, sacrifice, and biochemical analyses

Rats in group A (control) were treated intraperitoneally (IP) with normal saline (0.2 mL) daily for 5 days. Rats in groups B1 to B3 were treated IP with Se with 0.125, 0.25, and 0.50 mg/kg daily for 5 days, respectively. Rats in group C were treated IP with 5-FU (20 mg/kg) daily for 5 days. Rats in groups D1-D3 were treated IP with Se with 0.125, 0.25, and 0.50 mg/kg before treatment with 5-FU (20 mg/kg) daily for 5 days, respectively. On day 6, the rats were anesthetized with diethyl ether; blood samples were collected directly from the heart in sample containers and were allowed to clot. Serum samples were removed by centrifugation at 3000 g for 15 min and collected in sterile containers and analyzed for biochemical indexes. Subsequently, the rats were dissected, liver specimens were collected rinsed in cold saline, and homogenized with 0.05 M sodium phosphate buffer (pH=7.0). The homogenates were centrifuged at 1200 g for 15 min and the supernatants were collected and evaluated for biochemical indexes. Serum and liver Gamma-Glutamyl Transferase (GGT), Lactate Dehydro-

genase (LDH), conjugated bilirubin (CB), alkaline phosphatase (ALP), aspartate aminotransferase (AST), total bilirubin (TB), and alanine aminotransferase (ALT) were analyzed using standard test kits according to manufacturer's protocol. Liver protein was assessed according to the method described by Gonall et al. [13]. Catalase (CAT) was assessed according to the method described by Aebi [14]. Glutathione (GSH) was analyzed using the method described by Sedlak and Lindsay, [15] whereas superoxide dismutase (SOD) was assayed as performed by Sun and Zigman [16]. Glutathione peroxidase (GPx) was evaluated using the method described by Rotruck et al., [17] and malondialdehyde (MDA) was assayed using the method described by Buege and Aust [18].

### Histopathological analysis

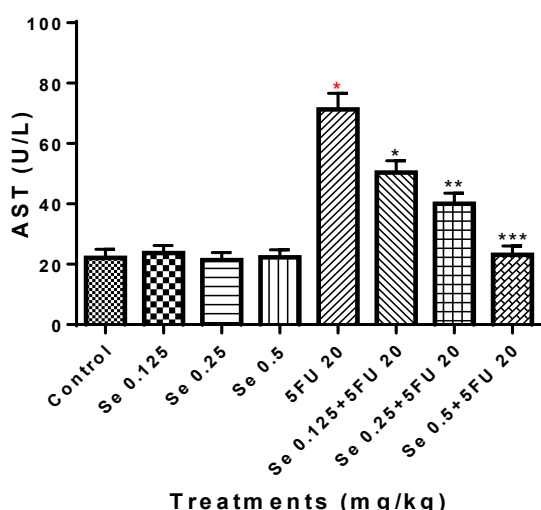
Liver specimens were fixed in 10% buffered formalin for 24 h after which they were dehydrated in graded concentrations of alcohol solutions. Liver tissues were prepared and embedded in paraffin. Then, 4- $\mu$ m thickness sections were prepared and paraffin removed. The sections were mounted on slides, stained with hematoxylin and eosin, and examined under a light microscope for histopathology.

### Statistical analysis

The results are expressed as Mean $\pm$ SEM (n=5 in each group). Differences among groups were evaluated using a 1-way analysis of variance (ANOVA) followed by Tukey's post hoc test. P values of <0.05; <0.01; <0.001 were considered statistically significant for different comparisons.

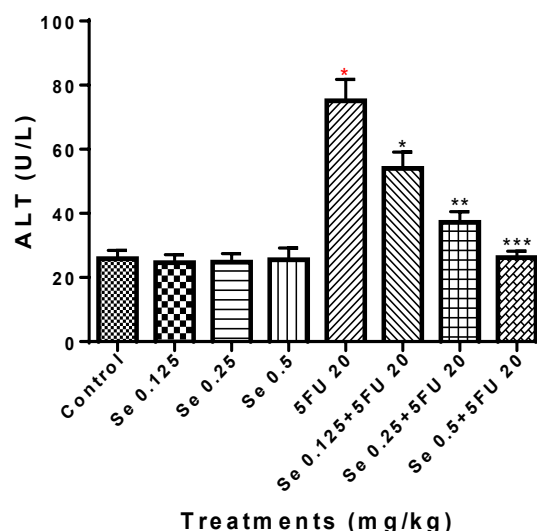
### 3. Results

The serum and liver levels of CB, TB, GGT, AST, ALT, ALP, and LDH were normal (P>0.05) in rats treated with Se when compared to those in the control. In contrast, elevated levels of CB, TB, GGT, AST, ALT, ALP, and LDH (P<0.001) were seen in 5-FU-treated rats (Figures 1-7, Table 1). However, the above hepatic function markers decreased in a dose-dependent fashion in rats supplemented with Se (0.125 mg/kg, P<0.05; 0.25 mg/kg, P<0.01; and 0.50 mg/kg, P<0.001) compared with the rats treated with 5-FU (Figures 1-7, Table 1). Furthermore, liver levels of GPx, SOD, GSH, CAT, and MDA were normal (P>0.05) in rats treated with Se when compared to the control (Table 2). On the contrary, liver GPx, SOD, GSH, CAT were decreased significantly (P<0.001) and the MDA level increased significantly (P<0.001) in rats treated with 5-FU compared with the control (Table 2). However, liver GPx, SOD, GSH, CAT levels were high whereas MDA level was low in a dose-



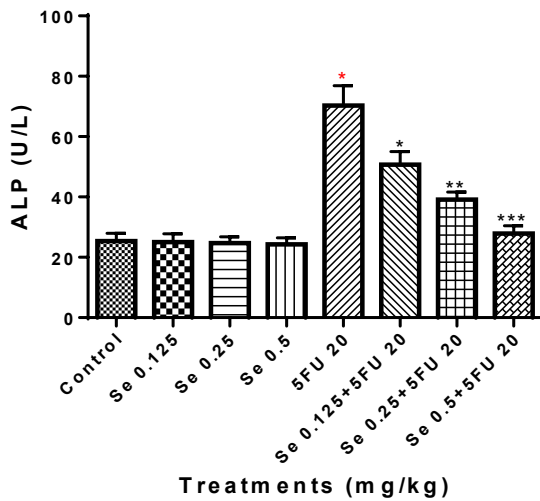
**Figure 1.** Effect of selenium on serum aspartate aminotransferase of 5-fluorouracil treated albino rats

AST: aspartate aminotransferase; Se: selenium; 5-FU: 5-fluorouracil. n=5. Data are expressed as Mean $\pm$ SEM. \*P<0.001 when compared to the control. \*P<0.05: when compared to the 5-FU group. \*\*P<0.01: when compared to the 5-FU group. \*\*\*P<0.001: when compared to the 5-FU group.



**Figure 2.** Effect of selenium on serum alanine aminotransferase of 5-fluorouracil treated albino rats

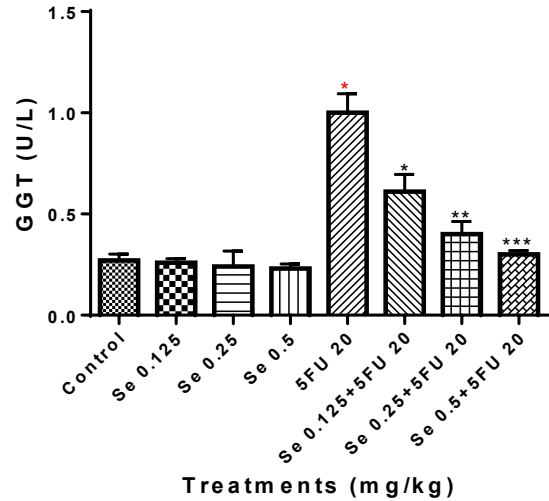
ALT: alanine aminotransferase; 5-FU: 5-fluorouracil. n=5. Data are expressed as Mean $\pm$ SEM. \*P<0.001: when compared to the control group. \*P<0.05: when compared to the 5-FU group. \*\*P<0.01: when compared to the 5-FU group. \*\*\*P<0.001: when compared to the 5-FU group.



**Figure 3.** Effect of selenium on serum alkaline phosphatase of 5-fluorouracil treated albino rats

ALT: alkaline phosphatase; 5-FU: 5-fluorouracil. n=5. Data are expressed as Mean±SEM. \*P<0.001: when compared to the control group. \*P<0.05: when compared to the 5-FU group. \*\*P<0.01: when compared to the 5-FU group. \*\*\*P<0.001: when compared to the 5-FU group.

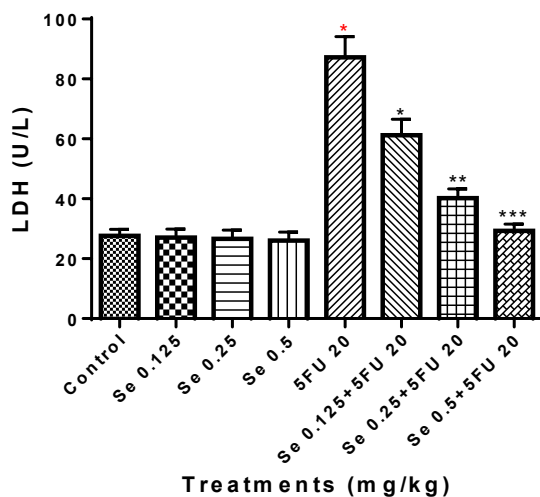
dependent fashion in rats supplemented with Se (0.125 mg/kg, P<0.05; 0.25 mg/kg, P<0.01; and 0.50 mg/kg, P<0.001) compared to the rats treated with 5-FU (Table 2). Normal hepatocytes were observed in the liver of the control rats whereas hepatocyte necrosis was observed



**Figure 4.** Effect of selenium on gamma-glutamyl transferase of 5-fluorouracil treated albino rats

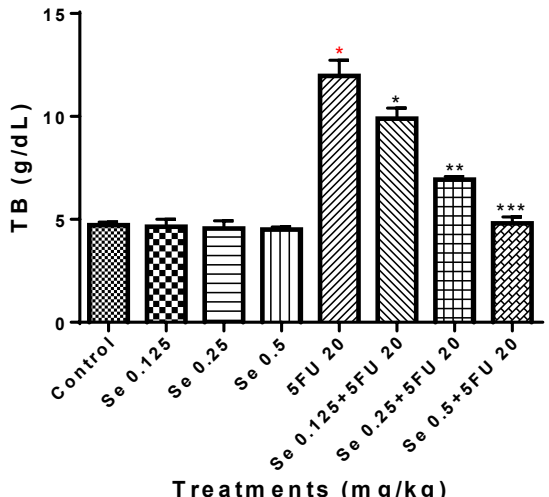
GGT: gamma-glutamyl transferase; 5-FU: 5-fluorouracil. n=5. Data are expressed as Mean±SEM. \*P<0.001: when compared to the control group. \*P<0.05: when compared to the 5-FU group. \*\*P<0.01: when compared to the 5-FU group. \*\*\*P<0.001: when compared to the 5-FU group.

in the liver of rats treated with 5-FU (Figure 8a and b). However, the liver of rats supplemented with Se (0.125 mg/kg) showed inflammatory cell infiltration (Figure 8c). On the other hand, rats supplemented with Se (0.25 mg/



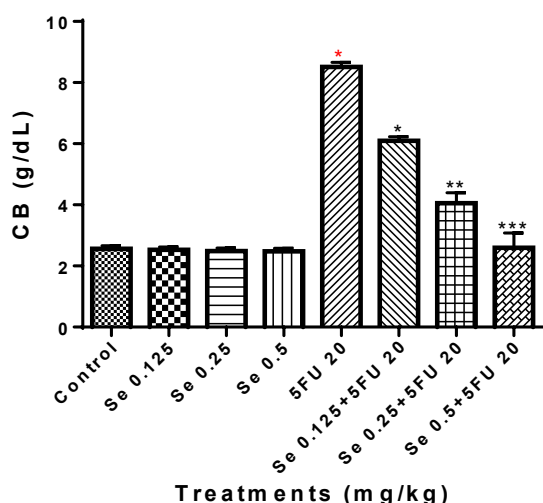
**Figure 5.** Effect of selenium on lactate dehydrogenase of 5-fluorouracil treated albino rats

LDH: Lactate dehydrogenase; 5-FU: 5-fluorouracil. n=5. Data are expressed as Mean±SEM. \*P<0.001: when compared to the control group. \*P<0.05: when compared to the 5-FU group. \*\*P<0.01: when compared to the 5-FU group. \*\*\*P<0.001: when compared to the 5-FU group.



**Figure 6.** Effect of selenium on total bilirubin of 5-fluorouracil treated albino rats

TB: conjugated bilirubin; 5-FU: 5-fluorouracil. n=5. Data are expressed as Mean±SEM. \*P<0.001: when compared to the control group. \*P<0.05: when compared to the 5-FU group. \*\*P<0.01: when compared to the 5-FU group. \*\*\*P<0.001: when compared to the 5-FU group.



**Figure 7.** Effect of selenium on conjugated bilirubin of 5-fluorouracil treated albino rats

CB: Total bilirubin; 5-FU: 5-fluorouracil. n=5. Data are expressed as Mean±SEM. \*P<0.001: when compared to the control group. \*P<0.05: when compared to the 5-FU group. \*\*P<0.01: when compared to the 5-FU group. \*\*\*P <0.001: when compared to the 5-FU group.

kg, Figure 8; 0.50 mg/kg, Figure 8e) showed normal hepatocytes.

#### 4. Discussion

The therapeutic benefit derived from the clinical use of 5-FU can be maximized by preventing the hepatotoxic consequence that may arise in the course of therapy. The

current study assessed the potential protective effect of Se on hepatotoxicity induced by 5-FU in albino rats. Serum AST, ALT, ALT, GGT, LDH, CB, and TB are essential markers used to ascertain the health status of the liver. AST is present in mitochondria and cytoplasm, whereas ALT is found in the cytoplasm. ALT is more sensitive than AST; therefore, it is the primary hepatic marker used to diagnose a drug-induced liver injury. Bilirubin is often produced by the liver at a constant rate and its level in the serum correlates with liver function. Elevation in serum bilirubin and aminotransferases can give a vivid diagnostic picture of hepatotoxicity [19]. This study observed normal serum and liver AST, ALT, ALT, GGT, LDH, CB, and TB levels in rats treated with Se. In contrast, the aforementioned parameters were severely elevated in rats treated with 5-FU. The observation in rats treated with 5-FU attests to hepatotoxicity which has been previously documented [20]. However, the levels of AST, ALT, ALT, GGT, LDH, CB, and TB were restored in a dose-dependent fashion in Se-supplemented rats. This finding showed the ability of Se to restore and stabilize hepatic function after intoxication by 5-FU.

Studies have speculated that increased free radical production culminating in OS is involved in the initiation and progression of hepatotoxicity caused by anticancer agents [21]. The liver has an inherent detoxification mechanism fortified with antioxidants such as CAT, GSH, GPx, and SOD that detoxifies the activities of excess free radicals. However, with the advent of free radical accumulation, the activities of the aforementioned antioxidant can be surmounted culminating in OS, lead-

**Table 1.** Effects of Selenium on liver biochemical parameters of 5-fluorouracil-intoxicated rats

Treatment (mg/kg)	AST (U/L)	ALT (U/L)	ALP (U/L)	GGT (U/L)	LDH (U/L)
Control	241.5±15.8	212.8±12.3	227.8±13.9	26.9±3.28	247.8±15.7
SE 0.125	225.5±13.4	211.5±11.5	217.8±15.3	24.3±4.32	224.7±12.5
SE 0.25	236.5±15.1	215.2±15.3	222.4±14.2	24.2±3.31	239.5±16.9
SE 0.50	223.9±14.0	214.8±12.9	210.1±12.8	24.5±2.37	223.8±13.2
5-FU 20	874.4±16.6*	877.2±17.6*	830.5±20.4*	96.9±7.02*	870.9±20.2*
SE 0.125 + 5-FU 20	533.6±13.9*	573.3±14.1*	531.4±17.8*	63.2±5.88*	537.3±15.2*
SE 0.25 + 5-FU 20	357.9±15.2**	370.7±2.7**	318.6±12.3**	40.4±3.78**	325.9±14.5**
SE 0.50 + 5-FU 20	245.9±13.8***	226.7±14.6***	241.0±13.7***	26.9±3.73***	254.1±2.9***

ALA: alanine aminotransferase; AST: aspartate aminotransferase; ALP: alkaline phosphate; GGT: gamma-glutamyl transferase; LDH: lactate dehydrogenase; 5-FU: 5-fluorouracil. n=5. Data are expressed as Mean±SEM. \*P<0.001: when compared to the control group. \*P<0.05: when compared to the 5-FU group. \*\*P<0.01: when compared to the 5-FU group. \*\*\*P <0.001: when compared to the 5-FU group.

**Table 2.** Effects of Selenium on liver oxidative stress markers parameters of 5-fluorouracil intoxicated rats

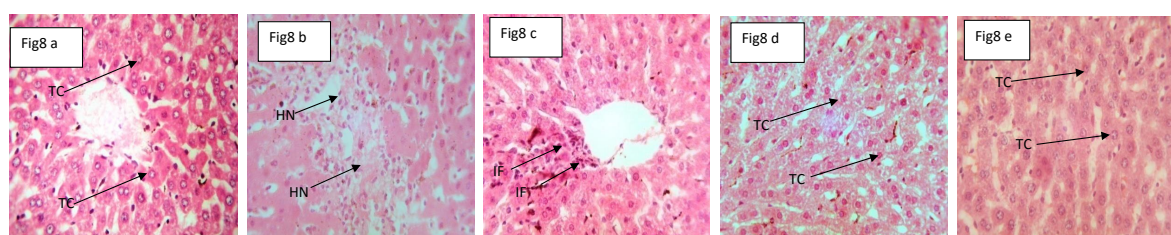
Treatment (mg/kg)	SOD (U/mg protein)	CAT (U/mg protein)	GSH ( $\mu$ mol/mg protein)	MDA (nmol/mg protein)	GPX (U/mg protein)
Control	32.3 $\pm$ 2.17	50.9 $\pm$ 3.88	13.5 $\pm$ 0.45	0.19 $\pm$ 0.03	16.5 $\pm$ 1.63
Se 0.125	32.5 $\pm$ 2.85	52.6 $\pm$ 4.76	16.4 $\pm$ 1.39	0.15 $\pm$ 0.01	15.6 $\pm$ 1.09
Se 0.25	32.6 $\pm$ 3.08	52.1 $\pm$ 4.19	15.9 $\pm$ 1.50	0.13 $\pm$ 0.01	14.7 $\pm$ 1.83
Se 0.50	33.4 $\pm$ 3.84	54.2 $\pm$ 3.98	17.4 $\pm$ 1.42	0.14 $\pm$ 0.02	15.6 $\pm$ 1.81
5-FU 20	10.2 $\pm$ 0.23*	17.9 $\pm$ 1.90*	5.74 $\pm$ 0.19*	0.84 $\pm$ 0.07*	4.33 $\pm$ 0.14*
Se 0.125 + 5-FU 20	15.9 $\pm$ 1.23*	26.7 $\pm$ 2.41*	7.61 $\pm$ 0.09*	0.82 $\pm$ 0.01*	6.76 $\pm$ 0.27*
Se 0.25 + 5-FU 20	21.5 $\pm$ 2.51**	35.8 $\pm$ 3.48**	11.0 $\pm$ 0.37**	0.38 $\pm$ 0.02**	8.89 $\pm$ 0.10**
Se 0.50 + 5-FU 20	32.5 $\pm$ 3.67***	53.4 $\pm$ 5.84***	17.3 $\pm$ 1.60***	0.22 $\pm$ 0.03***	12.3 $\pm$ 0.47***

MDA: malondialdehyde; GSH: glutathione; CAT: catalase; SOD: superoxide dismutase; GPX: glutathione peroxidase; 5-FU: 5-fluorouracil. n=5. Data are expressed as Mean $\pm$ SEM. \*P<0.001: when compared to the control group. \*\*P<0.05: when compared to the 5-FU group. \*\*\*P<0.01: when compared to the 5-FU group. \*\*\*\*P<0.001: when compared to the 5-FU group.

ing to hepatic biomolecular damage [22]. In the current study, normal cellular levels of CAT, GSH, GPx, and SOD were observed in the liver of Se-treated rats. On the other hand, severe depletions leading to decreases in liver CAT, GSH, GPx, and SOD levels occurred in rats treated with 5-FU. The finding in rats treated with 5-FU is a sign of OS which was reported previously [23]. Findings showed that free radical-induced LPO is a biochemical process that has to characterize the most adverse effects caused by anticancer agents [24]. LPO can culminate in the formation of lipoperoxyl radicals, lipid hydroperoxides (LOOHs), and some secondary products such as aldehydes, malondialdehyde (MDA), hexanal, and 4-hydroxynonenal. MDA is one of the primary markers for the assessment of LPO. It is a reactive aldehyde that interacts with deoxyguanosine and deoxyadenosine in DNA culminating in mutagenic DNA adducts [25]. This study observed normal MDA levels in rats treated with Se, but the MDA level was highly elevated in rats treated with 5-FU. The observation in

5FU-treated rats showed hepatic LPO which is consistent with previous findings [26]. On the other hand, the MDA level was normalized in rats supplemented with Se in a dose-dependent fashion.

Hepatic distortions such as liver necrosis, vacuolated cytoplasm, pyknotic nuclei congested hepatic sinusoids, and inflammatory cell infiltration are some features of hepatic damage caused by 5-FU [27]. This study observed hepatocyte necrosis in the liver of rats treated with 5-FU. However, hepatocyte necrosis was abrogated in rats supplemented with Se. Furthermore, some hypotheses by which 5-FU causes liver toxicity include the breakdown of 5-FU down to dihydro-fluorouracil which forms metabolites such as fluoro-beta-alanine (FABL) biotransforming in the liver. FABL can remain in the liver for a very long time after discontinuation of therapy, suggesting the saturation of pathways involved. These results indicating the reduced ability of the liver to metabolize fat and drugs leading to intracellular lipid

**Figure 8.** The photomicrographs of the livers of rats in the control group and the experimental groups

A: Livers of rats in the control group showing normal hepatocytes (TC); B: Livers of rats treated with 5-FU (20 mg/kg) showing hepatocyte necrosis (HN); C: Liver of rats treated with Se (0.125 mg/kg)+5-FU (20 mg/kg) showing inflammatory cell infiltration (IF); D: Liver of rats treated with Se (0.25 mg/kg)+5-FU (20 mg/kg) showing normal hepatocytes (TC); E: Liver of rat treated with Se (0.50 mg/kg) + 5-FU (20 mg/kg) showing normal hepatocytes (TC).

accumulation. 5-FU can stimulate mitochondrial damage-causing impaired fatty acids oxidation and elevated levels of free radicals leading to hepatic bimolecular damage [28]. The current study observed that supplementation with Se restored liver function and structure in rats treated with 5-FU in a dose-dependent fashion. This effect can be compared with the ameliorative potential of Se against hepatotoxicity induced by bisphenol A in rats [29]. Also, it is similar to the protective effect of selenium on tebuconazole-induced hepatotoxicity in adult rats [30].

This finding can be attributed to the antioxidant activity of Se in preventing OS thereby preserving the integrity of hepatocytes [31]. Se is a cofactor for antioxidant enzymes such as thioredoxin reductase and GPx [32]. GPx scavenges free radicals, prevents LPO, and maintains intracellular homeostasis and redox balance. Thioredoxin reductase plays a significant function in protecting against OS-induced injury, facilitating cell growth and transformation, and the inhibition of cellular apoptosis [33].

## 5. Conclusion

Selenium may have clinical use in hepatotoxicity caused by 5-fluorouracil.

## Ethical Considerations

### Compliance with ethical guidelines

All ethical principles are considered in this article. The participants were informed about the purpose of the research and its implementation stages.

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### Authors' contributions

Both authors equally contributed in preparing this article.

### Conflicts of interest

The authors declared no conflict of interest.

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