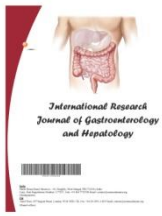

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Hepatotoxic Nature of Potash (Kaun) in Wistar Rats

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Authors' contributions

This work was carried out in collaboration among all authors. Authors FCO and AIA conceptualized and designed the study. Author AIA also wrote the manuscript. Authors OJO and SAE managed the analyses of the study. Authors APA and AUM managed the literature searches. Author EBA wrote the protocol while author AAA performed the statistical analysis. All authors read and approved the final manuscript.

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ABSTRACT

Background: The use of potash as food additive without a recourse to its adverse effect is on the increase in Nigeria.
Aim: This study is designed to assess its effect on hepatic indices of Wistar rats.
Methodology: Potash was locally sourced in a market in Owerri, Imo State, Nigeria. Thirty Wistar rats were acclimatized for seven days, grouped into five and it comprises of six animals

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respectively. Group A were given distilled water, whereas the treatment groups received 250 mg/kg, 500 mg/kg, 750 mg/kg and 1000 mg/kg body weight of potash for twenty-eight days via oral route of administration. The Wistar rats were anaesthetized using diethyl ether, sacrificed then whole blood needed for the study were obtained through cardiac puncture. Biochemical parameters needed for liver function test were analyzed using standard protocol from the manufacturer.

Results: It was revealed that potash administration at higher dose is toxic and perturbs hepatic biomarkers.

Conclusion: From the results of this study, potash is hepatotoxic; therefore, discontinuation of potash consumption needs to be recommended.

Keywords: Food addictive; hepatotoxic; potash.

1. INTRODUCTION

Liver serves as one of the major organs that perform some essential functions for proper body functionality such as ability to metabolized and detoxified various compounds, balance homeostasis, ensured essential growth coupled with provision of adequate energy and nutrient needed by the body [1]. Injury of the liver could be as a result of toxicity produced by various toxicants and infectious agents that do affect the liver [2]. Many diseases ensued from the hepatic dysfunction such as jaundice, cirrhosis and fatty liver, these have greatly threatened the health of so many people across the world [3]. The occurrence of lingering liver diseases across the world is about 18.5%, with cirrhosis taken about 4.5-9.5% which eventually led to the death of about 2 million people yearly. What we consumed serves as one of the contributing factors to the occurrence of liver problem.

Potash refers to one of the salts that been mined; which is made up of potassium that can easily dissolve in water, of which the name pot ash was obtained from the plant burn to ashes which is then dissolved in water housed by a pot, and this serves as the major production method on before the use of technology [4]. Potash production was so great globally with is annual production greater than 30 million tonnes because of is widely application as fertilizer. The major component of various kinds of fertilizer-potash is made up of potassium. And the first production of potassium was achieved through electrolysis of caustic potash (potassium hydroxide) in the year 1807 [5]. Ash burners are the set of people that do produces pot ash called potassium carbonate with the use of an old method by burning wood into ashes, leach and evaporate it in a big pot with deposit of a white residue tagged as pot ash [6]. Most of the wood that are burn into ashes, 10% of it are recovered back as pot ash. Recently development led to the name potash as an acceptable name worldwide rather than potassium salts and byproducts [7]. Potash refers to "*Kaun*" or "*Akanwu*'' by some ethnics group in Nigeria which is majorly used for cooking food. Beans are one of the foods that need the used of potash to make it done quickly [8]. And also, to increase the texture and retain the green colour of some vegetables soup such as *ewedu* and Okro [9]. But yet there is still no recommended dosage of potash to be consume in a daily meal of every Nigerian. Hence, this study was designed to examine the likely effect of potash on the liver.

2. METHODOLOGY

2.1 Experimental Design

Potash was locally sourced in a market in Owerri, Imo State, Nigeria and was carefully preserved to avoid contamination. A thirty Wistar rat with a weight range between 145 and 160 grams were purchase and allowed to acclimatized for seven (7) days with free access to food and drinkable water. They were housed in a clean and well-ventilated cage environment under a standard atmospheric condition for laboratory animals. This treatment was in accordance with the guide prepared for experimental animals by the National Academy of Science [10]. The animals were selected into five (5) major groups according to their body weight; with group A received only distilled water while the treatment groups were given 250 mg/kg, 500 mg/kg, 750 mg/kg and 1000 mg/kg body weight of potash for good twenty-eight days through oral administration. The Wistar rats were anaesthetized using diethyl ether, sacrificed, then whole blood needed for the study were obtained through cardiac puncture.

2.2 Determination of Hepatic Indices

Commercially available enzyme Randox kits were purchased to determine the activities of

Aspartate Aminotransferase (AST) and Alanine Aminotransferase (ALT) according to the procedure described by Reitman and Frankel [11]. Alkaline Phosphatase (ALP) activity was determined by Phenolphthalein Monophosphate method described by Babson et al. [12]. Amylase inhibition assay was determined by the method of Bernfield [13]. Biorex diagnostic kit was used to quantified activities of lipase based on the method stated by Lorentz [14]. Total bilirubin concentration was determined by diazo method described by Royden and Alfred [15]. Conjugated bilirubin concentration was determined by the method of Compernolle [16]. Subtraction of conjugated bilirubin from total bilirubin produced the quantity of unconjugated bilirubin to be quantified.

2.3 Statistical Analysis

One way analysis of variance was used to compare mean while the results were expressed as mean ± standard deviation and the graph were drawn using Graph Pad Prism software version 5.00. The results were considered to be significant when $p<0.05$.

3. RESULTS

The results of this study are presented in Figs 1-11. No significant difference was observed when the activities of ALT and AST in animals treated with lower doses (250 and 500 mg/kg) of potash were compared with those in the control group at $P<0.05$. A significant increase was however observed in the activities of ALT and AST in animals treated with higher doses (750 and 1000 mg/kg) of potash when compared with those in the control group (Figs 1 and 2). ALP activity was observed to increase in experimental animals when compared with those of the control animals. This elevation was however not significant when animals treated with 250 mg/kg body weight of potash were compared with the control group at $P<0.05$ (Fig. 3). No significant difference was observed in the concentrations of total protein and albumin in animals treated with lower doses (250 and 500 mg/kg) of potash when compared with that of the control group at $P<0.05$. A significant increase was however observed in the concentrations of total protein and albumin in animals treated with higher doses (750 and 1000 mg/kg) of potash when compared with those in the control group (Figs 4 and 5). The concentration of globulin was only significant when animals treated with 500 and 1000 mg/kg body weight of potash were compared with those of the control animals (Fig. 6). Administration of potash increased total bilirubin concentration when compared with those in control animals. The increase was significant when animals treated with 500 and 1000 mg/kg of potash were compared with those in the control group at $P<0.05$ respectively (Fig 7). No significant difference was observed in the levels of conjugated bilirubin in experimental animals when compared with those in control group at $P<0.05$ (Fig. 8). A significant increase was observed in the level of unconjugated bilirubin (except the group treated with 750 mg/kg) when compared with those in control group (Fig 9). The potash was observed to inhibit the activities of amylase and lipase (Figs 10 and 11) respectively in a dose-dependent manner.

4. DISCUSSION

Administration of potash to Wistar rat for 28 days revealed significant elevation of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) activities (Figures 1, 2 and 3) at higher dose of 750 mg/kg and 1000 mg/kg body weight than at lower dose of 250 mg/kg and 500 mg/kg body weight. It has been reported that an increase in the enzymatic activity of ALT, AST and ALP in the serum directly reflects hepatocellular damage [17]. These findings, therefore suggested that potash may be hepatotoxic at high doses, which is consistence with the finding of Iweka *et al*. [8] who revealed in their findings that as the dosage of potash increases, concomitantly increases activities of AST, ALT and ALP after administration for 21 days. This could be that exposure of animals to potash stimulated the transcription of the genes involved in glucose uptake, glycolysis and lipogenesis [18]. It has been documented that synthesis of cyclic adenosine monophosphate (cAMP) can be inhibited through the repression of inducible operon by glucose such as Lac operon [19]. Allosteric protein needs the help of nucleotide called cAMP for proper activation to effectively binds to the promoter catabolite activator protein (CAP) site, then enhances the joining of ribonucleic acid polymerase with the promoter to start transcription processes and then, the presence of cAMP is needed before joining CAP, this needs to join deoxyribonucleic acid to enable transcription processes [20]. Adenylase cyclase (AC) activity is blocked when glucose is available, hence the production of cAMP from Adenosine Triphosphate (ATP) needed the activity of AC [21]. But low synthesis

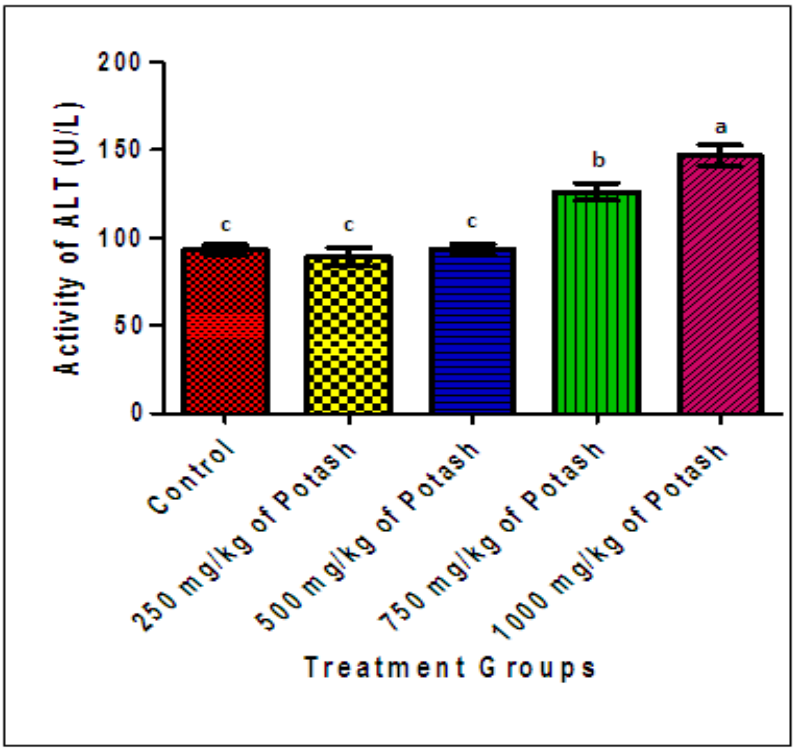

Fig. 1. Effect of potash on the activity of Alanine Amino Transferase (ALT) of animals after 28 days of treatment

Results are presented as mean ± SD with n = 6. Bars with different letters are significantly different at P<0.05

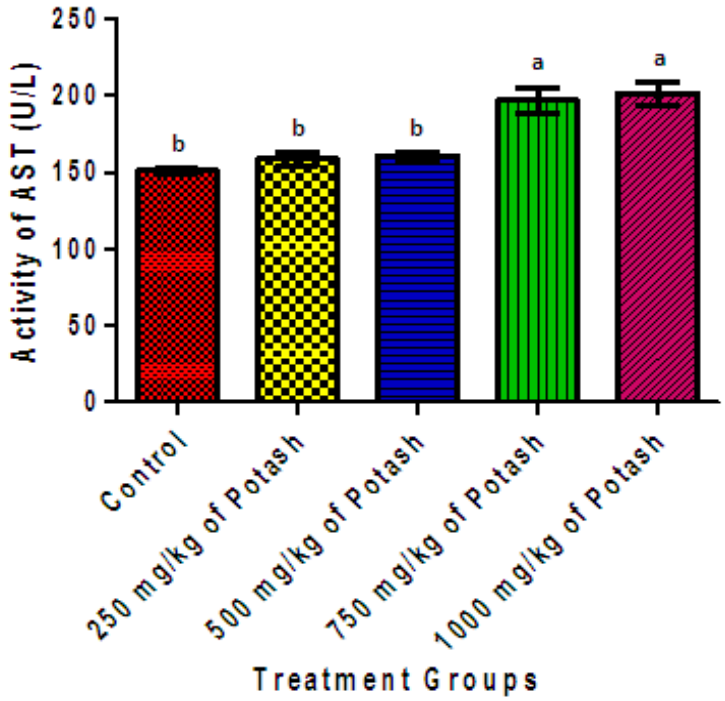

Fig. 2. Effect of potash on the activity of Aspartate Amino Transferase (AST) of animals after 28 days of treatment

Results are presented as mean ± SD with n = 6. Bars with different letters are significantly different at P<0.05

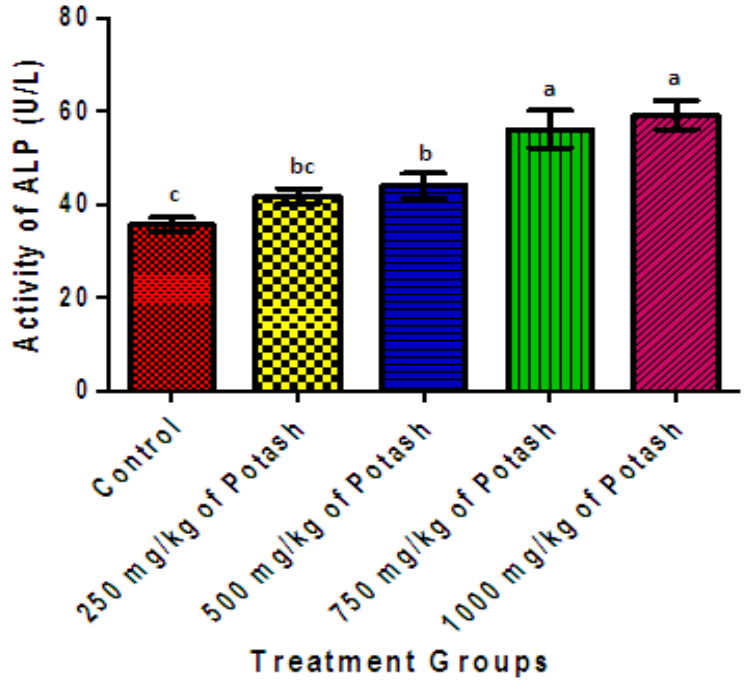

Fig. 3. Effect of potash on the activity of Alkaline Phosphatase (ALP) of animals after 28 days of treatment

Results are presented as mean ± SD with n = 6. Bars with different letters are significantly different at P<0.05

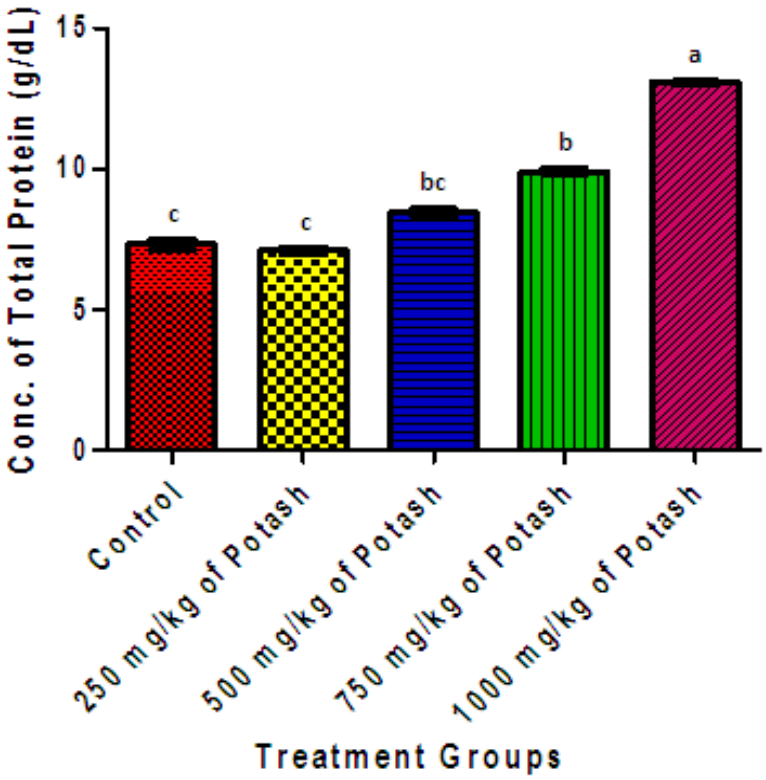

Fig. 4. Effect of potash on the concentration of Total Protein of animals after 28 days of treatment

Results are presented as mean ± SD with n = 6. Bars with different letters are significantly different at P<0.05

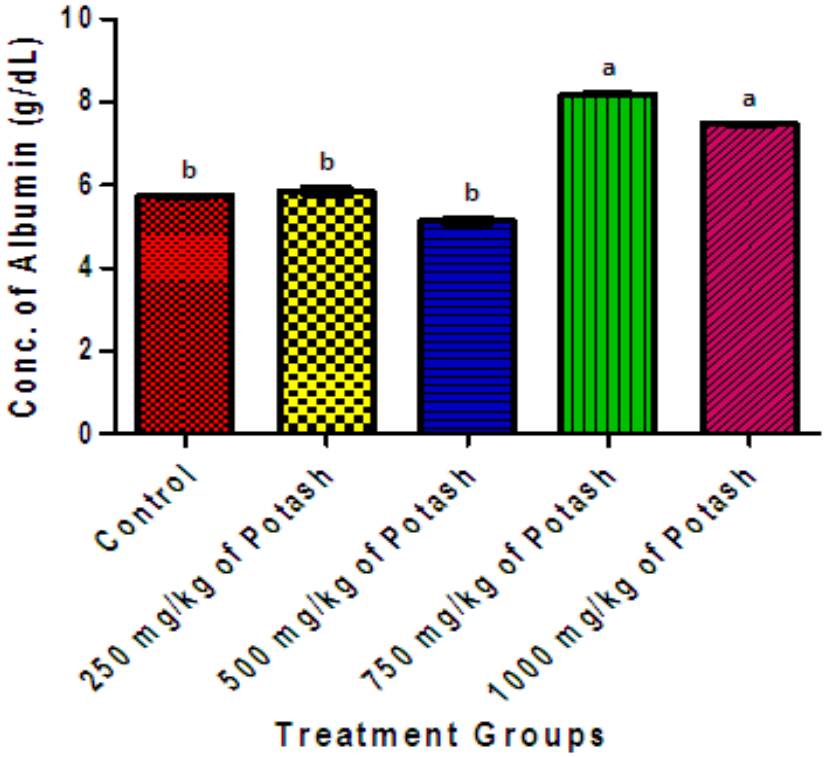

Fig. 5. Effect of potash on the concentration of Albumin of animals after 28 days of treatment
Results are presented as mean ± SD with n = 6. Bars with different letters are significantly different at P<0.05

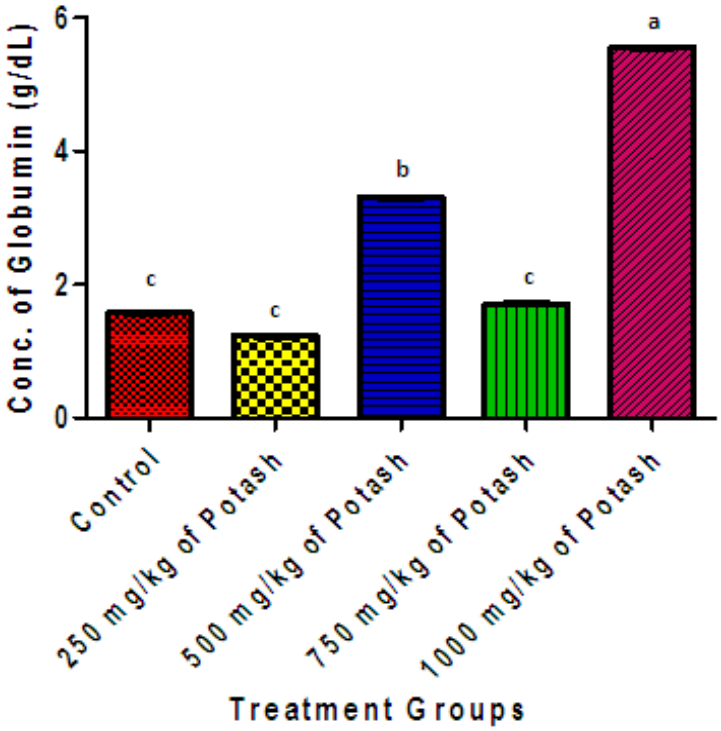

Fig. 6. Effect of potash on the concentration of Globulin of animals after 28 days of treatment
Results are presented as mean ± SD with n = 6. Bars with different letters are significantly different at P<0.05

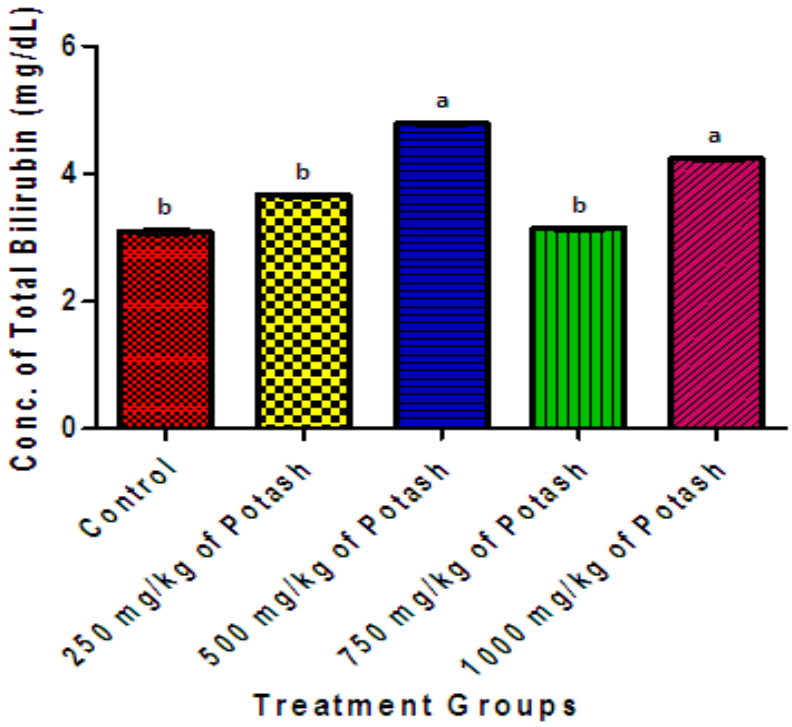

Fig. 7. Effect of potash on the concentration of Total Bilirubin of animals after 28 days of treatment

Results are presented as mean ± SD with n = 6. Bars with different letters are significantly different at P<0.05

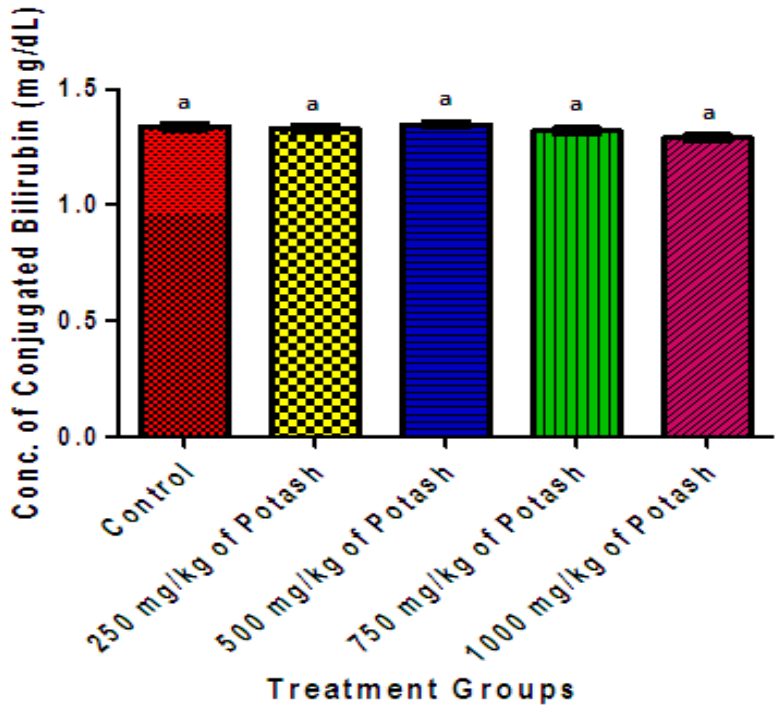

Fig. 8. Effect of potash on the concentration of Conjugated Bilirubin of animals after 28 days of treatment

Results are presented as mean ± SD with n = 6. Bars with different letters are significantly different at P<0.05

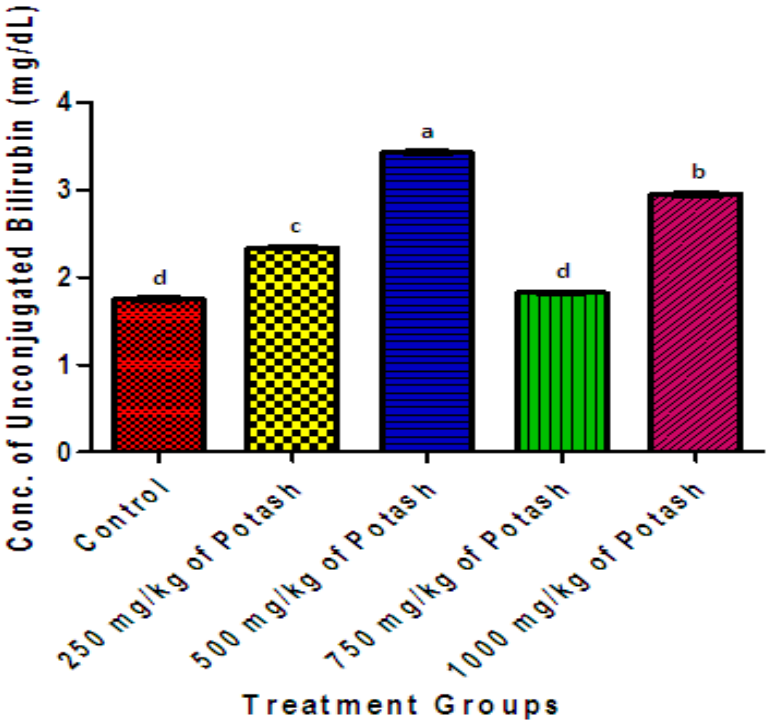

Fig. 9. Effect of potash on the concentration of Unconjugated Bilirubin of animals after 28 days of treatment

Results are presented as mean ± SD with n = 6. Bars with different letters are significantly different at P<0.05

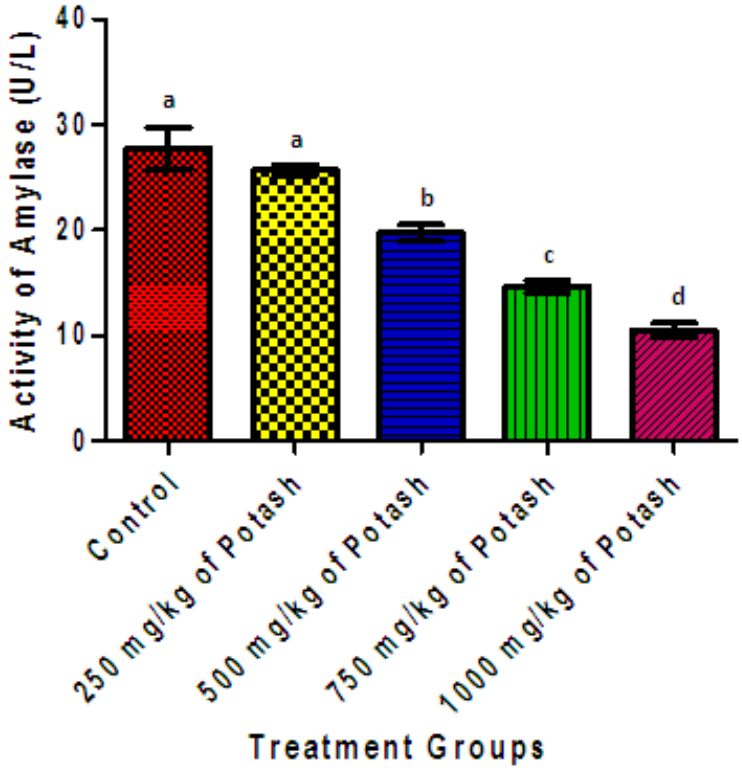

Fig. 10. Effect of potash on the activity of Amylase of animals after 28 days of treatment

Results are presented as mean ± SD with n = 6. Bars with different letters are significantly different at P<0.05

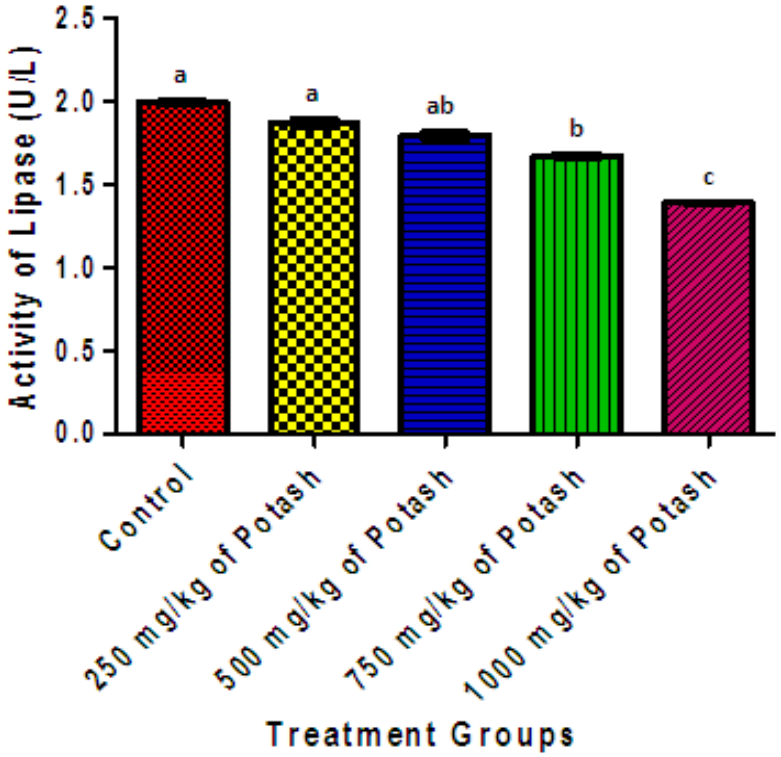

Fig. 11. Effect of potash on the activity of Lipase of animals after 28 days of treatment
Results are presented as mean ± SD with n = 6. Bars with different letters are significantly different at P<0.05

of cAMP will eventually lead to CAP inactiveness therefore, altered the processes of transcription. Thus, these inducible enzymes are reduced through direct effect on glucose, therefore caused reduction of cyclic AMP level. Administration of potash at high doses might have elevated cAMP in treated rats, thus the significant increase in these inducible enzymes. ALT is considered as one of the major biomarkers for liver damage because it is solely confined to the liver, unlike AST which is also abundantly present in other body organs such as the kidneys, brain, and hearts [22,23]. The significant increase in the activities of ALT, AST and ALP in animals treated with high doses of potash revealed that potash is considered toxic at high dose to the liver.

Similarly, concentrations of total protein and albumin were significantly elevated in animals treated with high dose of potash. This elevation suggested that metabolizing ability of the liver ensued from the administration of potash at higher doses has been compromised. This might have increased the functional activity of the liver by interfering with the state of synthesis, degradation, removal or clearance of total protein and albumin from the animals' body systemic circulation [24]. This increase in total protein could lead to dehydration which is harmful to the cellular homeostasis [25]. And have a negative effect on the metabolic activities of the liver, which will eventually result in disturbances of the health of the animal. The major function of albumin includes binding and transportation of metal ions, bilirubin, and drugs. Its quantification is required to evaluate the synthetic function of the liver [26]. Significant increase in the level of these parameters indicates that potash stimulated their synthesis in the liver at a higher dosage of 750 mg/kg and 1000 mg/kg body weight**.** Serum protein levels are controlled through synthesis in the liver and its levels thus reflect the synthetic ability of the liver [26].

Bilirubin refers to as one of the products produced during degradation of hemoglobin. However, high level of bilirubin in the blood lead is toxic to the body system, which we eventually cause different medical conditions such as jaundice, hyperbilirubinemia-induced auditory dysfunction and neurotoxicity resulting in brain damage [27]. Whereas slight elevation of unconjugated bilirubin in the blood serves as an antioxidant, that do safeguard the body from heart related diseases coupled with development

of tumor [28]. New investigation about reduction in the concentration of direct bilirubin may cause heart and brain defect. Serum bilirubin levels are often enhanced under a variety of clinical conditions. In the circulation of blood, bilirubin is bound to serum albumin, which prevents its potential toxicity thought to be caused by free bilirubin [29]**.** Despite its high-affinity of binding to albumin, bilirubin is rapidly and selectively taken up by the liver, biotransformed upon conjugation with glucuronate, and secreted into bile [28]. Thus, bilirubin is converted into bilirubin glucuronic acid in the liver and excreted along with bile. In this study, there was no significant change in the serum conjugated (direct) bilirubin concentrations. However, there was a significant increase in the serum levels of total and unconjugated (indirect) bilirubin in the serum of the Wistar that received potash. The elevation in the concentration of indirect serum bilirubin (unconjugated bilirubin) recorded might due to tissue injuries or damage caused by the toxic substance the animals were exposed to [30].

Administration of potash significantly reduced activities of amylase and lipase as the dosage of potash increases, and the lower is effect on each enzyme activities respectively. Indicating that at higher dose potash reduced amylase and lipase activities in the serum of the experimental animals. Amylase serves as one of the major enzymes needed during degradation of starch [20]. β-cells in the Islets of Langerhans are been destroyed by autoimmune reactions that occurred in the pancreas, which is facilitated by formation of reactive oxygen species in the leucocytes [31, 32]. During digestion and uptake of carbohydrates; when some of the key major enzymes were inhibited, this can easily reduce blood sugar level after taken carbohydrate diet. Hence, this may serve as one of the means of managing hyperglycemia associated with type 2 diabetes [33,34]. Whereas, Lipase is the enzyme responsible for digestion and absorption of triglycerides [20,26]. Its inhibition is one of the widest studied methods used to determine the potential activity of natural products to inhibit dietary fat absorption. Decrease in energy intake from dietary fat through inhibition of this enzyme may be an excellent strategy to prevent and treat obesity [35].

5. CONCLUSION

It was revealed in our findings that potash administration at higher dose is toxic and perturbs hepatic biomarkers, therefore discontinuation of potash consumption needs to be recommended.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

It is not applicable.

ETHICAL APPROVAL

As per international standard or university standard written ethical approval has been collected and preserved by the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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