

RESEARCH PAPER

THE EFFECT OF POTASH ON LIVER FUNCTION OF WISTER RATS

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ABSTRACT

This study was aimed at investigating the effect of potash on liver function of wistar rats. Thirty two adult albino wistar rats divided into eight (8) groups were used for this study. Group A was the control, while B, C and D were test groups given 0.4g/ml, 0.6g/ml, and 0.8g/ml of potash respectively for 21 days. The weights of both the test animals and the control were monitored. The results on acute toxicity tests showed that LD50 was greater than 2.5g/kg body weight. The activities of GGT increased in group B (1.63 ± 0.54 U/L), C (3.20 ± 0.92 U/L) and D (3.41 ± 0.81 U/L) when compared with the control (1.00 ± 0.36 U/L). The activities of AST increased in group B (0.83 ± 0.09 U/L), C (0.90 ± 0.05 U/L) and D (0.93 ± 0.09 U/L) when compared with the control (0.76 ± 0.13 U/L). ALT activities in group B (0.57 ± 0.18 U/L), C (0.67 ± 0.14 U/L) and D (0.87 ± 0.07 U/L) increased in comparison with the control (0.55 ± 0.17 U/L). ALP activities increased in group B (4.18 ± 1.99 U/L), C (6.86 ± 2.04 U/L) and D (6.00 ± 1.34 U/L), in comparison with control (3.80 ± 1.83 U/L). Our study revealed that Potash altered the functionality of the liver as well as the physical activity of the wistar rats and that the effect is dosage dependent.

Keywords: Potash, Liver, Toxicity, Rats, Dose

INTRODUCTION

Potash is any of various mined and manufactured salts that contain potassium in water-soluble form, the name derived from *pot ash*, refers to plant ashes soaked in water in a pot, the primary means of manufacturing the product before the industrial era (Davy, 1808). It is produced worldwide at amounts exceeding 30 million tonnes per year, mostly for use in fertilizers. Various types of fertilizer-potash constitute the single largest global industrial use of the element potassium. Potassium was first derived by electrolysis of caustic potash (aka potassium hydroxide), in 1807 (Knight, 1992).

The old method of making potassium carbonate (K_2CO_3) was by collecting or producing wood ash (an occupation carried out by ash burners), leaching the ashes and then evaporating the resulting solution in large iron pots, leaving a white residue called *pot ash* (Dennis, 2006). Approximately 10% by weight of common wood ash can be recovered as pot ash. Later, *potash* became the term widely applied to naturally occurring potassium salts and the commercial product derived from them (*The World Potash Industry, 2000*).

Locally known as “kaun” or “Akanwu”, Potash is used commonly for culinary purposes. It is used for cooking pulses like beans, akidi (black Mexican beans), fiofio (cowpea beans etc in order to tenderize the pulses easily. Akanwu is



also added in ewedu and okro soup (a Nigerian delicacy) during preparations in order to increase the greenness and texture of the vegetables (Okpala, 2015). No data exist about the quantity or dosage of potash consumed in the average daily meal of Nigerians.

The liver is a large, meaty organ that sits on the right side of the belly. Weighing about 3 pounds, the liver is reddish-brown in colour and feels rubbery to the touch. Normally you can't feel the liver, because it's protected by the rib cage. The liver is a vital organ of vertebrates and some other animals (Hirschfield and Gershwin, 2013). In the human, it is located in the upper right quadrant of the abdomen, below the diaphragm. The liver has a wide range of functions, including detoxification of various metabolites, protein synthesis, and the production of biochemicals necessary for digestion (Abdel-Misih and Bloomston, 2010). The liver is a gland and plays a major role in metabolism with numerous functions in the human body, including regulation of glycogen storage, decomposition of red blood cells, plasma protein synthesis, hormone production, and detoxification (Abdel-Misih and Bloomston, 2010). It is an accessory digestive gland and produces bile, an alkaline compound which aids in digestion via the emulsification of lipids. The gallbladder, which is a small pouch that sits just under the liver, stores bile produced by the liver. The liver's highly specialized tissue consisting of mostly hepatocytes regulates a wide variety of high-volume biochemical reactions, including the synthesis and breakdown of small and complex molecules, many of which are necessary for normal vital functions. Estimates regarding the organ's total number of functions vary, but textbooks generally cite it being around 500 (Tortora and Derrickson, 2008).

Liver function tests (LFTs or LFs) are groups of blood tests that give information about the state of a patient's liver. These tests include Alkaline Phosphatase (ALP), Aspartate Aminotransferase (AST) and Alanine Aminotransferase (ALT) which are useful biomarkers of liver injury in humans and animals with some degree of intact liver function (Lee, 2009). Most liver diseases cause only mild symptoms initially, but these diseases must be detected early. Hepatic (liver) involvement in some diseases can be of crucial importance (Johnston, 1999). This testing is performed on blood samples. Some tests are associated with functionality (e.g., albumin), some with cellular integrity (e.g., transaminase), and some with conditions linked to the biliary tract (gamma-glutamyl transferase (GGT) and alkaline phosphatase (ALP)) (McClatchey, 2002). No data exist about the quantity or dosage of potash consumed in the average daily meal of Nigerians, hence making it difficult to assess the potential toxicity in humans.

Although potash and related compounds such as potassium bromate are used for certain food preparations however, studies by Bankole *et al.*, (2015) and Kurokawa *et al.*, (1990) have revealed that it is not suitable for our health thus curtailing the level of consumption is highly advisable. Hence, the aim of this study was to determine the possible effect of *kaun*-Potash on the liver by using animal model to study its toxicity and relate it to the dose.

MATERIALS AND METHODS

Research Design: A total of thirty two (32) adult Albino wistar rats of comparable sizes were used for this study. They were divided into four equal groups of eight (8) rats each. Group A served as the control and the rats were given distilled water and normal feed (grower's mash). The test groups B, C and D rats were given 0.4g/ml, 0.6g/ml, and 0.8g/ml of potash respectively, alongside normal feed (grower's mash). The substance administration was given for 21 days (3weeks) the weights of both the test animals and the control were monitored before and after administration of potash. After administration, the rats were put under light chloroform anaesthesia and blood samples were collected for estimation of ALT, AST, ALP and GGT activities. ANOVA was used to analyze the results of the weight and differences was considered significant at $P < 0.05$ level of confidence. All data was expressed as Mean \pm Standard error of mean (SEM).

Geographical Description of the study area: This study was carried out in the Histology Laboratory at the College of Medical Sciences, Ambrose Alli University Ekpoma, in Esan land, Edo State. Esan land comprises 5 local government areas of Esan west, Esan central, Esan north-east, Esan south-west and Igueben in Edo State, Nigeria. Esanland is located on a plateau; we have the top and bottom of sections of the plateau (Segynola, 2015). This area is located between latitude $6^{\circ} 10'$ and $6^{\circ} 45'$ north of the equator and between longitudes $6^{\circ} 10'$ and $6^{\circ} 30'$ east of the Greenwich Meridian (Akinbode, 1983). The 2006 national census put the population of the study area at 591,534 people (NGSA, 2006). Projected to 2015 at 2.8 percent national growth rate, the 2015 population of the study area is 740,601 people.



Experimental Animals/ Housing Condition: Thirty two (32) Adult Albino Wistar rats of comparable sizes and weights ranging from 0.25kg to 0.36kg were procured from the animal farm, College of Medicine, Ambrose Alli University Ekpoma, Edo state and transferred to the Histology laboratory where they were allowed two (2) weeks of acclimatization. They were kept in wire mesh cages with tripod that separated the animals from the faeces to prevent contaminations. During this period of acclimatization, the rats were fed with growers' mash and water provided ad libitum. The animals were maintained and utilized in accordance with the standard guide for the care and use of Laboratory animals by the University Ethic committee.

Animal Grouping: The experimental animals were separated into four groups (A-D). Each group contained eight (8) rats each (n=8) using 4 big cages to house them. Group A served as the control, while groups B-D served as the test groups. Group B-D received graded doses of Potash prepared accordingly and weighed to determine the quantity to be administered. Group A received only the normal feed (grower's mash) and water with no administration of Potash.

Study Duration: The preliminary studies, animal acclimatization, ingredients procurement (potash preparation, actual animal experiment and evaluation of results lasted for a period of five months (July, 2015 to November, 2015). However, the actual administration of potash to the test animals lasted for 3 weeks.

Substance Preparation: Considerable quantities of Potash were obtained commercially from the open Ekpoma market. The potash purchased was carefully poured on a clean dry plastic container. From this container it was measured using an Electric balance and packaged in small plastic envelopes and then stored pending usage. The substance preparation process was performed with maximum care in order to avoid any form of contamination.

Acute toxicity test: This was done using the method described by Lorke, (1983). The study was conducted in two phases using a total of sixteen male rats. In the first phase; the wistar rats were divided into three (3) groups of three rats each. The wistar rats were treated with potash at doses of 0.01kg, 0.1kg and 10kg extract/kg body weight. Each rat was given a single dose after 5 days of adaptation. They were observed for 24hours for signs of toxicity including death. In addition, a fourth group of three rats was set up as the control and the animals in this group were not given the extract. In the second phase, wistar rats were divided into three (3) groups of one rat each and treated with potash at doses of 0.6g, 1.0g and 2.5g per kg body weight orally. All animals were observed for signs of acute toxicity for two weeks. The LD50 was determined accordingly to observable signs of toxicity.

Substance Administration: The rats were weighed before the administration of Potash and before they were sacrificed. The administration of potash was performed by giving orally as follows:

Group A (Control) received 150g of normal feed (growers' mash) and distilled water daily for 21days with no administration of Potash.

Test group B received 0.4g/ml of Potash plus 150g of feed daily and water was given ad libitum for 21days.

Test group C received 0.6g/ml of Potash plus 150g of feed daily and water was given ad libitum for 21days.

Test group D received 0.8g/ml of Potash plus 150g of feed daily and water was given ad libitum for 21days.

Sample Collection: Blood samples (5mls) of each rat was obtained through the femoral artery at the end of the administration (3 weeks) under light chloroform anaesthesia and dispensed into lithium heparin containers labeled appropriately (A, B, C and D). The samples were separated using a centrifuge for 5 minutes at 3000 rpm within two hours of collection into clean dry plain containers which were labeled corresponding to the initial blood sample container. Laboratory estimation of the ALT, AST, ALP and GGT was then carried out on the samples.

Analytical Method: Alanine Amino Transferase (ALT) and Aspartate Amino Transferase (AST) activity was determined using the method described by Rietman and Frankel, (1957), while the activities of Alkaline Phosphatase (ALP) and Gamma Glutamyl Transferase (GGT), were determined using the method described by Rec. Gsc (DGKC) (1972) and the method described by Szasz, (1969) respectively.

Statistical Analysis: The data generated from the study (both control and test groups) was subjected to basic statistical measurement using parametric analysis of variance (ANOVA) using the computer SPSS (Statistical Package for the



Social Sciences) version 21.0 windows application at 95% level of confidence. All results were reported as mean \pm standard error of mean).

RESULT

Table 1 shows the results of acute toxicity test. The results showed that no animal died within 24 hours after treatment with the extract and the LD50 was greater than 2.5g / kg body weight. There was no death recorded among all the dose groups throughout the two weeks experimental period.

Table 1: Acute toxicity test of Rats fed with Potash

Experiment	Dose g/kg bw	No of Deaths After 24 hours	Treated Rats After 24 hours
Phase 1 (n=9)	0.01	0/3	0/3
	0.1	0/3	0/3
	1.0	0/3	0/3
Control (n=4)	0	0/4	0/4
Phase 2 (n=3)	0.6	0/1	0/1
	1.0	0/1	0/1
	2.5	0/1	0/1

Table 2 presents the summary of physical observations and average feed consumption rate during and at the end of the study. There was no observable change in the fur colour of both the test and control animals. On the other hand, there were no recorded changes in skin surfaces on the feet, hand, tail, mouth, ears and eyes. However, test animals in group C and D presented signs of aggressiveness as part of their behaviour. Similarly, there was sign of diarrhoea in the faeces of test animals in groups B, C and D. There was no death recorded in both control and test groups. It was observed that test groups C and D rejected water intake within the period of study. It was also observed that the control group and the test group B gave birth within the second week of the study. The feed intake was observed to be higher in the control group particularly when compared with the test groups. This observation was statistically significant ($p < 0.05$) at groups B ($75.78 \pm 6.03g$), C ($74.29 \pm 5.06g$) and D ($104.76 \pm 4.12g$).

Table 2: Physical Observations and Average Feed Consumption of Rats Fed with Potash

Observations	Control	B (0.4g Pot)	C (0.6g Pot)	D (0.8g Pot)
Fur colour	-	-	-	-
Behavioral changes	-	-	+(aggressive)	+(aggressive)
Skin changes	-	-	-	-
Diarrhoea	-	+	+	+
Death	-	-	-	-
Water rejection	-	-	+	+
Birth	+	+	-	-
Physical agility	Active	Active	Weak	Weak
Feed intake (g)	139.29 ± 1.87^a	75.48 ± 6.03^b	74.29 ± 5.60^b	104.76 ± 4.12^b

Key: + = present; - = negative; Pot= Potash; Gp= group. Values are mean \pm standard error of mean. Values in a row with a different superscript are significantly different at $p < 0.05$



Table 3 shows the body weight changes in wistar rats fed with potash for 21 days (3 weeks). In this study it was observed that the mean weight of both test groups and control increased after the administration of potash. This increase in weight after acclimatization was noticeably observed in test group C. Also there was significant decrease in the body weight after the administration of potash. This significant reduction was observable in Group B and C that received the 0.4g/ml and 0.6g/ml of potash respectively.

Table 3: Body weight changes of rats fed graded doses of Potash at various intervals

Weight (kg)	Control (n=8)	B(0.4g Pot) (n=8)	C(0.6g Pot) (n=8)	D(0.8g Pot) (n=8)	F-value	P-value	Significance
WBA	0.26±0.04 ^a	0.23±0.01 ^a	0.36±0.06 ^b	0.23±0.03 ^a	13.58	0.00	Significant
WAA	0.33±0.07 ^a	0.25±0.04 ^b	0.43±0.01 ^b	0.28±0.03 ^a	9.60	0.00	Significant
WAPA	0.36±0.04 ^a	0.24±0.12 ^b	0.24±0.05 ^b	0.27±0.04 ^a	2.73	0.04	Significant

Key: WBA: weight before acclimatization; WAA: weight after acclimatization; WAPA: weight after potash administration; Values are mean ± standard error of mean; Wt=weight (Kilograms); n=number of samples; Pot=Potash. Values in a row with a different superscript are significantly different at P<0.05

Table 4 shows the liver function of rats fed with graded doses of potash for 21 days. The results obtained in this study shows that rats fed with potash induced an increase in the levels of GGT, AST, ALT and ALP when compared to the values in the control rats. This increase in liver function parameters were dosage dependent; though not statistically significant (P>0.05).

Table 4: Liver Function of Wistar Rats fed with graded doses of Potash for 21days.

Parameters (U/L)	Control (n=8)	B(0.4g Pot) (n=8)	C(0.6g Pot) (n=8)	D(0.8g Pot) (n=8)	F-value	P-value	Significance
GGT	1.00±0.36 ^a	1.63±0.54 ^a	3.20±0.92 ^a	3.41±0.81 ^a	2.91	0.067	Not significant
AST	0.76±0.13 ^a	0.83±0.09 ^a	0.90±0.05 ^a	0.93±0.09 ^a	0.62	0.62	Not significant
ALT	0.55±0.17 ^a	0.57±0.18 ^a	0.67±0.14 ^a	0.87±0.07 ^a	1.00	0.99	Not significant
ALP	3.80±1.83 ^a	4.18±1.99 ^a	6.86±2.04 ^a	6.00±1.34 ^a	0.64	0.64	Not significant

Key: Values are mean ± Standard error of mean; Pot=Potash; Wt=weight (Kilograms); n: Number of sample; GGT=Gamma glutamyl transferase; AST=Aspartate amino transferase; ALT=Alanine amino transferase; ALP=Alkaline phosphatase. Values in a row with the same superscript are not significantly different at P<0.05.

DISCUSSION

The acute toxicity of potash showed that no animal died within 24 hours after treatment with potash. The major signs of toxicity noticed within 24hours included loss of appetite and general weakness. The signs were not seen in 10 mg/kg b.w. dose group but progressed and became increasingly pronounced as the dose increased towards 2.0g/kg b.w. The LD50, being greater than 2.5g/kg b.w. is thought to be safe as suggested by Lorke (1983). Again, the absence of death among rats in all the dose groups throughout the two weeks of the experiment seems to support this claim. According to Material Safety Data Sheet (2004), it was reported that the LD50 of potash (potassium chloride) was 2600mg/kg and this corresponds with the findings in the study. Similarly, it was also reported that the LD50 of potash in the form of potassium chloride was 2.6g/kg (Material Safety Data Sheet, 2004). The oral LD50 value in this study suggests that potash is relatively non toxic substance when taken in relatively small proportion.



The results obtained showed that the administration of potash induced a significant reduction in the weight of wistar rats fed orally for 21 days when compared with the control groups (<0.05). This reduction is dosage dependent. This finding is in agreement with the study carried out by Kurokawa *et al.*, (1990) in which they reported that the reduction in body weight could be as a result of the chemical nature of potash and probably as a result of the chemical nature of potash and probably as a result of decreased feed intake resulting from the undesirable taste of potash. This finding is also supported by the work done by Okalie and Ikewuchi (2004) who reported a significant reduction in body weight of rabbits that received potassium bromate. Furthermore, the result on weight reveals that the intake of potash caused some characteristic physical changes in adult wistar rat as evident in the reduction of physical activity and feebleness. This is in agreement with the study of Oyewo *et al.*, (2013) who reported the alterations in wistar rats fed with potassium bromate.

In this study, the liver function of wistar rats fed with potash was evaluated. It was observed that treatment with potash induced changes in the serum levels of the liver parameters. However the levels of serum ALT, AST, ALP and GGT activities were higher in the test groups (group B, C and D) when compared to the control group, though this observation was not statistically significant ($P>0.05$). Measurement of enzymatic activities of AST, ALT, ALP and GGT is of clinical and toxicological importance as changes in their activities are indicative of liver damage by toxicants or in diseased conditions (Singh *et al.*, 2001). The observed increase in the activities of serum ALT, AST and ALP in the potash treated rats may be an indication of liver dysfunction.

No single liver function test alone is considered as a reliable index of liver function under all conditions, but increase in several of the liver enzymes could suggest liver damage. Most liver diseases usually show altered serum GGT levels. ALP comes mainly from the cells lining bile ducts, but is also in bone. The increase in AST, ALT and GGT suggests high permeability of hepatocytes due to inflammation or cell death. AST and ALT normally resides inside the liver cells (in cytoplasm), so raised levels suggests hepatocellular damage of the wistar rats, which gives an indication of the possible hepatotoxic effect of potash. The results obtained in this study supports earlier study by Singh *et al.*, (2001) where administration of plant extract resulted in increased activities of ALT and AST in rat serum. It is also in agreement with the observations of Azeez *et al.*, (2011); they reported increase in liver enzymes of rats treated with potassium nitrate.

The elevated levels of ALP as observed in this study may be of liver or bone origin, since the two main sources of ALP are liver and bone, although more studies will be required to further unveil the relationship. Therefore, the findings of this study supports the alternate hypothesis which states that potash consumption can alter the weight and liver function of test animals and the changes observed were dosage and duration dependent.

CONCLUSION

This study has revealed that Potash altered the functionality of the liver as well as the physical activity of the wistar rats. Though the intake of Potash in small quantities may not be toxic to the liver we suggest that the consumption of Potash be reduced to the barest minimum, and other natural dietary sources of potassium such as fruits (e.g. bananas) be encouraged.

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IWEKA, F.K.; Chief researcher, laboratory supervisor and content analyst. DIC-IJIEWERE, O.E.; Resource supervisor and result analyst. OAIKHENA, F.; Sampling/Laboratory analyst, BANKOLE, J.K.; Co-researcher. FESTUS, O.O.; Co-researcher. DADA, F.L.; Co-researcher.

