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Histopathologic and Biochemical Effect of Potash, Dye, and *Tamarindus indica* on selected organs

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ABSTRACT
Potash, Dye, and Tamarindus indica are commonly used to induced abortion in Africa, especially by individuals who cannot afford the conventional methods and such abortions are illegal. This study therefore, investigated the biochemical and histopathological changes in selected organs of albino rats. A total Albino rats (n= 60), weighing 80-100g aged between 5-8 weeks were divided into four groups of five animals each. A single dose of 6500mg/kg, 7500mg/kg and 8500mg/kg of potash, dye, and T. indica extract were administered to the albino rats orally for 14 days. The animals were euthanized, blood samples, the liver, kidney, and stomach were analyzed for biochemical and histopathologic changes. Results showed an increase in body weight, increase water consumption as well as urine output of the rats. The mean body weight of all experimental animal groups (Potash, Dye and T. indica) when compared with control increased after the administration of the substances. The effect of Potash shows statistically significant value (P<0.05) in all the biochemical parameters. The effect of dye shows statistically significant values only in AST, ALT, ALP (0.000, 0.002, 0.005) compared with the control. While T indica shows statistically significant increase (P<0.05) in serum Sodium (0.002), Potassium (0.035), creatinine (0.034), and AST (0.006) values in the treated animals when compare to the values in the control. The histologic sections of the liver in potash treatment group showed linear cord of hepatocytes with vesicular nuclei, some tightness of the sinusoids, ballooning of the cytoplasm of the hepatocytes and pyknosis of the nuclei and T. indica is sometimes binucleate. The histologic sections of the kidney in potash, dye and T. indica were generally unremarkable, the histologic section of the stomach seems to have little effect. Therefore, consumption of these substances in large amount for a long period of time can be harmful to the liver, kidney and stomach.
***Keywords**: Toxicity, Potash, Dye, and Tamarindus indica*

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INTRODUCTION

The use of Potash, Dye, and *Tamarindus indica* for inducing abortions is a significant issue in Africa, particularly Nigeria. The toxicity of plant extracts can also be life-threatening, manifesting as symptoms such as heart block, lightheadedness, and vomiting [1]. Consumption of large amounts of plant extracts can harm the liver, kidneys, and gastrointestinal tract [2,3,4,5].

A plant with a tree-like structure, the Tamarind (*Tamarindus indica* L) is a member of the *Fabaceae* family and Caesalpinioidae subfamily under the genus Tamarindus. The fruit of *Tamarindus indica* appears reddish-brown color at first, changing black or black-brown with time. In Nigeria, Tamarind [1] and is a versatile fruit commonly called Ajagbon in the Yoruba language. In northern parts of the country inhabited by the *Hausa -Fulani* tribes particularly, it is known as *Tsamiya*. According to report [6], Potash (Kanwa) is a white crystalline residue that is left over after an aqueous extract of ashes has evaporated. It is a mixture of potassium salt and an impure type of potassium carbonate. Potash has been produced and utilized for residual cleansing purpose from ancient times in a number of different countries around the world [7].

Dyes are introduced into the environment through industrial effluent from food, drug, cosmetic, textile and dyestuff factories [8,9,10]. Dyes are coloured organic compounds that are used to colour various substances like fabrics, food, hair, paper and drugs. Some of these chemicals and dyes are highly structured polymers that are very challenging for living things to degrade. This study therefore, investigated the biochemical and histopathological changes in selected organs of albino rats.

MATERIAL AND METHODS

Extraction of Plant Material

Fresh *Potash, Tamarindus indica and Dye* were bought from Katako market in Jos, processed to extraction at the Department of Biochemistry, National Veterinary Research Institute Vom, Plateau State by the method described by Anamika [11].

Animal Welfare Provision and Ethical Clearance

This study was conducted in accordance with current National Research Council [12] guidelines for animal welfare. The protocol was reviewed by the institutional Animal care. Ethical Clearance for the study was from Animal House, Department of Pharmacology, University of Jos.

Determination of Oral Lethal Dose

An oral acute toxicity study was carried out using the 'Up-and- Down' method of testing mice and a dose of 5000 mg/kg was administered via oral gavage following the Organization for Economic Development (OECD) guideline [13]. Animals were observed for 30 min for signs of toxicity and mortality. The same procedure was repeated for the remaining animals and observed for 14 days.

Experimental Design

A total of sixty (60) albino rats aged between 5-6 weeks with body weights ranging between 80-100g, were used for this study. Ten animals were used for acute toxicity study while fifty animals randomly divided into four groups of five animals each for Potash, Dye, and *Tamarindus indica* for the sub-acute toxicity study with a normal control. The animals were housed in the animal house, University of Jos and allowed for one week to acclimatize under standard laboratory conditions in a controlled room with 12 hours light; 12 hours dark cycle; (12 hours: 12 hours dark – light cycle) at room temperature of 21-25^{0}c and 52-58% humidity. The animals were fed with commercially prepared meal and water was given *ad libitum* throughout the study period. Animals were divided into four (4) groups consisting of 5 rats each. The weight of each was taken daily throughout the period of the study. The changes in the rate and quantity of food and water consumption were also recorded each day.

The grouping is as follows;

Group A: Negative control fed with Grower mash and water only.
Group B: Given 6500mg/kg *Potash* aqueous extract.
Group C: Given 7500mg/kg *Potash* aqueous extract.
Group D: Given 8500mg/kg *Potash* aqueous extract.
The same method was applicable for *Tamarindus Indica* and Dye.

Sacrifice and Histopathology study

All rats were painlessly sacrificed under chloroform anesthesia and subjected to standard necropsy procedures. The appearance and macroscopic changes in the rats' organs were observed and harvested, fixed in 10% formal saline. Tissue samples were processed using Leica automatic processing machine. Slides of 5µm were made, stained by the Haematoxylin and Eosin method and examined microscopically [14]. Photomicrographs were taken using a light microscope and the photomicrographs were taken with a Hirocam High Resolution Optics microscope digital camera system using X10 and X40 objectives.

Statistical analysis

The mean values of the rat body weight, organ weight, food and water consumption during the experiment and results were expressed in tables and bar charts. Values are given as means and data collected were analyzed using SPSS windows version 20.0 software and Microsoft excel 2013.

RESULTS

Table 2: General Observations

Treatment	Fur appearance	Alertness	Breathing	Feacal droppings	Colour	Urine output
Control	Smooth	Active	Normal	Formed	Brown	Normal
POTASH A to C	Rough	Less active	Fast	S/formed	Brown	Slight Increase
DYE A to C	Rough	Less active	Very fast	s/formed	Light-deep blue	Slight increase
T. INDICA A to C	rough	Less active	Fast	f/formed	brown	Slight increase

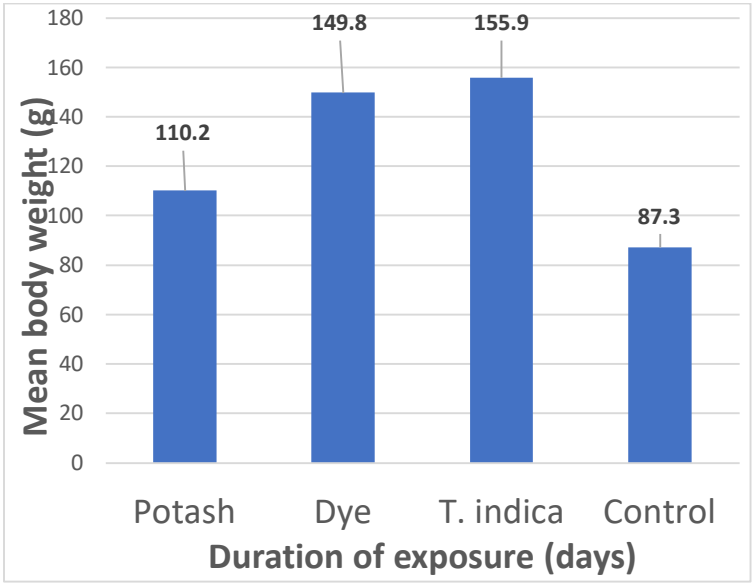


Figure 1: Mean body weight (g) of the albino rats during the duration of the experiment

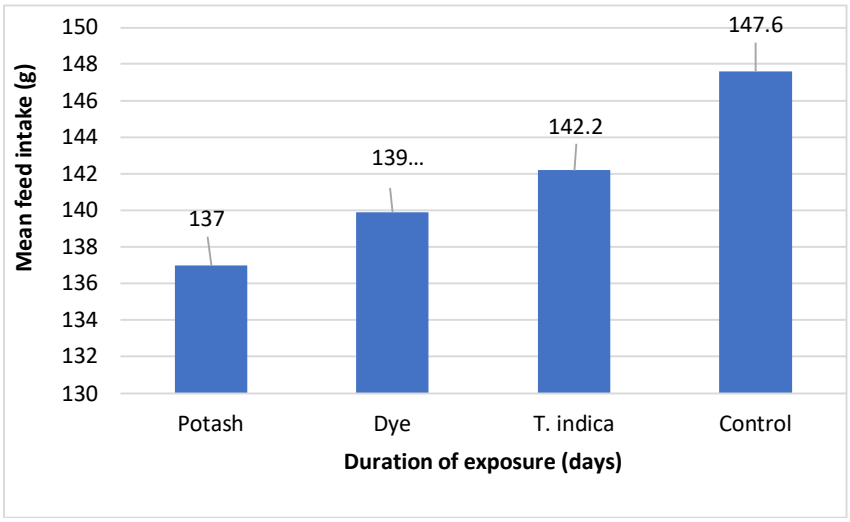


Figure 2: Mean feed intake (g) of albino rats during the duration of the experiment

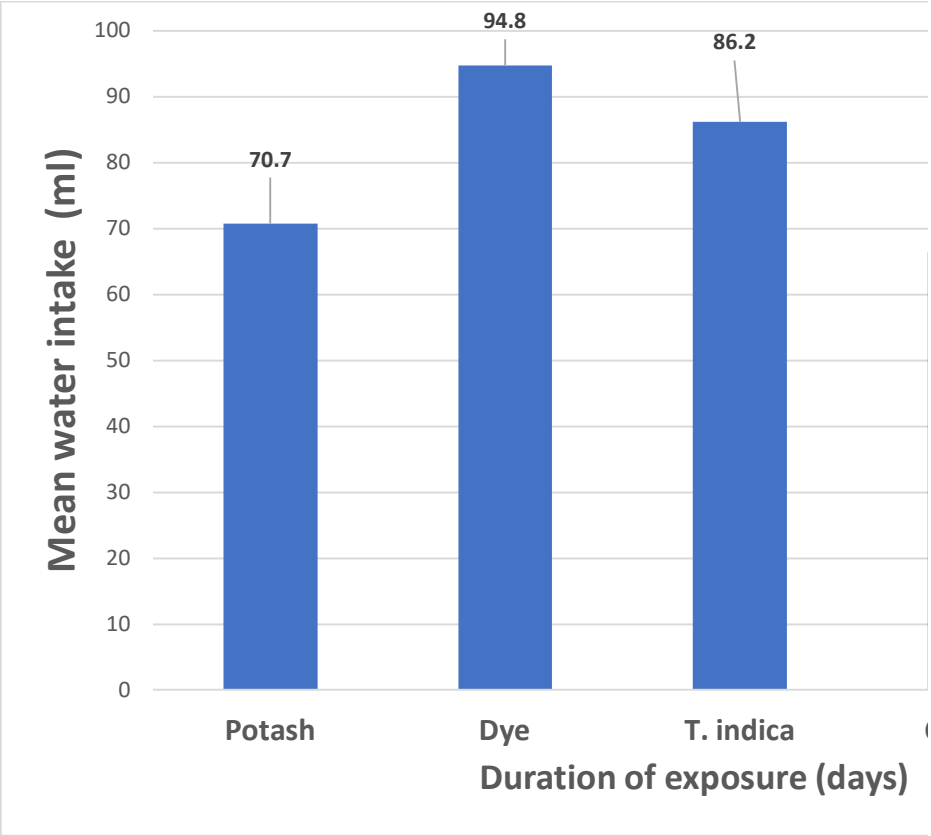


Figure 3: Mean water intake (ml) of albino rats during the duration of the experiment

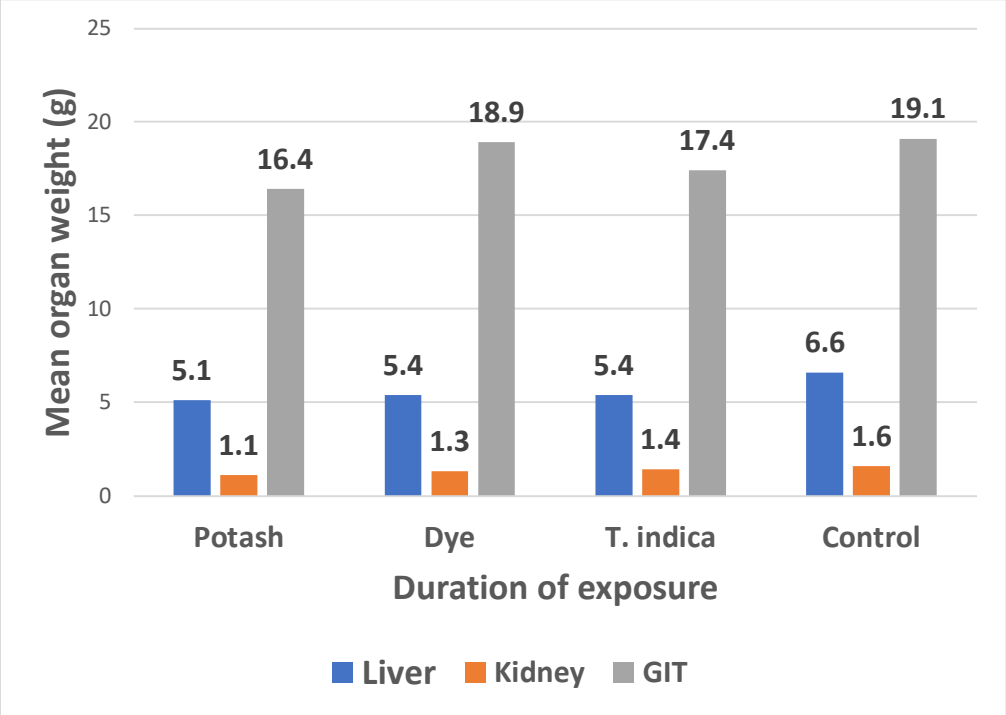


Figure 4: Mean organ weight (g) of albino rats during the duration of the experiment

Table 3.0: Effects of Potash on Electrolyte, Urea, Creatinine, and Liver Function Test of albino rats

Parameters/Groups	Potash	Control	t-test	P-value
Na^+	133.3±8.1	125±0.0	28.571	0.001
K^+	5.4±0.6	5.0±0.0	16.550	0.004
Cl^-	90.7±11.7	86.0±0.0	13.450	0.005
HCO_3^-	26.3±1.2	30.0±0.0	39.500	0.001
UREA	6.2±0.3	3.3±0.0	40.589	0.001
CREATININ	36.0±1.0	48.0±0.0	62.354	0.000
ALP	285.3±36.4	194.0± 0.0	13.591	0.005
ALT	94.7±7.5	92.0±0.0	21.846	0.002
AST	180.7±5.1	220.0±0.0	60.980	0.000

KEYS: Na^+- Sodium, K^+- Potassium, Cl -Chloride, HCO_3^- - Bicarbonate, ALP- Alkaline phosphatase, ALT- Alanine aminotransferase and AST- Aspartate aminotransferase

Table 4: Effect of Dye on Electrolytes, Urea, Creatinine and Liver Function Test on albino rats

Parameters/Groups	Dye	Control	t-test	P-value
Na^+	139.3±8.5	125±0.0	2.919	0.100
K^+	5.3±0.2	5.0±0.0	2.000	0.184
Cl^-	94.3±9.5	86.0±0.0	1.519	0.268
HCO_3^-	27.0±4.6	30.0±0.0	1.134	0.374
UREA	3.6±0.3	3.3±0.0	1.835	0.208
CREATININ	41.3±4.9	48.0±0.0	2.341	0.144
ALP	277.0±20.1	194.0± 0.0	7.161	0.019
ALT	87.3±4.9	92.0±0.0	1.639	0.243
AST	172.0± 15.7	220.0±0.0	5.290	0.034

Table 5.0: Effects of *Tamarindus Indica* on Electrolytes, Urea, Creatinine, and Liver Function Tests of albino rats.

Parameters/Groups	*T. indica*	Control	t-test	P-value
Na^{2+}	138.0±1.0	125±0.0	22.517	0.002
K^+	5.3±0.1	5.0±0.0	5.192	0.035
Cl	97.0±5.6	86.0±0.0	3.422	0.076
HCO3	28.7±3.2	30.0±0.0	1.718	0.547
UREA	4.5±0.6	3.3±0.0	3.554	0.071
CREATININ	41.7±2.1	48.0±0.0	5.270	0.034
ALP	251.3±35.4	194.0± 0.0	2.808	0.107
ALT	83.7±10.0	92.0±0.0	1.441	0.286
AST	144.7± 10.4	220.0±0.0	12.536	0.006

KEYS: Na^+- Sodium, K^+- Potassium, Cl^- ChlorideHCO_3^- - Bicarbonate, ALP- Alkaline phosphatase, ALT- Alanine aminotransferase and AST- Aspartate aminotransferase

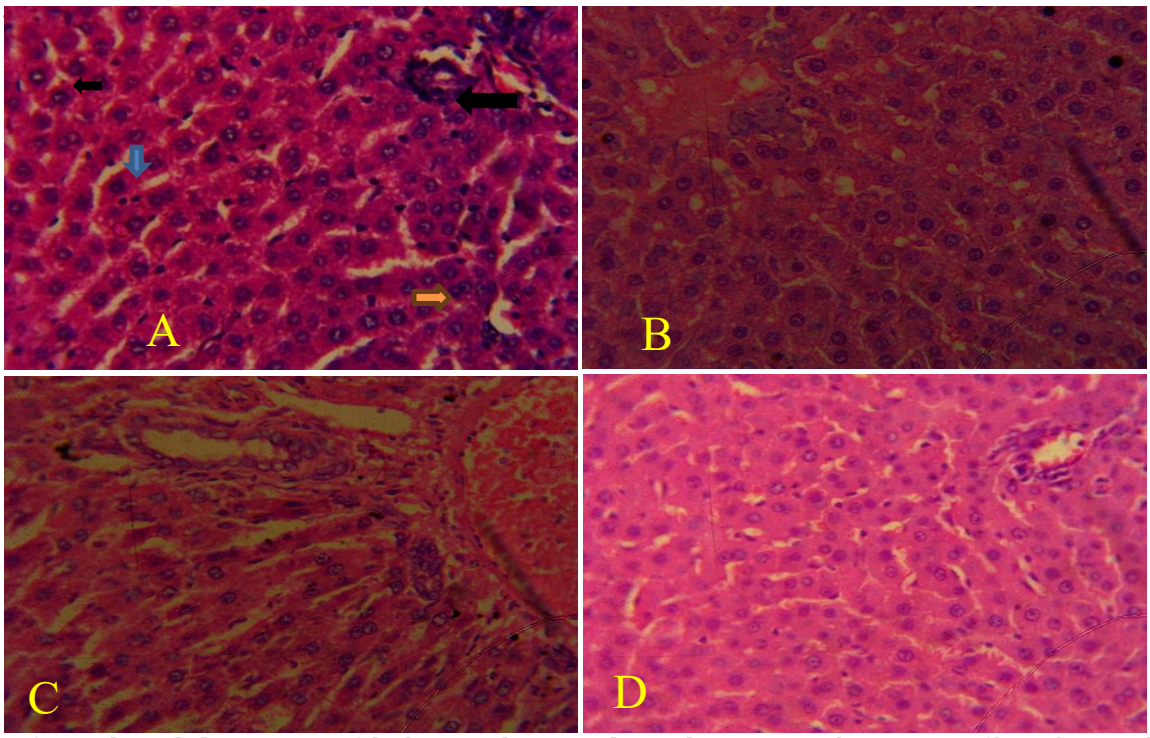


Fig. 5: Histological observations of the liver as shown in plate 1: [Group A Histologic section of liver showing the central vein (blue arrow), linear cord of hepatocytes (green arrow) with vesicular nuclei. While the black arrow and yellow arrow shows presence of kupffer cells lining the sinusoids and bile duct respectively. [Group B Potash 6500mg/kg body weight] Histologic section of Liver showing a measure of distortion of the arrangements of the hepatocytes and uptake of both H and E. [Group C –Potash 7500mg/kg body weight] Histologic section of Liver showing a measure of distortion of the arrangements of the hepatocytes and uptake of both H and E. Portal traid (right arrow) consisting with. Bile ducts (green arrow), portal artery (yellow arrow). [Group D –Potash 8500mg/kg body weight] Histologic section of Liver showing some tightness of the sinusoids, ballooning of the cytoplasm of the hepatocytes and pyknosis of the nuclei. Magnification X400, H and E Stain

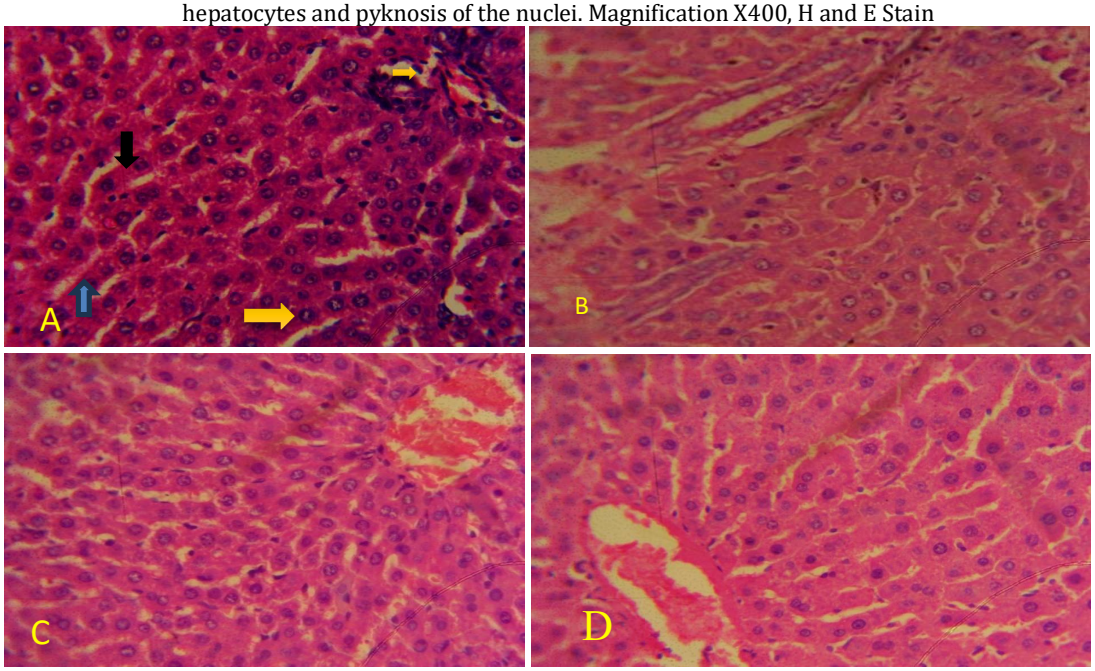


Fig. 6: Histological observations of the liver as shown in plate 2: [Group A Histologic section of liver showing the central vein (blue arrow), linear cord of hepatocytes (green arrow) with vesicular nuclei. While the black arrow and yellow arrow shows presence of kupffer cells lining the sinusoids and bile duct respectively. [Group B Dye 6500mg/kg body weight] Histologic section of Liver shows an architecture that slightly deviate from normal. Sinusoids are more obvious, nuclei slightly reactive (more uptake of haematoxylin). [Group C –Dye 7500mg/kg body weight] Histologic section of Liver showing distortion of the architecture is more prominent, altering the linear arrangement of the hepatocytes and distorting the sinusoids, nuclei are also reactive [Group D –Dye 8500mg/kg body weight] Histologic section of Liver seems to be less affected in general morphology, nuclei of hepatocytes are reactive, sometimes binucleate (yellow arrow)

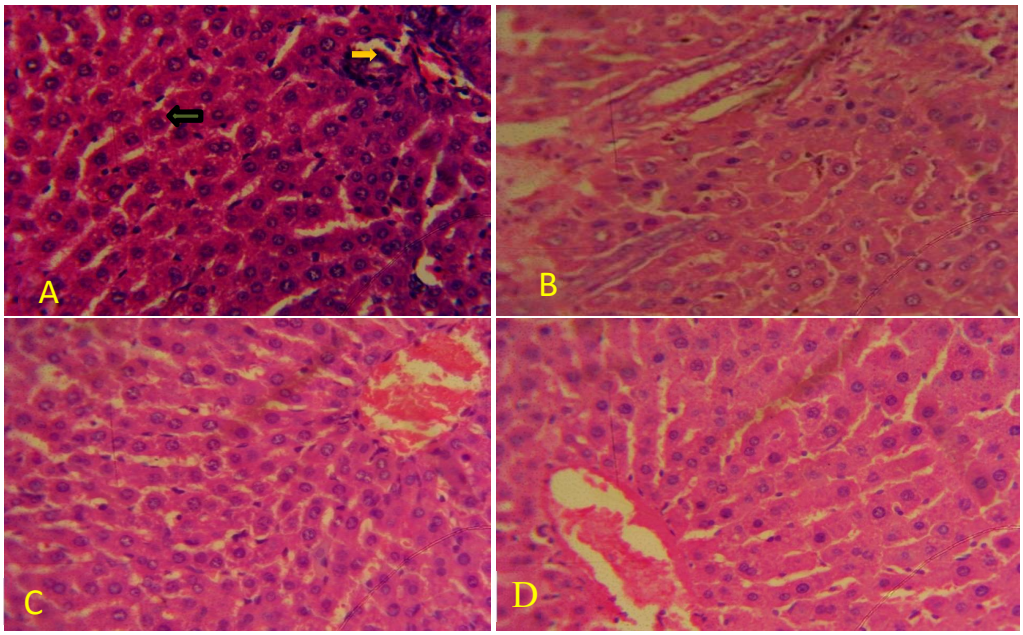


Fig. 7: Histological observations of the liver as shown in plate 3: [Group A- control] Histologic section of liver showing the central vein (blue arrow), linear cord of hepatocytes (green arrow) with vesicular nuclei. While the black arrow and yellow arrow shows presence of kupffer cells lining the sinusoids and bile duct respectively. [Group B *T. Indica* 6500mg/kg body weight] Histologic section of Liver shows an architecture that slightly deviate from normal. Sinusoids are more obvious, nuclei slightly reactive (more uptake of haematoxylin). [Group C –*T. indica* 7500mg/kg body weight] Histologic section of Liver showing distortion of the architecture is more prominent, altering the linear arrangement of the hepatocytes and distorting the sinusoids, nuclei are also reactive [Group D –*T. indica* 8500mg/kg body weight] Histologic section of Liver seems to be less affected in general morphology, nuclei of hepatocytes are reactive. Magnification X400, H and E

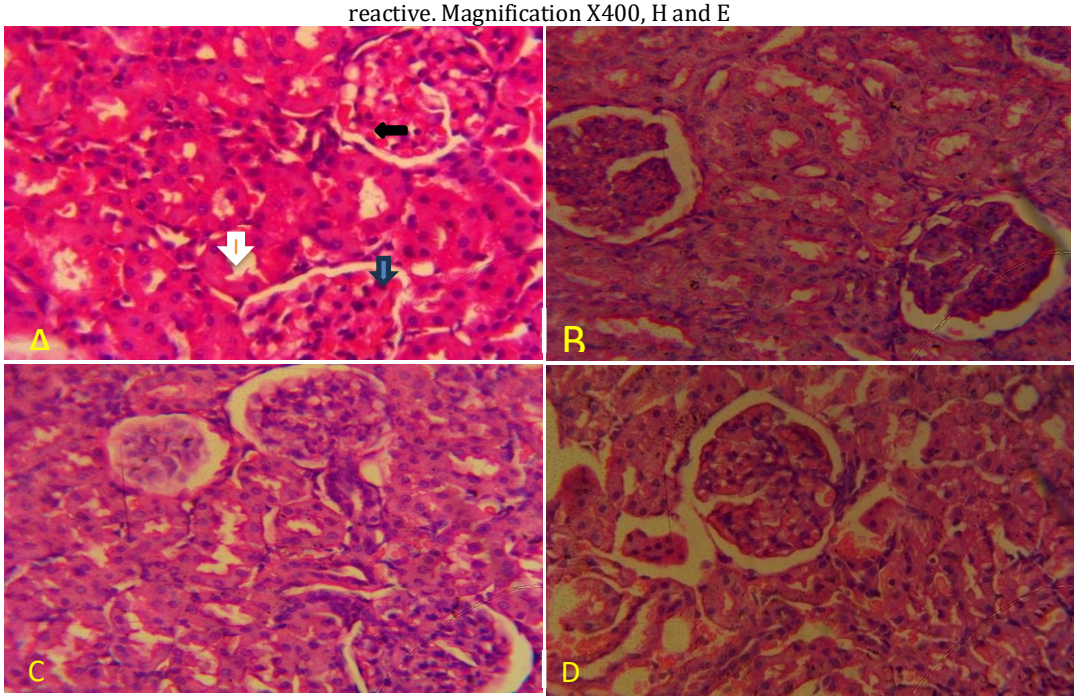


Fig. 8: Histological observations of the liver as shown in plate 4: [Group A- Control] Histologic section of Kidney showing Glomerular tufts (black arrow), encased in Bowmans capsule (yellow arrow), a section of the proximal tubule (blue arrow).[Group B Potash 6500mg/kg body weight] Histologic section of the Kidney showing the glomerular tufts but generally unremarkable [Group C –Potash 7500mg/kg body weight] Histologic section of Kidney showing the glomerular tufts, with the tubules but generally unremarkable. [Group D –Potash 8500mg/kg body weight] Histological section of Kidney showing the glomerular tufts but generally unremarkable. Magnification X400, H and E Stain.

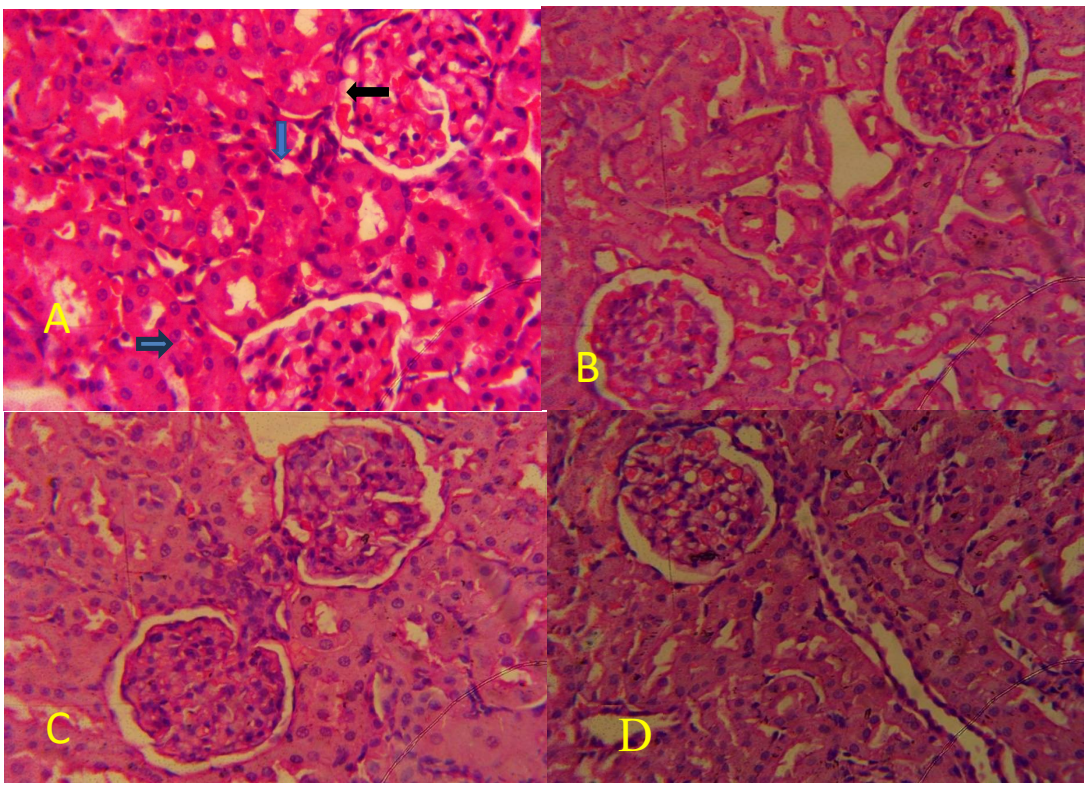


Fig. 9: Histological observations of the Kidney as shown in plate 5: [Group A- Control] Histologic section of Kidney shows glomerular tufts (black arrow), encase in bowmans capsule (yellow arrow), a section of the proximal tubule (blue arrow). [Group B Dye 6500mg/kg body weight] Histologic section of the Kidney showing the glomerular tufts but generally unremarkable [Group C –Dye 7500mg/kg body weight] Histologic section of Kidney showing the glomerular tufts, with the tubules but generally unremarkable. [Group D –Dye 8500mg/kg body weight] Histological section of Kidney showing the glomerular tufts but generally unremarkable. Magnification X400, H and E Stain

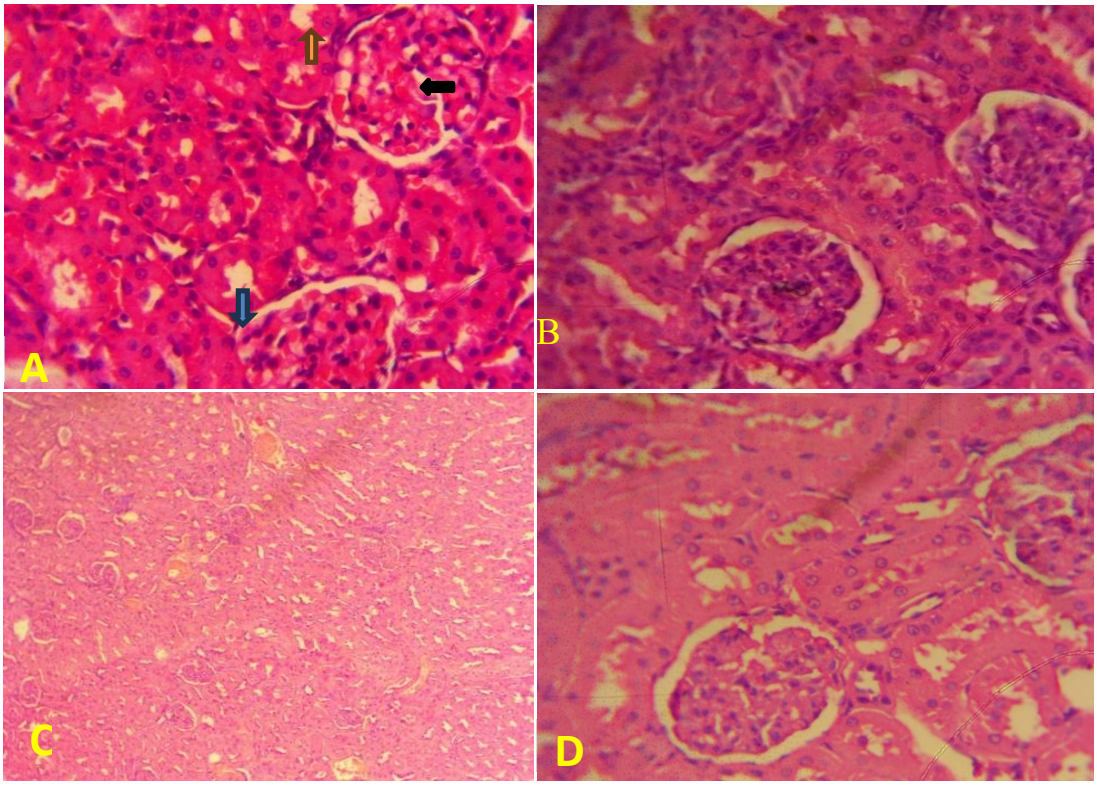


Fig. 10: Histological observations of the Kidney as shown in plate 6: [Group A- Control] Histologic section of Kidney shows glomerular tufts (black arrow), encase in bowmans capsule (blue arrow), a section of the proximal tubule (green arrow). [Group B *T. indica* 6500mg/kg body weight] Histologic

section of the Kidney showing the glomerular tufts but generally unremarkable [Group C –*T. indica* 7500mg/kg body weight] Histologic section of the Kidney showing the glomerular tufts, with the tubules but generally unremarkable. [Group D –*T. indica* 8500mg/kg body weight] Histologic Section of Kidney showing the glomerular tufts but generally unremarkable. Magnification X400, H and E Stain

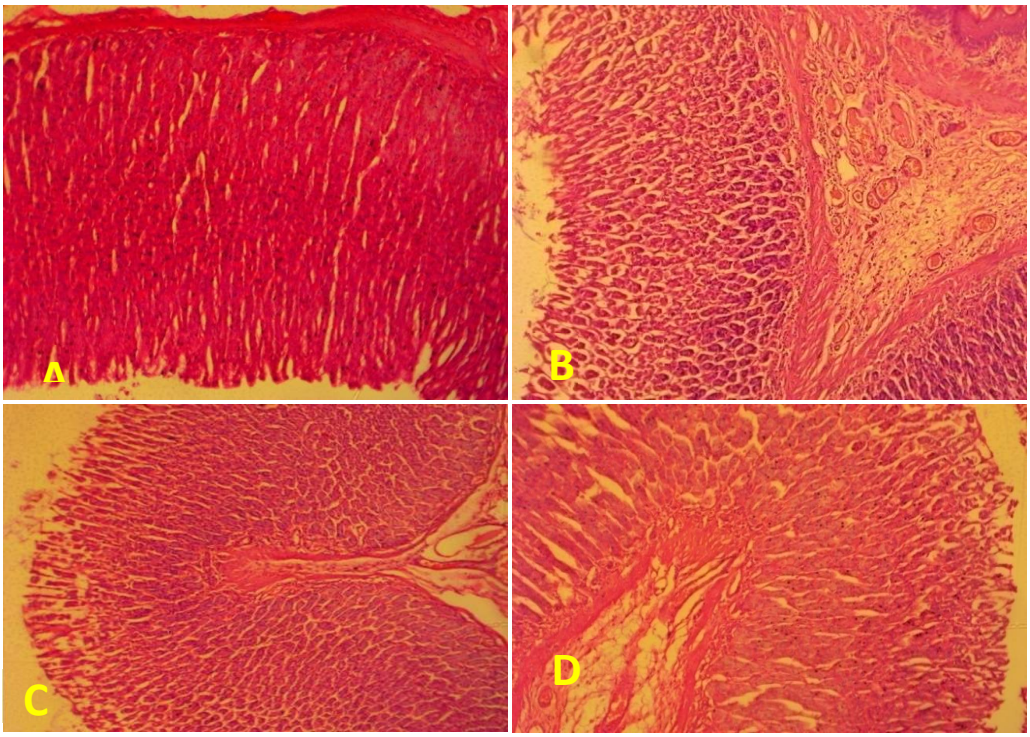
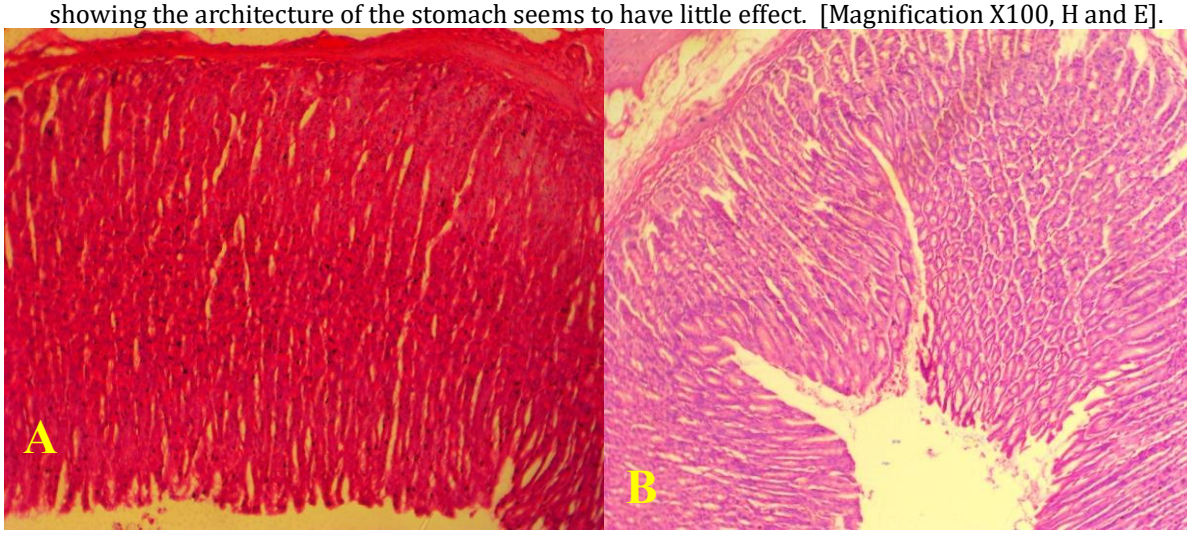


Fig. 11: Histological observations of the GIT as shown in plate 7: [Group A- Control] Histologic section of stomach showing several glandular epithelia, muscular layer [Group B Potash 6500mg/kg body weight, Group C – Potash 7500mg/kg body weight, [Group D –Potash 8500mg/kg body weight] Histologic section showing the architecture of the stomach seems to have little effect. [Magnification X100, H and E].



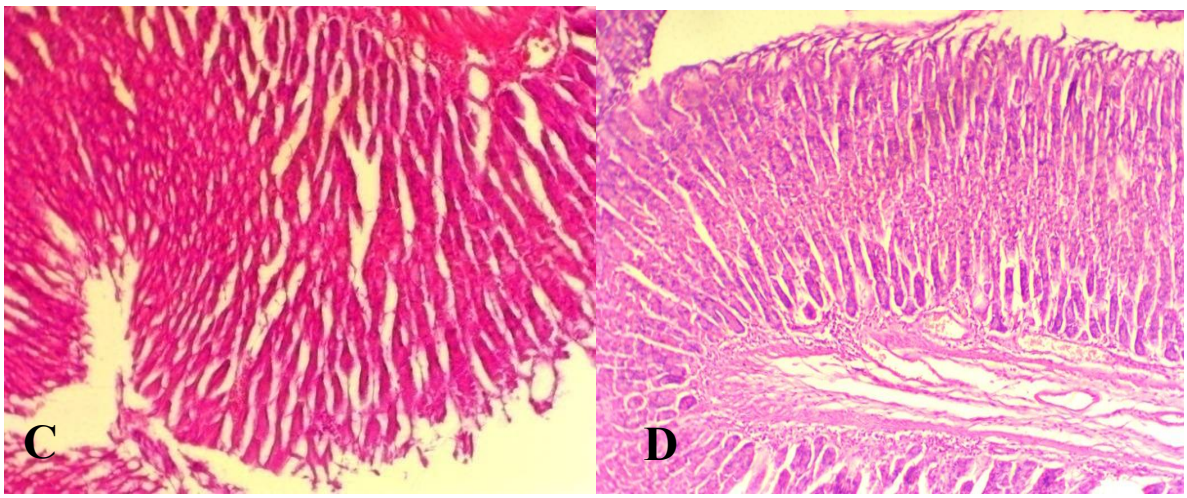


Fig. 12: Histological observations of the GIT as shown in plate 8: [Group A- Control] Histologic section of stomach showing several glandular epithelia, muscular layer [Group B Potash 6500mg/kg body weight, Group C – Potash 7500mg/kg body weight, [Group D –Potash 8500mg/kg body weight] Histologic section showing the architecture of the stomach seems to have little effect. [Magnification X100, H and E].

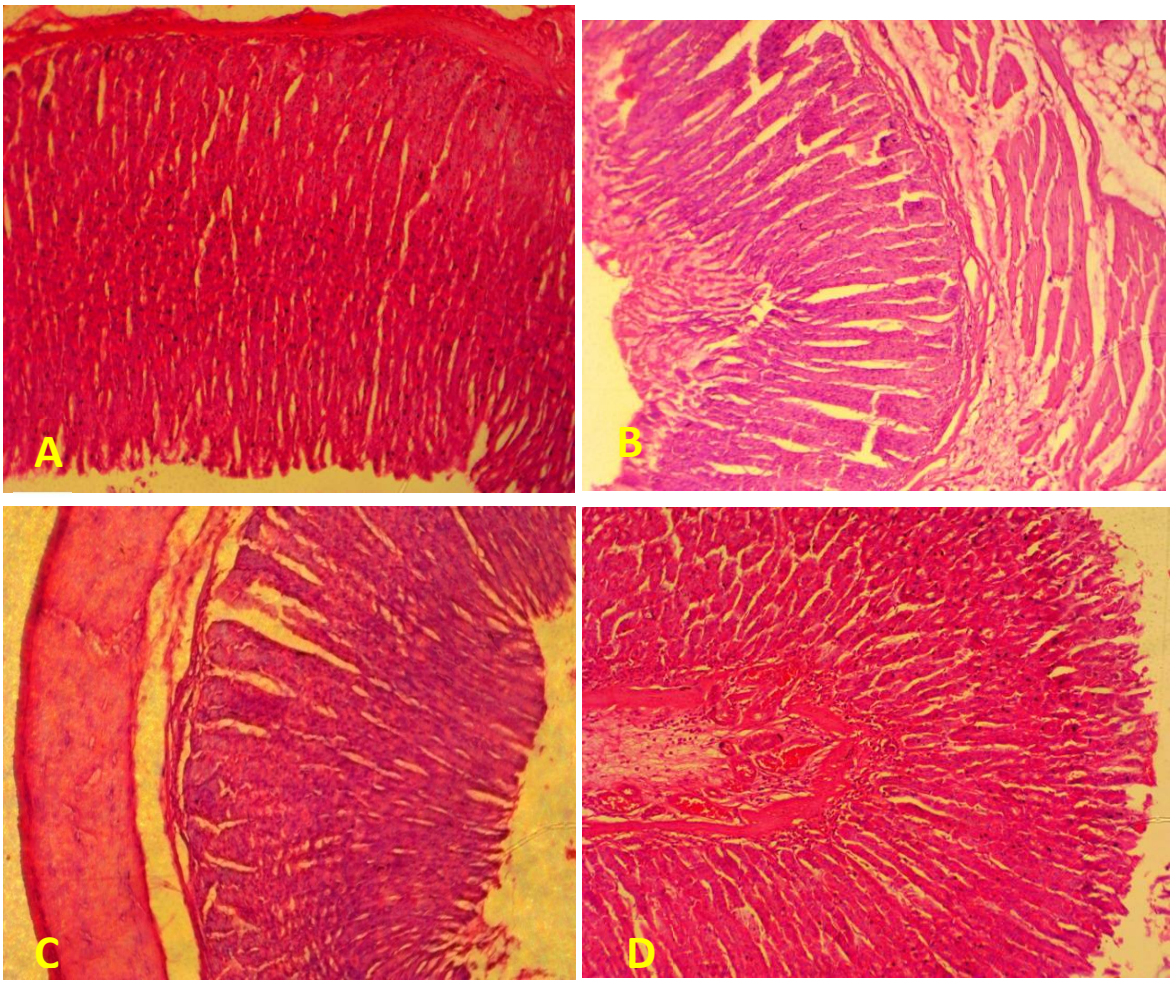


Fig. 13: Histological observations of the GIT as shown in plate 9: [Group A- Control] Histologic section of stomach showing several glandular epithelia, muscular layer. [Group B *T. indica* 6500mg/kg body weight], [Group C –*T. indica* 7500mg/kg body weight], [Group D –*T. indica* 8500mg/kg body weight] Histologic section of stomach shows the architecture of the stomach seems to have little effect. [Magnification X100, H and E].

DISCUSSION

The result of lethal dose 50, the dose that kills 50% of the animals when exposed to *Potash*, dye and *Tamarindus indica* extract showed no mortality. However, this work agreed to the previous report [15] on toxicological and biochemical investigations in rats administered "Kaun" (trona), a natural food additive used in Nigeria. The administration of potash, dye and *T. indica* to albino rats up to 6500 mg/kg resulted in no mortality of the experimental albino rats after 24 hours. Hence the LD 50 of this *Potash*, dye and *Tamarindus indica* extract was estimated to be greater than 6500 mg/kg.

In this study it was observed that the mean weight of all experimental animal groups (Potash, Dye and *T. indica*) and control increased after the administration of the substances. The weight gained in potash is lower than the weight gained in dye. However, the *T. indica* group lost weight. The weight loss could be attributed to the action of extracts. This finding does not support previous reports [16,17] 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. However, this is also not in agreement with the study of Oyewo *et al*., [2] who reported the alterations in body weight of wistar rats fed with potassium bromate. The results obtained in this study shows that there was significant increase ($P<0.05$) in the levels of serum Sodium, Potassium, Chloride, Bicarbonate, Urea and creatinin, and liver function values in the treated animals when compare to the values in the control (Table 2). These findings concurred with [6] who similarly noted noticeably increased plasma urea and creatinine levels in rats given potash orally. However, Khan *et al*., [6] reported that 125mgkg-1 body weight of potassium bromated given intra peritoneally to rats resulted in marked elevation Blood urea nitrogen and creatinine which correlate with the report of this study. Similar findings were reported [18]. However, the results obtained in this study (Table 3) shows statistically significant values ($P>0.05$) only in AST, ALT, ALP (0.000, 0.002, 0.005) compared with the control. While, the results obtained in this study (Table 4) shows statistically significant increase ($P<0.05$) in serum Sodium (0.002), Potassium (0.035), creatinine (0.034), and AST (0.006) values in the treated animals when compare to the values in the control. These statistically significant differences in the mean values of these parameters were dosage dependent.

The histologic findings in the liver showed extract causes a measure of distortion in the architecture of the linear cord of hepatocytes and uptake of haematoxylin and eosin. However, plate 1 seems to be more affected with evidence of pyknosis which is an indication of cell death compared to plate 2 shows some tightness of the sinusoids, ballooning of the cytoplasm of the hepatocytes and pyknosis of the nuclei and plate 3 which also slightly deviate from normal. Histologic findings in the kidney were generally unremarkable. The kidney showed normal histology. This study support the work of Markham et al. [19] who reported that findings in relation to the effect of *Tamarindus Indica* where unremarkable**.** The histologic findings in the gastrointestinal tract. The result in plate 7, 8 and 9 (Group A (control), showed normal gastrointestinal tract histology with the epithelium, mucosa layer. Plate 7, 8 and 9 (B, C and D) seems to have little effect. This is in agreement with the previous study conducted [20], that reported the histologic sections showed no visible lesion and physical examination of the intestines revealed there was no apparent congestion or any sign of haemorrhage.

CONCLUSION

Based on our study, consumption of potash, dye, and *Tamarindus indica* extract for a long period of time could be harmful to the liver, kidney and stomach. Hence, as an abortive substance are discouraged.

Ethics approval: Approved by the Institution Ethic Committee for the use of laboratory animals

Consent for publication: Not applicable

Availability of data and material: Data are available from the corresponding author upon request

Competing interests: The authors declare no conflict of interest

Funding: The study did not receive any additional funding.

Author's contributions: GJT, APU and JOA conceptualized, performed, and write the original draft, edited the manuscript as well as performed the critical literature search and laboratory analysis with SMG. All authors read and approved the final manuscript.

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