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Oral Selenium Supplementation Ameliorated Khat-Induced Hematotoxicity, Hepatotoxicity and Nephrotoxicity in a Mouse Model

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Abstract
Khat abuse in Kenya by young people is rapidly rising despite the much-publicized toxicological effects of khat. Furthermore, little research has focused on mediation strategies against khat-induced toxicities. Chemicals present in khat have been associated with multiple forms of toxicities. This study investigated the impact of khat on physiological and biochemical parameters and the putative role of selenium, a powerful antioxidant in mitigating khat-induced toxicities. Twenty-four, four weeks old male Balb/c mice were randomly divided into four groups, n=6. The first group was the control; groups two, four and three received 1500 mg/kg of khat or 200 mg/kg of selenium or both respectively. Exposure to khat resulted in increased relative organ weight of the spleen and brain; the liver, and kidney were unaffected. Khat-induced leukopenia was normalized in the presence of selenium. In addition, selenium treatment attenuated Khat-induced derangement of the WBC subtypes; neutrophils, monocytes and basophils. Khat-induced anemia was noted as depicted by decreased levels of RBCs, HGB and HCT when mice were exposed to khat. Importantly, selenium ameliorated the khat-driven anemia. Besides, khat administration led to increased serum levels of blood urea nitrogen and creatinine suggesting impaired renal function of excreting the metabolites; a phenomenon that was reversed in the presence of selenium. Furthermore, selenium assuaged khat-driven elevation of serum aspartate aminotransferase (AST), alanine aminotransferase (ALT,) total bilirubin and alkaline phosphatase (ALP). The findings for the first time indicated that selenium administration prevented the khat-induced alterations in relative organ weights, stabilized hematological parameters, and normalized livers and kidney function markers.
Keywords: Selenium; Khat; hepatotoxicity; hematotoxicity; nephrotoxicity

1. Introduction
Catha edulis Forsk is also known as Miraa, Jaba, Muguka, and Veve. It is a woody shrub and a natural stimulant commonly found in Eastern Africa, Yemen, and Madagascar. In Kenya, it is majorly grown in Meru (Aden et al., 2006) It grows best at high altitudes (1800m above sea level).

Young, fresh leaves are masticated principally for their stimulant and ecstatic outcomes (Hoffman and Al'Absi, 2010). Khat constitutes about forty alkaloids, amino acids, minerals, glycosides, tannins, and vital amines. Cathine is a milder stimulant than cathinone which is the vital component of khat. However, it is unstable in the existence of O2 it perishes to cathine within a few days of picking thus fresh leaves are the best cathinone source (Masoud et al., 2016).

Approximately seven million people worldwide abuse khat daily. Khat has been listed as a substance that creates addiction in people by the World Health Organization (WHO) and has linked it to many side effects including mood changes, increased alertness, aggressiveness, anxiety, and constipation. Severe side effects include heart attack, lung problems, kidney damage, changes in sex drive, dysphoria, lethargy, paranoia, psychosis, insomnia, tachycardia, and inability to get an erection; impotence (Al-Motarreb et al., 2010).

Hematoxicity which leads to kidney injury following long-term khat exposure in rodents and humans has been shown. Hematological disturbances following khat consumption in humans have also been reported (Alsalahi et al., 2012). Case studies have also shown a close correlation between khat use and derangement of hematopoiesis and hematological indices leading to macrocytic anemia, leukopenia, and a decrease in Total Leukocyte Count (Ismaeel et al., 2014a). Khat-induced anemia can be characterized as microcytic and macrocytic which is determined by levels of red cell indices. Other blood abnormalities associated with khat abuse are leukocytopenia and thrombocytopenia. These khat-induced derangements suggestively activate apoptotic pathways causing organ injury (Kennedy et al., 2020).

Khat-induced kidney injuries have been shown (Chepukosi et al., 2021; Ismaeel et al., 2014). Khat-induced alterations of kidney function are further extrapolated by Alsalahi et al., 2012 in their research which showed that it causes upregulated creatinine levels and blood urea nitrogen in treatment groups relative to the controls. The conventional markers of nephrotoxicity are blood urea nitrogen and creatinine levels (Al-Naimi et al., 2019). These will be used to evaluate the functionality of the kidney because most nephrotoxic substances elevate blood urea nitrogen and serum creatinine (Kimutai and Godfrey, 2017).

Oxidative stress is one of the mechanisms by which khat induces its toxicity (Al-Zubairi et al., 2003). This arises when there is an imbalance between the levels of free radicals and the cell's antioxidant systems (Rosy and Goyal, 2016).This imbalance further activates apoptotic pathways leading to cell death (Abdelwahab et al., 2018; Kennedy et al., 2020a). Khat reportedly induces loss of appetite (Al-Motarreb et al., 2002; Elmi, 1983; Hoffman and Al'Absi, 2010; Kalix, 1984). When eating becomes less routine, it may lead to selenium deficiency since its bioavailability depends on dietary intake and thus it was hypothesized to reduce the activity of glutathione peroxidase which requires it as a cofactor to regulate cellular oxidant status directly through the elimination of hydroperoxides and via oxidation of glutathione (Xia et al., 2005). Khat has also been shown to deplete the body's natural antioxidants reserve (Naji et al., 2015) thus need to improve antioxidant action.

Selenium is a mineral antioxidant system and is a cofactor for glutathione peroxidase, thus playing a major role in the detoxification system and providing protection against oxidative stress (Fulvio and Alberto, 1979). Its synergistic effect with vitamin E amplifies the body's defense mechanisms and it is also involved in the recycling of vitamin C from its spent form to its active form, allowing for greater antioxidant protection (Hoekstra et al., 1975). Selenium is therefore hypothesized that it can reverse khat toxicity because it possesses antioxidant and anti-inflammatory functions.

Fewer studies have been done to find intervention strategies for khat toxicity and no study has been done on the ameliorative effect of selenium against khat toxicity. The current study evaluated a variety of physiological and biochemical alterations following exposure and co-exposure to selenium and khat. This study pioneered to investigate the impact of oral selenium supplementation against khat-induced toxicity.

2. Methods, techniques, studied material and area descriptions

2.1 Ethical approval

All procedures involving use of mice were approved by the Institute of Primate Research (IPR) ethics committee (approval number IRC/03/16).

2.2 Preparation of khat

Khat extract was constituted by cutting 15 g of khat leaves into small portions and then they were blended in deionized water (15 g/10 ml). Filtration was conducted, and the resulting filtrate was administered after one hour. Selenium was prepared by crushing the tablets and dissolving 200mcg in 10ml of deionized water.

2.3 Experimental design and dosages

Twenty-four Balb/c mice aged 4 weeks were grouped randomly into 4 groups n=6. The first grouping acted as the control and had only access to food and water; the second grouping was treated with 1.5 g/ml of

hydro khat extract per kg per day; the third grouping was treated with selenium 200 mg/kg; and finally, the fourth group was given 1.5 g/mL of khat extract per kg of body weight and was treated with Selenium 200 mg/kg, this dose has been proven to be a therapeutic dose for selenium supplements. The determination of this dosage (1500 mg/kg) was based on the average amount of khat chewers consume and the dosage utilized in similar studies (Al-Zubairi et al., 2008; Alsalahi et al., 2012; Gitonga et al., 2017; Ismaeel et al., 2014; Chepukosi et al., 2020) The exposure period was 28 days.

2.4 Sample collection

After body weight measurement the dosage of ketamine was determined to be 0.2ml. The mice were anaesthetized by intraperitoneal administration of 0.2ml ketamine. The mice were surgically sectioned prilumbarly and via cardiac puncture blood was drawn. The heart was perfused with 5ml of potassium buffered saline to remove blood from organs. The organs were harvested for determination of relative organ weight.

2.5 Determination of body weights and relative organ weights

Body weights were measured before and after the experiment respectively, and the difference of the means was denoted as body weight change. The organ weights were measured at the end of the experiment and used to calculate relative organ weights for standardization. Weights were determined using an electronic analytical precision Balance.

2.6 Sample collection and biochemical analysis

Blood drawn by cardiac puncture was collected into EDTA tubes. An automatic analyzer {what analyzer} was used to measure hematological indices such as levels of erythrocytes, leukocytes, thrombocytes, and their subtypes. Blood was drawn by cardiac puncture into microcentrifuge Eppendorf tubes, and it was centrifuged at 10000 rpm for 10 mins at 4°C to obtain serum. Biochemical assays of serum of liver and kidney markers were measured using an automated analyzer.

2.7 Statistical analysis

Data analysis was conducted utilizing the GraphPad Prism (Version 9.0). Comparison of the treatment groups with controls was done using One-way ANOVA. Internal comparisons were done utilizing Tukey's post hoc. Analyzed results were presented as a means ± SEM at $p<0.05$ level of significance.

3. Results

3.1 Selenium administration ameliorated khat-induced splenomegaly

There was evident splenomegaly when mice were exposed to khat as depicted by the increased relative organ weights of the spleen ($p<0.01$) (Fig. 1A). The relative organ weight of the kidney ($p<0.05$) (Fig. 1B) was also increased following exposure to khat. The liver (Fig. 1C) and brain (Fig. 1D) were unaffected by khat.

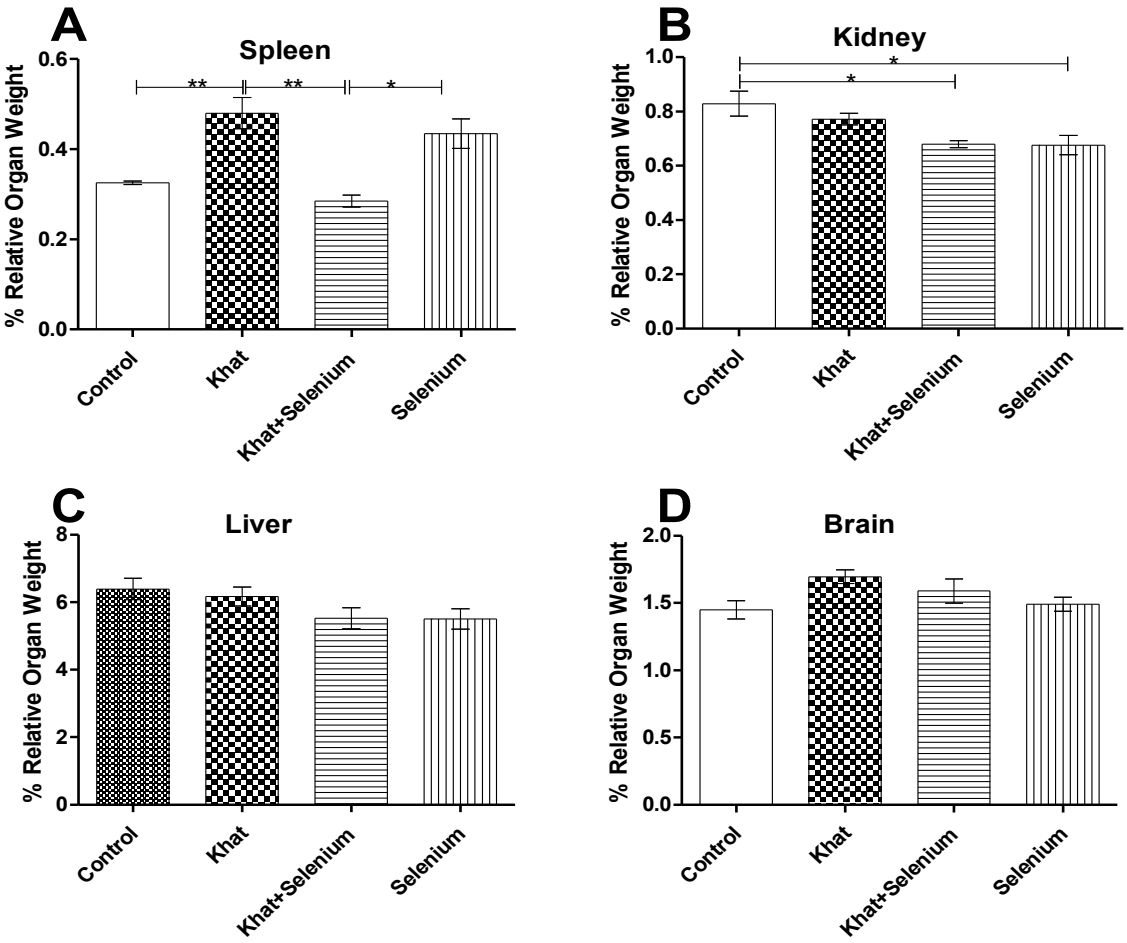

Figure 1: Comparison of the impact of Khat and selenium administration on relative organ weight (%) in male Balb/c mice. The figures show the change in relative organ weight of the spleen (A), kidney (B), liver (C), brain (D). Analysis between various groups and the control group was done by one way ANOVA with Tukey multiple comparisons post hoc test for internal comparisons. (Indicated level of significance *p<0.05, **p<0.01, ***p<0.001). Bars represent mean ±SEM.

3.2 Selenium administration significantly reversed khat-induced neutrophilia, monocytosis and basophilia

Khat administration significantly led to elevated levels of neutrophils ($p<0.01$) (Fig. 3A), monocytes ($p<0.05$) (Fig. 2B), and basophils ($p<0.01$) (Fig. 2C). Khat administration also resulted in depletion of lymphocytes $p<0.05$) (Figure 2D), eosinophils $p<0.01$) (Fig. 2E) a clear indicator of cytotoxic effect of khat. Selenium administration stabilized the levels of WBC subtypes (neutrophils, monocytes, basophils). Finally, there was significant depletion in the levels of white blood cells $p<0.001$) (Fig. 2F) in the Khat treatment group a clear indicator of khat driven leukopenia.

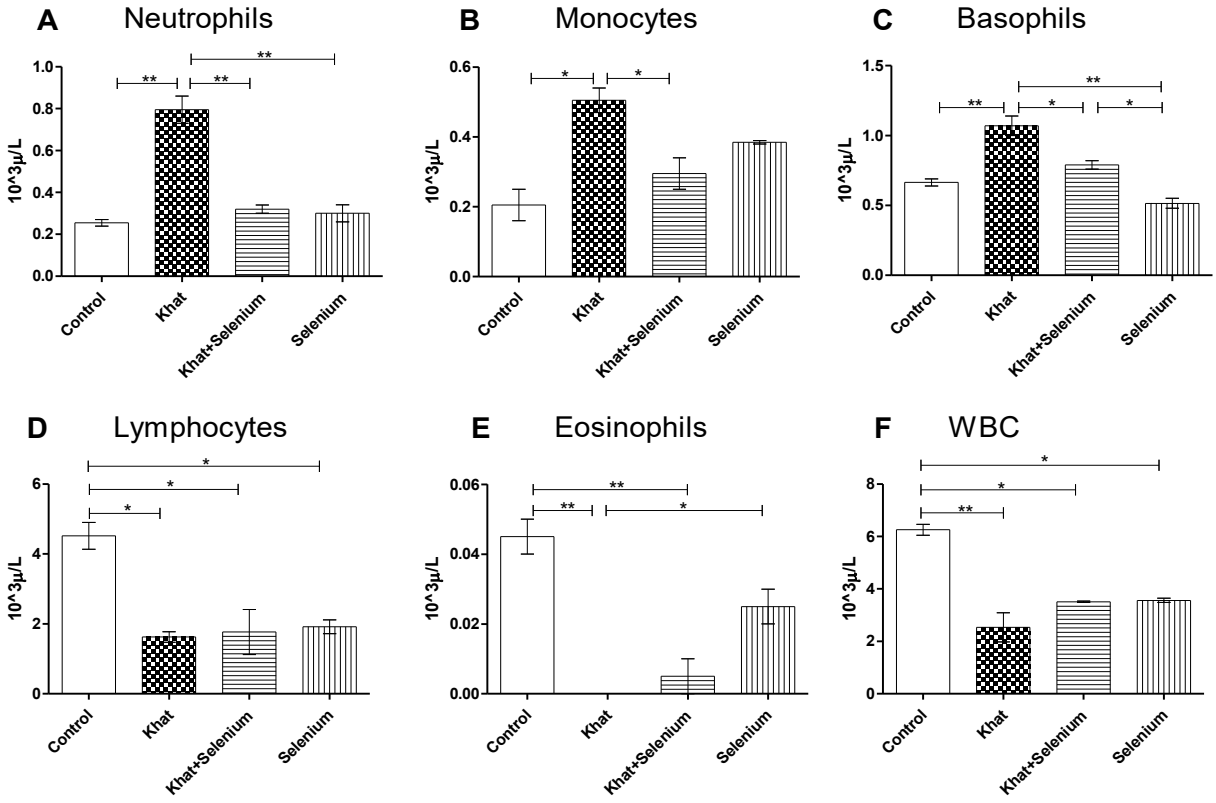

Figure 2: The impact of khat and selenium supplementation on WBC subtypes in male Balb/c mice. Figure A: Effect on Neutrophils, B: Monocytes, C: Basophils, D: lymphocytes, E: Eosinophils and F: WBC. Analysis between various groups and the control group was done by one way ANOVA with Tukey multiple comparisons post hoc test for internal comparisons. (Indicated level of significance *p<0.05, **p<0.01, ***p<0.001). Bars represent mean ±SEM.

3.3 Selenium administration abrogated khat-induced anemia in mice

Khat administration resulted in suppressed levels of red blood cells (RBCs) ($p<0.05$) (Fig. 3A), hemoglobin (HGB) ($p<0.05$) (Fig. 3B) and hematocrit (HCT) ($p<0.05$) (Fig. 3C), a clear indicator of anemia. Selenium administration led to the stabilization of RBCs, HGB and HCT.

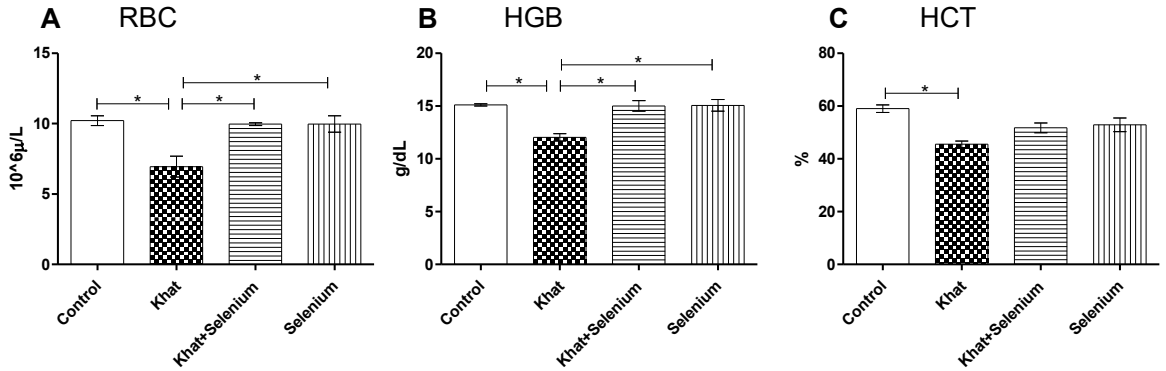

Figure 3: Comparison of the impact of khat and selenium administration on the mean number of RBC (A) HGB (B) and HCT (C) from blood of male Balb/c mice. Analysis between various groups and the control

group was done by one way ANOVA with Tukey multiple comparisons post hoc test for internal comparisons. (Indicated level of significance *p<0.05, **p<0.01, ***p<0.001). Bars represent mean ±SEM.

3.4 Selenium supplementation nullified khat-induced derangement of RBC indices

Administration of khat led to decreased levels of MCV (p<0.05) (Fig. 4A), MCH (p<0.05) (Figure 4B), MCHC (p<0.01) (Fig. 4C), RDW-SD (p<0.05) (Fig. 4D) and RDW-CV (p<0.05) (Fig. 4E) a clear indicator of microcytic anemia. Upon supplementation with selenium, the levels of the MCV, MCH, MCHC, RDW-SD and RDW-CV were stabilized to levels comparable to their respective controls.

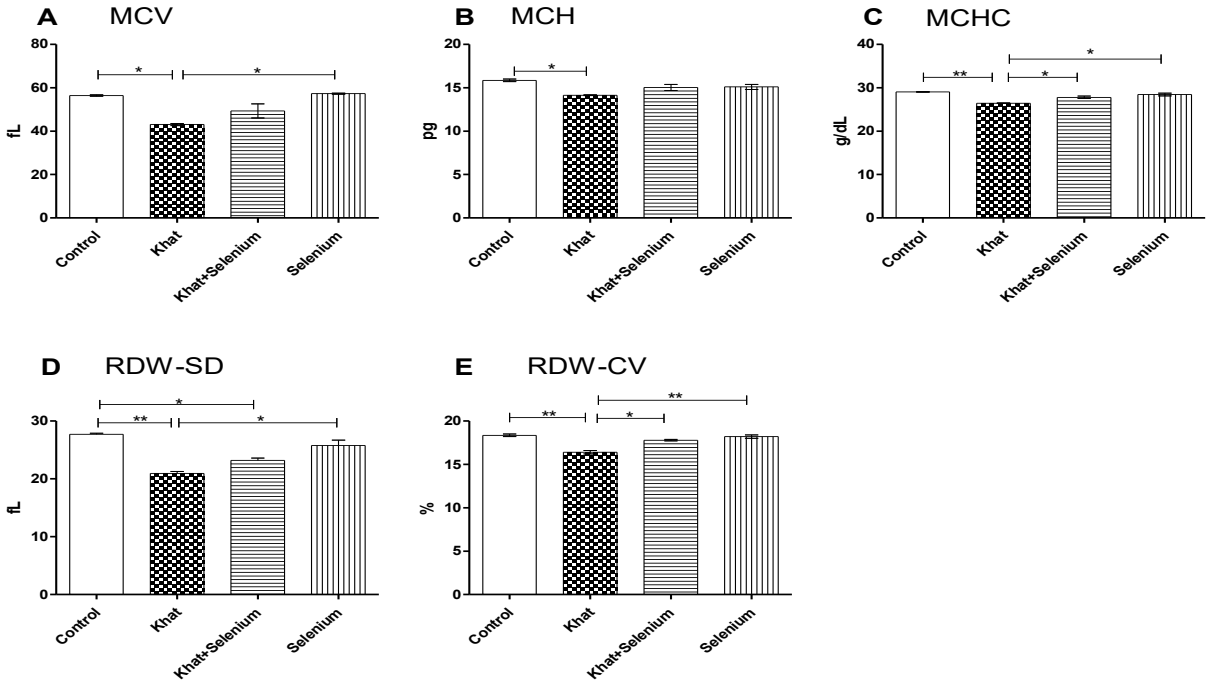

Figure 4: Comparison of the impact on red blood cell subtypes indices following selenium and khat administration in male Balb/c mice. The figures show MCV (mean corpuscular volume) (A), MCH (mean corpuscular hemoglobin) (B), MCHC (mean corpuscular hemoglobin concentration) (C), RDW-SD (red cell distribution width –standard deviation) (D) and RDW-CV (red cell distribution width –coefficient of variation) (E). Analysis between various groups and the control group was done by one way ANOVA with Tukey multiple comparisons post hoc test for internal comparisons. (Indicated level of significance *p<0.05, **p<0.01, ***p<0.001). Bars represent mean ±SEM.

3.5 Selenium supplementation alleviated khat induced thrombocytopenia

Khat administration led to thrombocytopenia as denoted by the reduced levels of platelets (p<0.01) (Fig. 5A). Further scrutiny of the platelet subtypes indices revealed a decrease in the levels of platelet large cell ratio (P-LCR) p<0.001) (Fig. 5D), Platelet distribution width (PDW) (p<0.01) (Fig. 6B), plateletcrit (PCT) (p<0.05) (Fig. 5E) and mean platelet volume (MPV) (p<0.05) (Fig. 5C). Upon selenium co-supplementation the levels of platelets and its subtypes were stabilized to levels comparable to their respective controls.

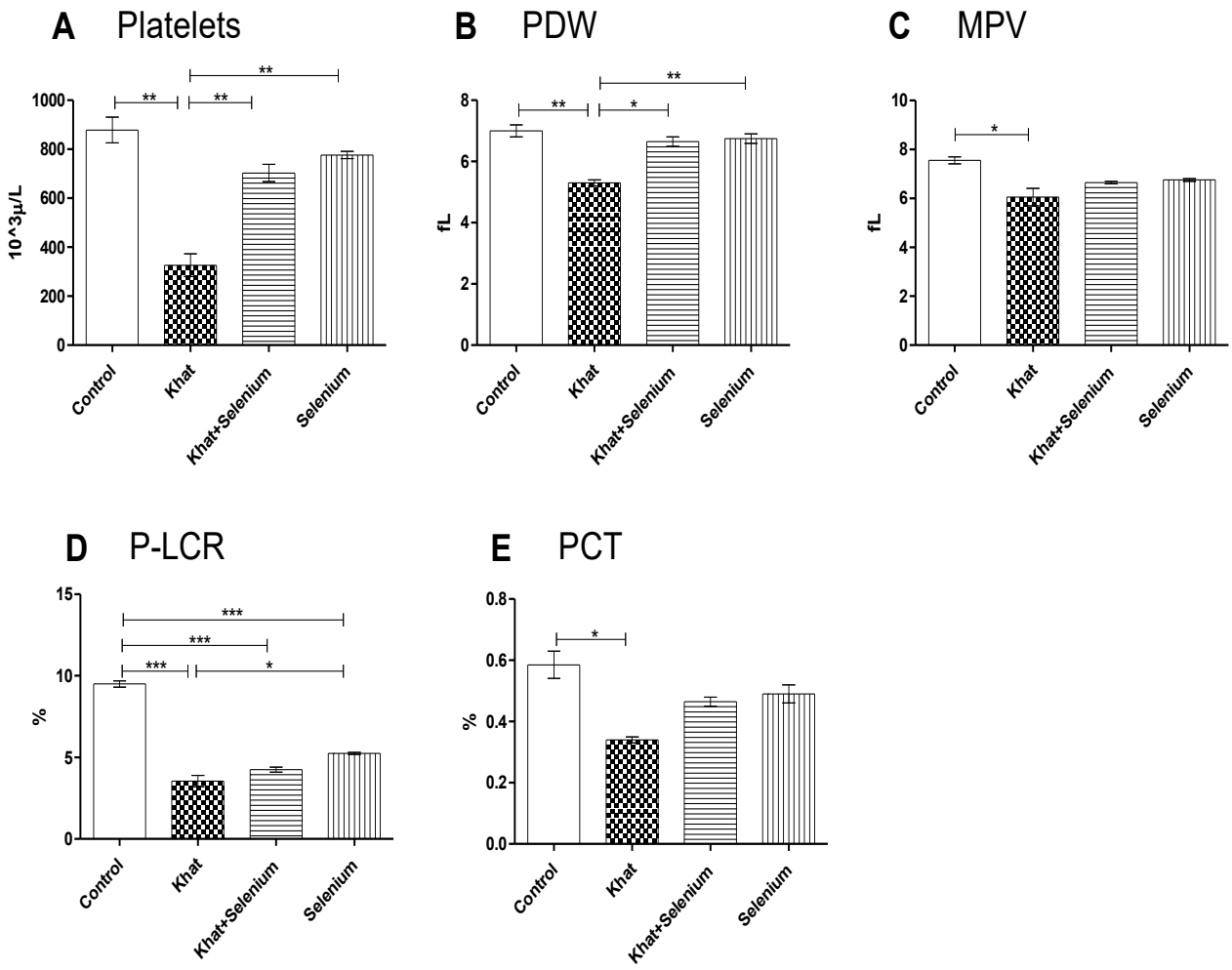

Figure 5: Comparison of the impact of khat and or Selenium supplementation on the platelets and platelet subtypes. Figure A: Effect on PLT (platelets), Figure B: PDW (platelet distribution width), Figure C: MPV (mean platelet volume), Figure D: P-LCR (platelet large cell ratio) and Figure E: PCT (plateletcrit). Analysis between various groups and the control group was done by one way ANOVA with Tukey multiple comparisons post hoc test for internal comparisons. (Indicated level of significance *$p<0.05$, **$p<0.01$, ***$p<0.001$). Bars represent mean ±SEM.

3.6 Selenium administration down-regulated khat-induced kidney injury

Khat administration led to significant elevations in blood urea nitrogen (BUN) ($p<0.01$) (Fig. 6A) and creatinine ($p<0.001$) (Fig. 6B), signaling khat-induced kidney injury. In addition, the levels of chloride ions were elevated ($p<0.01$) following exposure to khat (Fig. 6C). On the contrary, the levels of sodium (Fig. 6D) were comparable across all the treatment groups. Besides, the serum levels of potassium were significantly elevated following khat exposure ($p<0.01$) (Fig. 6E). Remarkably, selenium co-administration resulted in stabilized levels of BUN, creatinine, and electrolytes which suggests improved renal function.

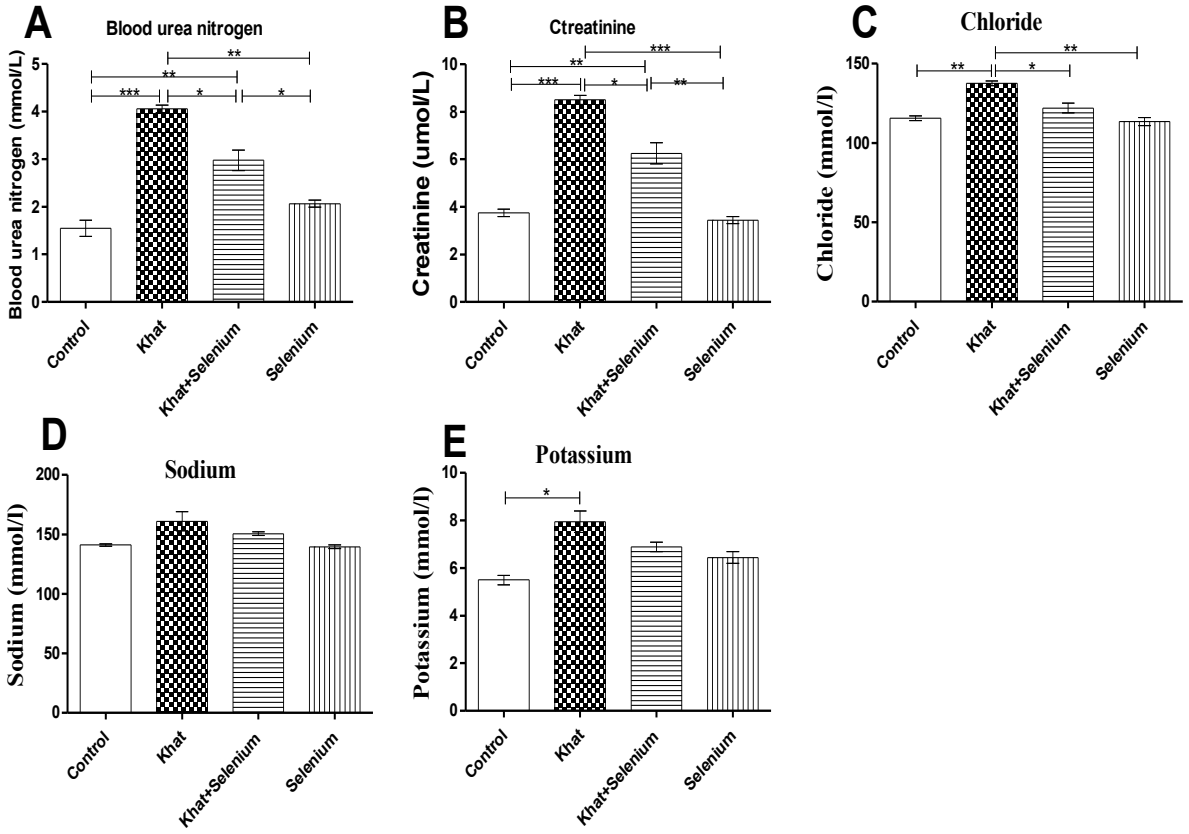

Figure 6: Comparison of the impacts of Khat and selenium administration on serum levels of creatinine, blood urea nitrogen and kidney electrolytes. The figures show the change in serum levels of the BUN (A), Creatinine (B), Chloride (C), sodium (D) and potassium (E). Analysis between various groups and the control group was done by one way ANOVA with Tukey multiple comparisons post hoc test for internal comparisons. (Indicated level of significance *p<0.05, **p<0.01, ***p<0.001). Bars represent mean ±SEM.

3.7 Selenium supplementation nullified khat-induced elevation of liver biomarkers AST, ALT, ALP and total bilirubin

Exposure to khat resulted in significant increase of the liver biomarkers aspartate aminotransferase (AST) (p<0.001) (Fig. 6A), alanine aminotransferase (ALT) (p<0.001) (Fig. 6B), alkaline phosphatase (ALP) (p<0.001) (Fig. 6C) and total total bilirubin (p<0.001) (Fig. 6D), denoting khat-induced liver damage. Importantly, the khat-induced elevations were nullified in the presence of selenium.

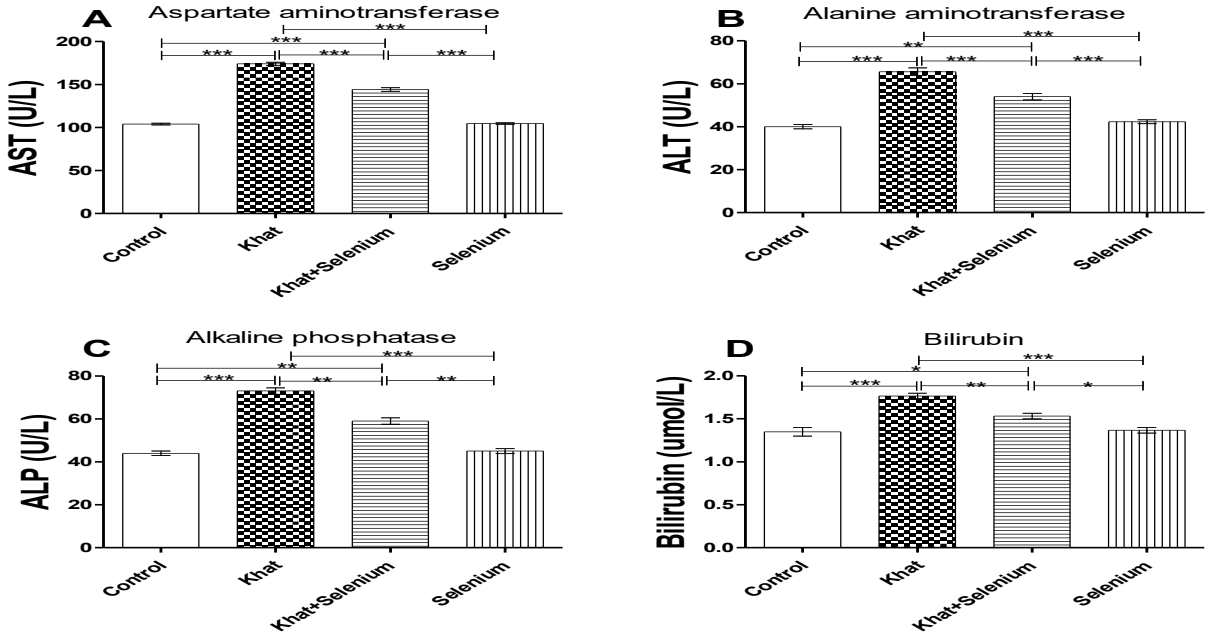

Figure 6: Comparison of the impacts of Khat and selenium administration on serum levels of liver biomarkers. The figures show the change in serum levels of the AST (A), ALT (B), ALP (C) and Bilirubin (D). Analysis between various groups and the control group was done by one way ANOVA with Tukey multiple comparisons post hoc test for internal comparisons. (Indicated level of significance *$p<0.05$, **$p<0.01$, ***$p<0.001$). Bars represent mean ±SEM.

4. Discussion

This study's findings show that khat administration negatively impacted physiological and biochemical parameters in mice which were mitigated by selenium supplementation. Moreover, findings from this study established that oral exposure to selenium alone did not have a negative effect on the physiological and biochemical parameters, perhaps because the dosage used in this study was therapeutic.

White blood cells are vital in fighting off infections. Monocytes, basophils, and neutrophils counts were significantly increased while eosinophils and lymphocytes were decreased following khat administration. Upon selenium supplementation the levels of WBC subtypes were restored to normal levels comparable to that of the controls. The khat-driven increase of neutrophils may account for the latter shown splenomegaly due to clonal expansion. Results comparable by a previous study (Kennedy et al., 2020b). The current study demonstrates immune enhancing properties of selenium in improving the activation and proliferation lymphocytes which may be attributed to the stabilized levels of lymphocytes in khat and selenium co-administered groups. WBCs were significantly lowered which may be attributed to khat-induced cytotoxicity. Suppression of WBC following khat exposure has been reported (Ismaeel et al., 2014b). Since WBCs play a significant protective role in immune function, it is plausible that khat-driven leukopenia may compromise diagnostic efforts in diseases that heavily rely on WBC and its subtypes.

Increase in the relative organ weights of the spleen in the khat-exposed mice is likely an immunological response due to toxic components of khat since the spleen is peripheral immune organ. The spleen was significantly distended following khat exposure. A similar observation was noted in the brain organs of the khat-exposed mice. This observation may be linked to the edema inducing potential of khat. Remarkably, this study is the first to demonstrate that concomitant administration of khat with selenium nullified khat induced tissue pathology.

Hematopoiesis is a vital process that results in continuous production of cells and components of cells. The current study presents evidence of khat-induced derangement of hematopoiesis indices by significant suppression of hemoglobin, hematocrit, and erythrocytes. The noted decrease in the levels of HCT, HGB and RBCs following khat exposure in the current study indicates anemia, this corroborates findings from other studies (Chepukosi et al., 2020b; Ketema et al., 2015b; Ketema et al., 2015d). Upon selenium supplementation the levels of HCT, HGB and RBCs were stabilized. Decrement in the levels of RBCs and HGB may be due to impaired erythropoiesis and accelerated hemolysis (RBC lysis). A notable finding is that selenium administration protected the mice from khat induced suppression of the RBCs, HCT and HGB and its subtypes.

Red blood cell indices are vital parameters in characterization of anemia. In this study, khat-driven microcytic hypochromic anemia was recorded, as denoted by the significant decrease in the levels of MCH, MCHC, RDW-SD. Similar findings have been reported in other studies where mice were exposed to khat (Ismaeel et al., 2014b; Chepukosi et al., 2020b). Selenium restored the levels of RBCs indices; demonstrating the capacity of selenium to restore the integrity of RBCs.

Khat administration resulted in depleted levels of platelets and its indices. Some studies have reported similar results (Ismaeel et al., 2014b; Ketema et al., 2015d). Worth noting in this realm is that platelets play a significant role in blood clotting cascade, preventing bleeding, and enhancing wound healing other than having a role in inflammatory response. Therefore, it is plausible to assume that subjects chronically exposed to khat may develop wound healing and blood clotting complications when physically injured. Khat suppresses platelets through the induction of caspase 1 and 8 dependent apoptosis, as reported by previous studies (Dimba et al., 2004). The khat-driven thrombocytopenia was reversed in the presence of selenium, hence, there is a likelihood that selenium modulates the above-mentioned caspase dependent apoptosis. This hypothesis warrants further scrutiny.

Serum creatinine and blood urea nitrogen are markers commonly used to assess glomeruli filtration rate as well as concentrating and diluting capacity of tubular functions of the kidney. An increase in the values of these markers beyond normal ranges may indicate development and extent of renal tubular damage (Bacha, et al., 2015b). Increased creatinine levels have been linked to decreased glomerular filtration, impaired waste products elimination and kidney inflammation (Ismaeel et al., 2014b).

In the present study khat administration led to elevated levels of creatinine and blood urea nitrogen (BUN), suggesting impaired kidney ability to handle the wastes, a clear indicator of khat-driven kidney injury (Al-Naimi et al., 2019). This results corroborates previous studies which associated increased serum blood urea nitrogen, creatinine, and electrolytes with kidney injury (Husain et al., 2014a, 2014b; Ismaeel et al., 2014b; Ketema et al., 2015b). In the present of selenium, the levels of BUN and creatinine were normalized. This outcome demonstrates the renoprotective role of selenium when given in therapeutic dosage.

Khat administration led to significant elevation of the electrolytes Na-C, and K-C a clear indicator of khat-induced metabolic acidosis. Elevations in kidney electrolytes following khat administration have been linked to increased catabolism which cause renal failure and lead to acidosis (Alam et al., 2014). Acidosis results in anorexia which in turn decrease oral food intake which eventually lead to body weight loss (Ketema et al, 2015b). Oral administration of selenium nullified exacerbated levels of BUN, Creatinine, and kidney electrolytes, which may be attributed to its antioxidant and anti-inflammatory functions. Oral exposure to khat causes serious detrimental effects in physiological and biochemical parameters in mice which were mitigated by selenium administration, a finding that was demonstrated for the first time. Selenium has anti-inflammatory and antioxidant properties which nullified khat induced organ injury and cell death perhaps via oxidative stress.

Investigation of the liver and kidney biomarkers is of profound importance in determining the extent of liver and kidney damage induced by toxins (Aldenborg et al., 2006). In the current study an assay of the liver enzyme biomarkers aspartate amino transferase (AST), alanine amino transferase (ALT), alkaline

phosphatase and total bilirubin were evaluated. Consequently, exposure to khat resulted in significant elevation of the liver enzymes AST, ALT, ALP and total bilirubin. This outcome clearly shows that acute exposure to khat potentially induces liver damage. The outcome further corroborates findings from previous studies following acute exposure to khat (Abdul-Mughni et al., 2018; Pantano et al., 2016). Intriguingly, in the presence of selenium, the khat-induced elevations of liver enzyme markers was abrogated. The role of selenium in modulating the khat-induced liver pathology may partly be attributed its role as a powerful antioxidant and anti-inflammatory.

5. Conclusion

Acute administration of khat, resulted to significant alteration of relative organ weight of the spleen and kidney, hematological profile derangement and damage to the liver and kidney. The findings from the present study indicates for the first time that exposure to selenium prevented the khat-induced derangement in hematological parameters, and kidney damage. Therefore, selenium may be beneficial in reversing khat-induced body weight loss, hematoxicity hepatotoxicity and nephrotoxicity with practical clinical implications.

6. Acknowledgements

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