Original Research Article

Comparative study of Fe, Mn and Ni contamination potential of Disc and Hammer Milling Equipment and their toxic effect on rat kidney.

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ABSTRACT

Aim: To investigate the effect of hammer and disc milling equipment on the levels of Fe, Mn and Ni contents in maize (Zea mays) flour and the consequent impact on the kidney of albino rats.

Study design: The rats were randomly divided into groups of five rats per group. Six of the groups were fed with the maize flour milled with hammer mill and the other six groups were fed with the maize flour milled with disc mill, while the thirteenth group, which served as the control, was fed with the crushed flour in mortar and pestle. After a period of 14^{th} and 28^{th} days, the rats were sacrificed by decapitation under anesthesia.

Place and Duration of Study: Departments of Biochemistry and Histology laboratories of the University of Jos, Nigeria, between January to June, 2021.

Methodology: A portion of the maize grains was milled with a hammer mill, another portion with a disc mill both for six consecutive days while a third portion was crushed into flour with a wooden mortar. The levels of Fe, Mn and Ni in the maize flour were determined using the Inductive Coupled Plasma mass spectrophotometric method. Blood samples were collected carefully by allowing the blood to flow on the wall of the test tube to the bottom to avoid lysing of the blood. The blood was allowed to clot under room temperature, centrifuged, serum collected and stored frozen until needed for biochemical analysis. The rats' kidneys were excised, washed and fixed in chloroform until needed for histopathological studies.

Results: The results show that both plate and hammer milling machines contained a very high level of Fe (325.16 ± 30.00 mg/kg and 205.05 ±30.20 mg/kg) and Ni (20.92 ± 5.92 mg/kg and 18.00 ± 2.70 mg/kg) respectively. The levels of Fe and Mn contained in the maize flour milled by disc mill was higher than those in the one obtained from the hammer milling machine. The values of serum urea, creatinine, K^+, Na^+ and Cl^- of rats in all the groups were significantly higher than the control. The histopathological analysis suggests that the kidney tissues of all the rats in both the control group and the groups fed with the hammer-milled flour were normal except group 1 of disc mill which showed mild damage.

Conclusion: The kidney tissue of the Day 1 group fed with disc-milled flour showed a dense cast, atrophy and loss of nuclei. The results of the study show that the hammer milled maize flour is less harmful than the disc milled maize flour as shown by the histopathology of the kidney.

Keywords: Comparative, Iron, Manganese, Nickel, Contamination, Maize flour, Grinding plates, Histopathology.

1. INTRODUCTION

Food processing methods involve several operations, which include size reduction of the food stuff by milling (grinding) into coarse or fine particles. Grinding of foodstuff like dry grains in the past was done by using traditional methods, which include stones, bricks, wooden mortars and pestle. These methods were effective but rather slow, energy sapping, time consuming and unhygienic. As the need of the people for food increased, new technologies were developed and modern methods of grinding foods were invented such as blenders, mills and crushers. (Oniya *et al.*, 2018).

Commonly used milling machines in Jos include Hammer and Disc (plate) Mills. Hammer mills consist of a series of hammers (usually four or more) hinged on a central shaft and enclosed within a rigid metal case. It produces size reduction by impact. The materials to be milled are struck by these rectangular pieces of hardened steel (ganged hammer) which rotates at high speed inside the chamber (Pharmapproch, 2019). A disc mill is a type of crusher that can be used to grind, cut, shear, shred, fiberize, pulverize, granulate, crack, rub, curl, fluff, twist, hull, blend, or refine. It works in a similar manner to the ancient Burrstone mill in that the feedstock is fed between opposing discs or plates (Wikipedia,2018).

Maize is an important staple food grown in many parts of the tropics which serves as food for many people and animals. In Nigeria, it is one of the most popular of all grain crops and it is grown all over the country (Adebayo *et al*., 2010). Worldwide production of maize is 785 million tones with the Africa producing 6.5% and the largest African producer is Nigeria with nearly 8 million tones (IITA, 2011). Most maize production in Nigeria is rain fed and 95% of total maize production is consumed locally compared to the World regions that use most of its maize as animal feed. This is evident in Nigerian meal as consumption of maize cut across all economy class especially at its seasons as either boiled or roasted. Nutritionally, it has high content of carbohydrates, fats, proteins, and some of the important vitamins and minerals, the product has acquired a well-deserved reputation as a poor man's nutria-cereal (Punita, 2006). Maize is processed into corn flour which is used in the preparation of many local foods such as pate, *waina, kunu, akamu (pap), kenkey, donkunu, tuwo, zaafi, eko* and lots more (Lokko *et al*., 2004).

When these machines are in operation, the plates revolve and rub against each other as the food stuff is being crushed into powder or paste. The sliding process of the plates generates friction which leads to wear and tear thereby introducing contaminants into the milled foodstuffs (Oniya *et al*, 2018). These contaminants include heavy metals and other potentially toxic substances like grease and paintings used to cover the outer surface of the metals. Heavy metals can be defined as a group of metals and metalloids having atomic density greater than 4g/cm³, some of which at very low levels, can be useful in metabolic activities in the body but as their concentrations exceed permissible levels, they constitute varying degrees of health hazards to man (Ogunlalu, *et al.,* 2017). Examples are iron, manganese, nickel, zinc, lead, copper, cadmium, and chromium. Ingestion of heavy metals through food has been shown to have serious consequences on health and thereby economic development, associated with a decline on labour productivity as well as increased direct costs of treating illnesses such as kidney diseases, damage to the nervous system, diminished intellectual capacity, heart disease, gastrointestinal diseases, bone fracture, cancer and death (Yashim and Suleiman, 2016). Generally, heavy metals disrupt basic metabolic functions in human body in two ways: firstly, they disrupt the functioning of vital organs and glands such as the brain, kidney, or liver; they prevent uptake of nutrients that are essential for biological functions (Odusote, *et al.,* 2017).

However, with the current emphasis on eating more healthy diets which should not contain toxic metals, it is very essential to assess the chemical composition and heavy metal concentration in maize flour that is popularly consumed by Nigerians. This study is aimed at investigating the effect of hammer and disc milling equipment on the levels of some heavy metals contents in *Zea mays* flour and the consequent impact on the kidney of albino rat.

2.0. MATERIAL AND METHODS

2.1. Study area

Maize cobs, which have been allowed to dry out while still in their stalks, were acquired in sufficient quantity from farmlands around Mandung, Maikatako community of Bokkos Local Government Area of Plateau State. The cobs were de-husked, shelled and the seeds further sundried after which they were stored in plastic containers for further analysis.

2.1.2. Milling machines.

Both hammer and disc milling machines privately owned by individuals from Dadin-kowa first gate Jos-south, Plateau state were used, particularly when the plates have been recently sharpened up to the time the plates were needed to be changed

2.1.3. Mortar and pestle

Wooden mortar and pestle were used to crush some of the maize was used as control for the experiment.

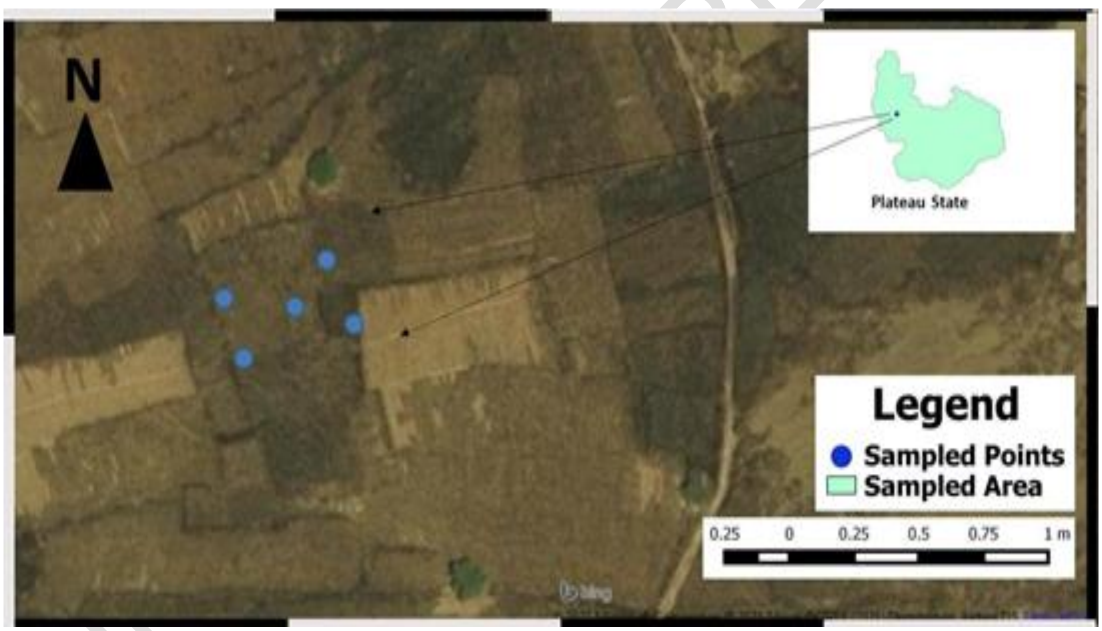


Fig. 1. Satellite image showing maize sample location within the study area.

2.1.4. Grinding plates.

The Used grinding plates from hammer and plate mill was obtained and thoroughly washed with de-ionized water after which 1.0g was cut out for elemental analysis of iron, manganese and nickel using Atomic Absorption Spectrometry Techniques

2.1.5. Experimental Animals.

Sixty-Five (65) male albino rats weighing between an average of 262.5g were obtained from the Animal House unit, University of Jos. The rats were housed in well-ventilated plastic rat cages and fed with standard rat pellet and tap water *ad libitum* throughout the study.

2.2 Methods Applied

2.2.1. Processing of maize grains into flour.

I. Preparation: The interior of the milling machines was thoroughly washed with distilled water in order to ensure that they are free from previously ground material. The mortar and pestle were equally washed with distilled water for the same purpose. The machines and mortars were air dried before the milling processes began.

II. Crushing: 1 kg of the maize was crushed using dry mortar and pestle until it was powdered for a period of three days at an interval of five (5) hours daily.

III. Milling: 3kg of the maize seeds were grind in both the hammer milling machine and the disc-milling machine until the maize flours are obtained. For both hammer and disc milling machines, samples were collected each day for 6 days.

IV. Sieving: The maize flour samples obtained after the crushing and milling, were sieved for uniform particle size and stored in well-labelled plastic containers as described by (Ebenezer, *et al.*, 2018).

2.2.2. Analysis of maize flour and Soil Samples.

2.2.2.1. Digestion of the Maize Flour and Soil Samples added to each sample to leach in a heating block of hot water bath Samples 0.5g of the maize flour samples were digested according to the method and protocol as carried out in Acme laboratory, Vancouver, Canada. All the milled maize samples were leached in cold nitric acid and then digested in a hot water bath. After cooling, a modified Aqua Regia solution of equal volume parts of concentrated HCl, HNO_3 and DI H_2O were made to to mark with dilute HCl and then filtered with Whatman filter paper No.42.

A B C

Figure 2: Grinding methods: (A) mortar and pestle; (B) Hammer mill and (C) Disc Mill.

A B

Figure 3: Grinding Machine Discs: (A) Hammer plates; (B) Disc plates

2.2.2.2. The Determination of Metal Contaminant.

The Nickel, Iron and Manganese metals in the digested samples were determined according to the manual of each instrument carried out in Acme laboratory, Vancouver, Canada. The concentrations of Nickel, Iron and Manganese metals in the digested maize flour were determined using NexION 300 Inductively Coupled Plasma Mass Spectrometry (ICP- MS).

2.2.3. Digestion protocol adopted for the grinding Plates

The 1.0 g cut out of each plate sample were put into 37% HCl for 30 minute and then washed with de-ionized water. This is done to avoid any external contamination due to handling and exposure to dust. The plate sample was left in 25ml (95% HCL) for 3 days to dissolve. It was digested with aqua-regia (3 ml 65% HNO3: 1ml 37% HCl) in high borosilicate glass vessel on a hot plate at 95°C for 3 hours. The digested samples were allowed to cool at room temperature. The sides of the beakers were washed with de-ionised water and made to a final volume of 30 ml. The

digested solutions were filtered with Whatman Paper No.42 into test tubes and kept for elemental analysis (VARIAN. Publication No 85- 100009-00 Revised March 1989).

2.2.4. Experimental design

A total of 65 albino rats were used for this experiment. The rats were randomly divided into thirteen groups of five rats per group. Six of the groups were fed with 2g each of the maize flour milled with hammer mill and the other six groups were fed with 2g each of the maize flour milled with disc mill while the thirteenth group, which served as the control, was fed with the 2g of the crushed maize flour in mortar and pestle daily. All the animals were given water and feed *ad libitum* for a period of 28 days.

2.2.5. Determination of urea, creatinine, Na+, K+, Cl- and histopathological analysis of the kidney

Serum urea was determined by the method of Fawcett and Scott (1960), using Randox commercial kit, while Serum creatinine was determined by the method described by Tietz (1995), using Randox commercial kit. Serum Sodium ion concentration was determined by the method of Trinder (1951), while Serum Potassium were determined using the method described by Egbung *et al*. (2019). Serum Chloride and Bicarbonate were determined by Mercuric Nitrate titrimetric method of Skeegs and Hochestrasser (1964).

The rats were sacrificed by decapitation after anaesthesia. Kidney excised, washed with ice cold saline to remove blood and stored in saline until required for histopathological study.

2.3. STATISTICAL ANALYSIS

The data were expressed as mean (of 3 replicates) ± SEM. It was then subjected to statistical analysis using the Graphpad prism version 9.0. All significant differences were determined by one-way Analysis of Variance (ANOVA) and Post Hoc. Multiple comparisons were done using Tukey -Kramer multiple comparison test. The significance level was set at *p*<0.05, p<0.0001 and p<0.01 respectively.

3.0 RESULTS

3.1. Concentration of heavy metals obtained from the experimental disc and hammer milling machine plates used for the experiment.

The elemental concentrations of the grinding plates of both machines were presented below in figure 4. The result showed that Iron had the highest concentrations of (325.16 ±1.80mg/kg and 205.05 ±1.83mg/kg) in the plate's analysis for both disc and hammer mill while manganese had the lowest concentration of (6.30±0.31 mg/kg and 5.51±0.41 mg/kg) respectively for both milling plates. Nickel had the second highest concentration in both plates of (20.92±1.40mg/kg and 15.3 ±1.30mg/kg).

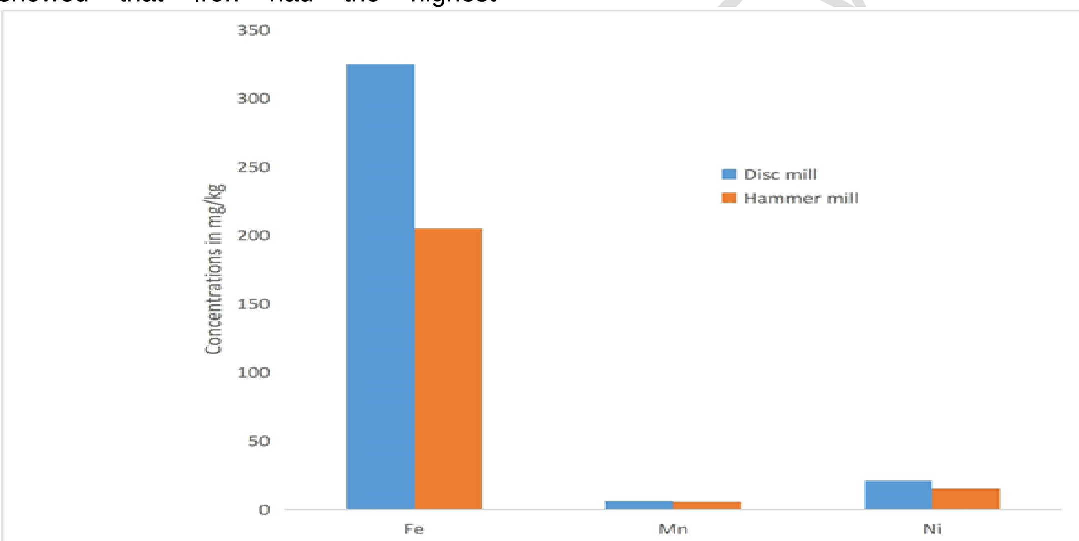


Figure.4. Mean concentration of Fe,Mn and Ni metals present in Disc and Hammer Milling plates.

3.2. The mean concentration of Fe in the maize flour samples obtained from hammer and disc milling machine for a period of 6days.

The concentration of iron in mg/kg in the maize flour samples obtained from hammer and disc milling machine for a period of six days were presented below on table Figure 5. The concentrations of Fe in the maize flour obtained from hammer mill on days 2, 3 and 4 were significantly (*p*< 0.05), (*p*< 0.01) and (*p*< 0.0001) respectively higher than that of the control. The results of the Fe concentration in maize flour milled with disc mill shows that day 1 was (*p*<0.001) while the values from days 2-6 were significantly (*p*< 0.0001) higher than that of the control.

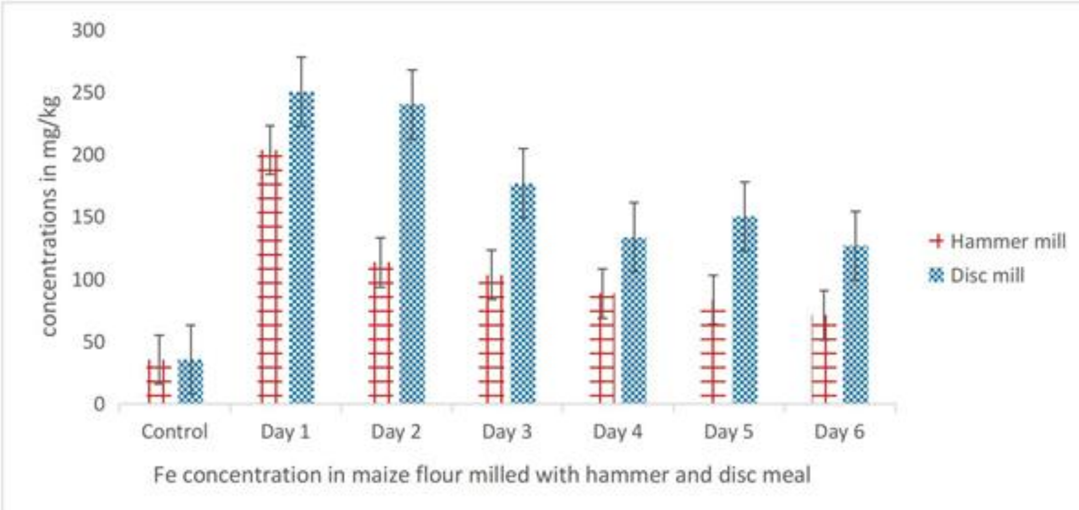


Figure 5. Fe concentration in maize flour milled with hammer mill and disc mill

3.3. The mean concentration of Mn in the maize flour samples obtained from hammer and disc milling machine for a period of 6 days.

The concentration of manganese in the maize flour samples obtained from disc milling machine over a period of six days are presented in figure 6 below. The concentrations of Mn in all the flour mills obtained from both milling machines were not significantly different from control.

Figure 6. Mn concentration in maize flour milled with hammer mill and disc mill

3.4. The mean concentration of Ni the maize flour samples obtained from hammer and disc milling machine for a period of 6 days.

The concentration of Nickel in the control maize flour and the maize flour samples obtained from disc and hammer milling plates over a period of six days is shown on fig.7. The concentration of Nickel increased on day 1 and day 2, which was significantly different ($p<0.005$) from control for both the disc and hammer milling plates. However, there was no significant difference ($p>0.005$) between day 3, 4, 5 and 6 with the control for both disc and hammer milling plates.

Figure 7. Ni concentration in maize flour milled with hammer mill and disc mill

3.5. Serum urea concentration in rats fed with the processed flour milled with Disc milling plates for 14 and 28 days.

Fig. 8 shows the result of urea concentration in rats fed control maize flour and the groups fed with the maize flour from disc plates of day 1 to 6 for 14 and 28 days. Result show that there was significant difference ($p<0.005$) between control group and the group fed with day 1 flour for 14 and 28 days, but there was no significant difference ($p>0.005$) between the urea concentration of day 14 and day28, although there was a decrease on day 28. A similar trend was observed for the groups treated with the four of day 2, 3, 4, 5 and 6 of the disc milling plates. The result show that the increase in number of feeding days beyond 14 to 28 days did not increase nephrotoxicity.

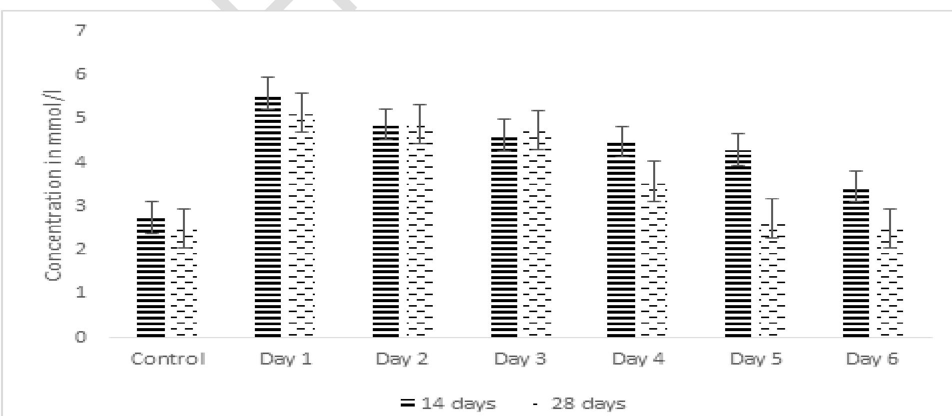


Figure 8. Urea concentration in rats fed with control maize flour and maize flour milled with disc milling plates for 14 and 28 days

3.6. Serum urea concentration in rats fed with the processed flour milled with hammer milling plates for 14 and 28 days.

Fig. 9 shows the result of urea concentration in rats fed control maize flour and the groups fed with the maize flour from hammer milling plates of day 1 to 6 for 14 and 28 days. Result show that there was significant difference ($p<0.005$) between control group and the group fed with day 1 and 2 flour for 14 and 28 days, but there was significant difference ($p>0.005$) between the urea concentration of day 14 and day 28. There were no significant difference between the control group and the groups treated with the four of day 3, 4, 5 and 6 of the hammer milling plates. This result show that the hammer milling plate is less nephrotoxic as only the groups treated with day 1 and 2 flour showed some level of damage but groups 3 to 6 were not significantly different ($p>0.005$) from control.

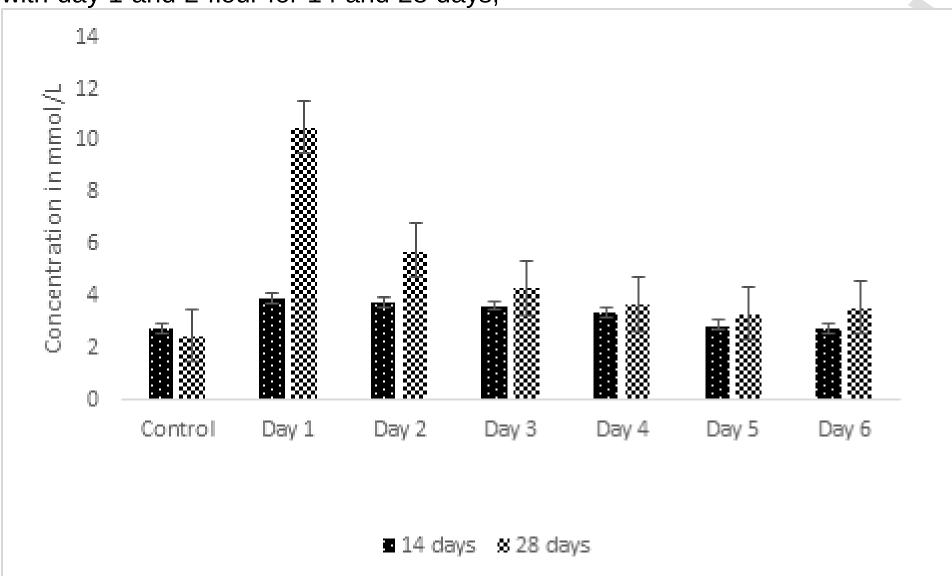


Figure 9: Urea concentration in rats fed with control maize flour and maize flour milled with hammer milling plates for 14 and 28 days

3.7. Serum K^+ concentration in the rats fed with the processed flour milled with disc milling machine.

Fig. 10 shows the result of $\mathbf{K^+}$ concentration in rats fed control maize flour and the groups fed with the maize flour from disc plates of day 1 to 6 for 14 and 28 days. Result show that there was significant difference ($p<0.005$) between control group and the group fed with day 1 flour for 14 and 28 days, but there was no significant difference ($p>0.005$) between the $\mathbf{K^+}$ concentration of day 14 and day 28, although there was an increase on day 28. A similar trend was observed for the groups treated with the flour of day 2, 3, 4, 5 and 6 of the disc milling plate. The result show that the increase in number of feeding days beyond 14 to 28 days did increase nephrotoxicity.

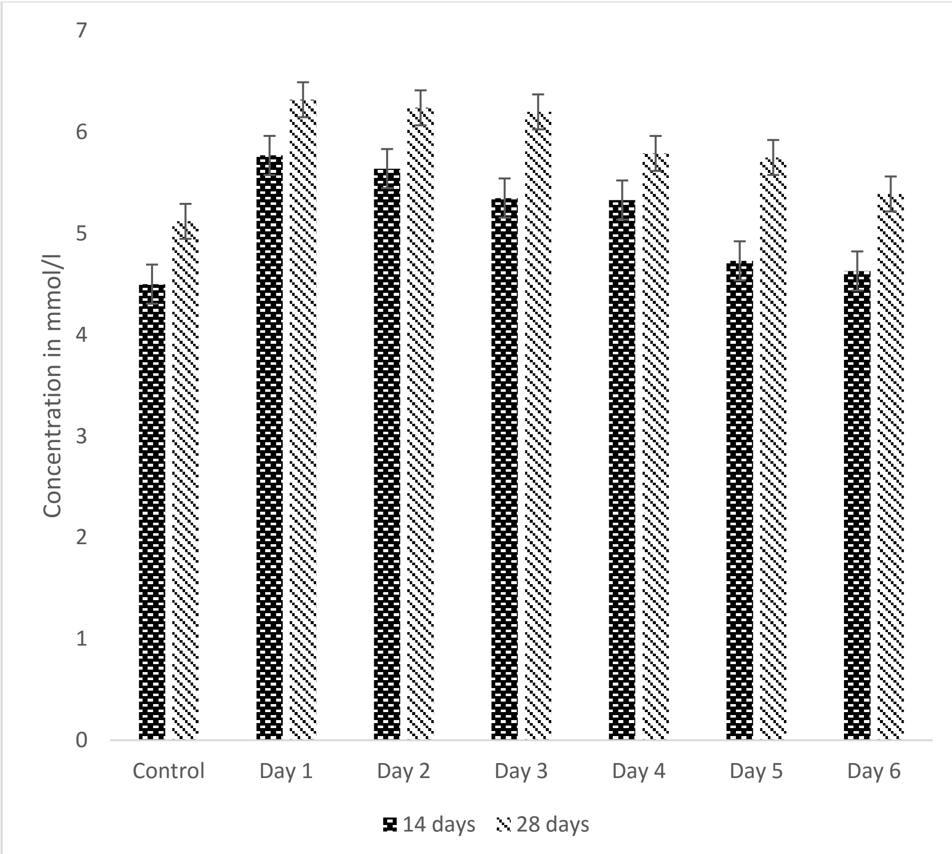


Figure 10: K^+ concentration in rats fed with control maize flour and maize flour milled with disc milling plates for 14 and 28 days

3.8. Serum K^+ concentration in rats fed with control maize flour and maize flour milled with hammer milling plates for 14 and 28 days.

Fig. 11 shows the result of K^+ concentration in rats fed control maize flour and the groups fed with the maize flour milled with hammer milling plates of day 1 to 6 for 14 and 28 days. Result show that there was significant difference ($p<0.005$) between control group and the group fed with day 1 and 2 flour for 14 and 28 days, but there was significant difference ($p>0.005$) between the K^+ concentration of day 14 and day 28. There was no significant difference between the control group and the groups treated with the four of day 3, 4, 5 and 6 of the hammer milling plates. This result show that the hammer milling plate is less nephrotoxic as only the groups treated with day 1 and 2 flour showed some level of damage but groups 3 to 6 were not significantly different ($p>0.005$) from control.

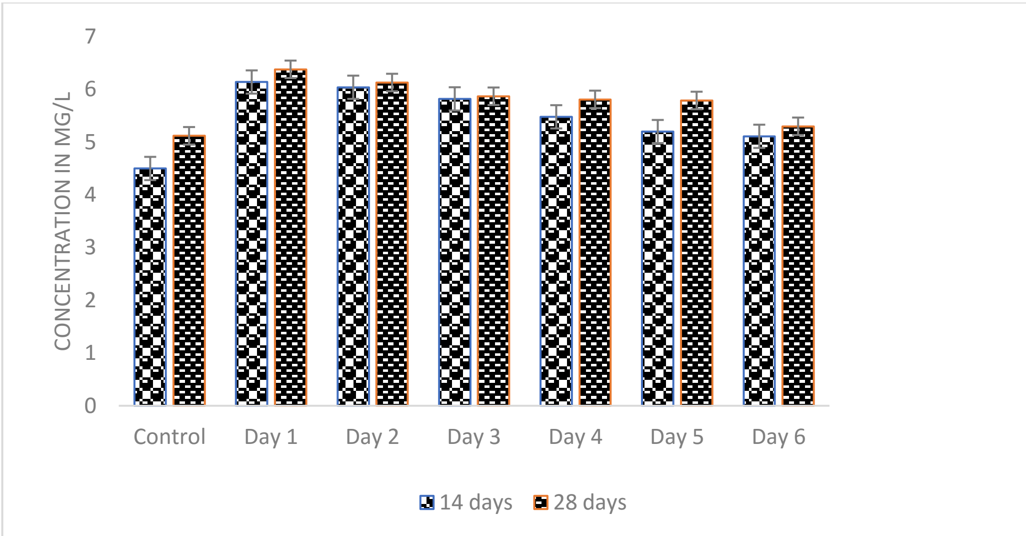


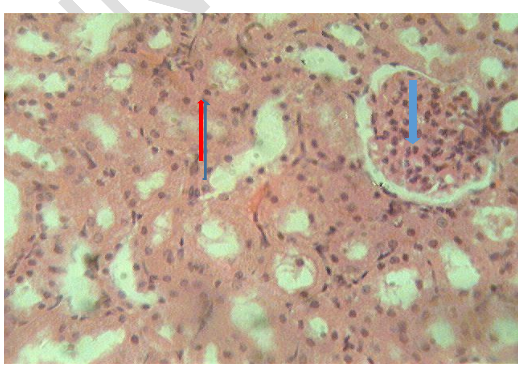
Figure 11: K^+ concentration in rats fed with control maize flour and maize flour milled with disc milling plates for 14 and 28 days

3.9. Serum Creatinine, Na^+ and Cl^- concentrations in rats fed with the processed flour milled with hammer and disc milling machine.

The results show that there was no significant difference between any of the groups with the control both after 14 days and between 28 days of feeding for both milling machines.

3.10. Histological analysis of the kidney of rats

Plate 1 shows the kidney section of the rats fed with maize flour crushed with mortar and pestle having normal glomerulus and normal convoluted tubule with normal nuclei arrangement (up-pointing arrow). Plate 2 is the Kidney section of the rats fed with maize flour from hammer milling plate showing relatively normal glomerulus and normal convoluted tubule with normal nuclei arrangement (up-pointing arrow). Plate 3 is the Kidney section of the rat fed with the flour from disc milling plates showing loss of nuclei in the convoluted tubule (up-pointing arrow) and acinic differentiation in the glomerulus (down-pointing arrow).



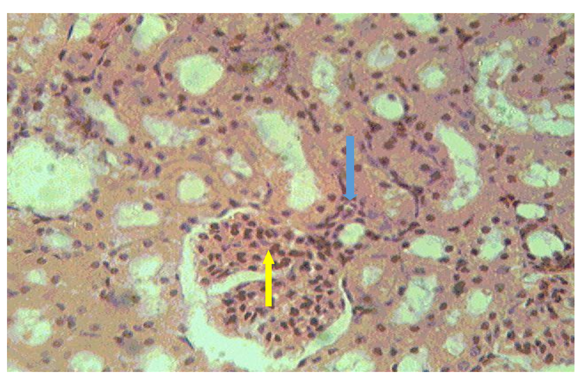


Plate I: Photomicrograph of the kidney tissue of the control group of rats fed with maize flour crushed with mortar and pestle (x40). Arrows up and down shows normal nuclei and glomeruli

Plate 2: Photomicrograph of the kidney tissue of rats fed with maize flour milled with hammer milling machine (x40). Arrows up and down shows normal nuclei and glomeruli.

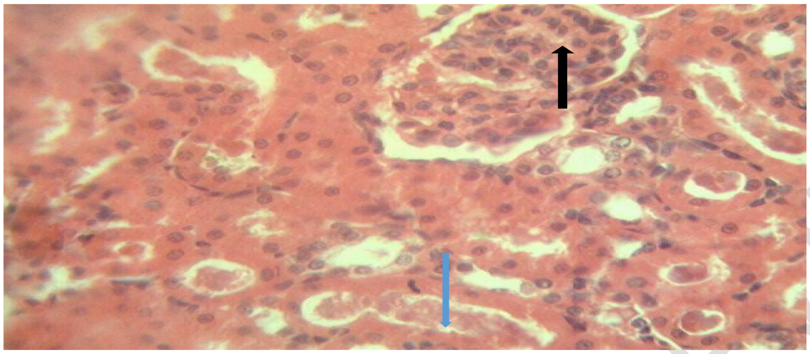


Plate 3: Photomicrograph of the kidney tissue of rats fed with maize flour milled with disc milling machine (x40). Arrows up and down shows loss of nuclei and dense glomeruli.

4.0. Discussion

Iron had the highest concentration in the disc and hammer milling plates. This means that if the tensile strength of the plates are low, there is the risk of iron contamination in food that is prepared by the use of these grinding machines. The Fe concentration recorded in all the milled maize flours were much higher than that obtained in the control maize flour. This shows that the milling process introduced some amount of Fe into the maize flour. The high difference between Fe content in the control of maize flour and that of the maize flour from the grinding plates can be because Fe forms the highest percentage concentration of the alloy used for the grinding plates, during grinding of the flour, the shear force applied by the machine caused the grinding disc to rub against each other, and possibly caused wear and tear on the grinding disc. This wear and tear of the grinding disc could be responsible for the high iron concentration in maize flour. Iron is an essential element in humans as it helps in oxygen transport and regulates cell growth and differentiation (Andrews, 1999). Deficiency of iron will therefore limits oxygen delivery to cells resulting in fatigue, poor work performance, and decrease immunity (Bhaskaram, 2001; Haas and Brownnlie, 2001) Nevertheless, excess iron intake can result in iron overload and toxicity, arrhythmia, heart failure, increased atherosclerosis risk, and increases the risk of liver, breast, gastrointestinal, and hematologic cancers (Araujo *et al*., 1995; Nelson *et al*., 1995; Sahinbegovic *et al*., 2010; Ellervik *et al*., 2012; Kallianpur *et al*., 2004; Dongiovanni *et al*., 2011; Kremastinos *et al*., 2011). Excess Fe is toxic and has been reported to inhibit the absorption of Zn (Solomons and Ruz, 1997) and ingestion of Fe could lead to increase adverse effect and death due to microbial infection such as malaria and other communicable diseases (Najat, 2008).

Manganese

The Mn concentration recorded in all the milled maize flours were higher than that obtained in the control of maize flour but with no significant difference. Similarly, the highest level of 6.33 mg/kg was recorded in the maize flour processed from disc mill and hammer mill machine on day 3. This shows that the milling process introduced some amount of the Mn metal into the maize flour. Generally, the concentration of Mn in the maize flour processed from Hammer mill were higher. Manganese is the least toxic of the essential metals and it is toxic to varying degrees, depending on the type of ion and its oxidation state. Growth retardation, nonspecific anemia, metal fume fever and psychic and neurological disorders are some of the symptoms of manganese intoxication. Chronic Mn toxicity in humans follows chronic exposure through inhalation, ingestion or parental administration to a high concentration of 75 mg/dl (Dorman *et al*., 2001) results in "Manganism", a disease of the central nervous system involving psychic and neurological disorder

Nickel

Ni in the grinding plates was 20.92 mg /kg and 15.3mg/kg. This shows that the milling process could introduced some amount of Ni into the maize flour. The Ni mean concentration ranges between 0.17-0.23 mg/kg respectively for both milling machines. In 2011, Abrefah *et al*., 2011, reported the concentration of 26.18 mg/kg in corn flour in Ghana. Higher concentration of nickel could cause serious harmful health effects, such as chronic bronchitis, reduced lung function, and cancer of the lung and nasal sinus (USEPA, 2009). There was no significant difference (p>0.005) between the two milling machine in mean manganese concentration and that of control. The manganese concentration in the maize flour was below the maximum permissible limit and hence, it is considered safe from the hazardous effect of nickel. Nickel has been considered an essential trace element for human and animal health. Plants also absorb Ni rapidly (Hjortenkrans, 2003).

Biochemical Indices

Among the waste products of metabolism excreted by the kidney are urea and creatinine, while electrolytes are reabsorbed to maintain body's homeostasis. Creatinine and urea are non-protein nitrogenous metabolites that are cleared by the kidney via glomerular filtration. The assessment of serum urea, creatinine and electrolytes (Na+, K+, Cl-, HCO_3^-) are vital and sensitive biochemical markers which are usually employed in the diagnosis of renal damage and failure (Agbasi *et al*.,2010).

Serum Urea

Urea is the major nitrogen-containing metabolic product of protein catabolism. Urea level in blood rises when there is kidney impairment, which prevents the kidneys from filtering urea out of the blood. The results implies that the rate of filtration and subsequent excretion of urea in the blood may have been affected by the high concentration of urea in the serum of the test groups as compared to the control group. Urea is a molecule produced in the liver as a bye product of protein metabolism, so blood levels of urea will rise when there is impairment of the kidney (Chatterjea and Shinde, 2012).

Serum Creatinine

Creatinine is produced endogenously in the muscle by a non-enzymic action on creatine phosphate. Creatinine clearance in the glomerulus of the kidney is a useful tool to assessing the functionality of the kidney (Delvin, 2011). More precise measure of the kidney function can be estimated by calculating how much creatinine is cleared from the body by the kidneys. This is referred to as creatinine clearance and it estimates the rate of filtration by the kidneys (glomerular filtration rate, GFR) (Charles *et al*.,2016). The results of creatinine obtained for both milling machines showed no significant difference between any of the groups with the control both on the 14th day and the 28th day of feeding. This indicates that there was no significant impairment of the kidney which could have been as a result of the copping strategy of the cell (Chatterjea and Shinde, 2012).

Serum Potassium (K^+)

Potassium ions play an important role in the way in which nerve impulses are propagated along the nerve cells and transmitted to receptor cells. In this study the significant ($p<0.0001$) decrease in serum potassium content may be attributed to an abnormal increase in blood pH and/or decreased reabsorption of potassium ions at the renal tubules. Since potassium ion is a major electrolyte in intracellular fluids, a consistent decrease in serum potassium levels may lead to hypokalemia, a condition implicated in some cardiac diseases as well. In humans, the reference range for serum potassium is 3.6 - 5.0 mmol/L. Potassium levels below 3.0 mmol/L are associated with arrhythmia (irregular heartbeat), tachycardia (rapid heartbeat) and cardiac arrest (Palmer, 2015).

Serum Na^+ and serum Cl^-

The result of Na^+ and Cl^- obtained in this study for both milling machines showed no significant difference between any of the test groups with the control group on the 14th day and the 28th day of feeding. This indicates that there was no significant injury done on the kidney. Elevated serum chloride may be observed in dehydration, hyperventilation, congestive heart valve, prostatic and urinary obstruction (Anderson and Scotti, 1980). Increased level of chloride indicates dehydration but can occur with other problems causing high blood sodium or hyperventilated (Anderson and Scotti, 1980).

Decreased levels of serum chloride also occur with disorders that result in low blood sodium, prolonged vomiting or gastric suction, chronic diarrhea, with loss of acid from the body (Young *et al*., 2001).
The histopathology of the kidney shows the kidney section of the rats fed with maize flour crushed with mortar and pestle had normal glomerulus and normal convoluted tubule with normal nuclei arrangement. The Kidney section of the rats fed with maize flour from hammer milling plate showed relatively normal glomerulus and normal convoluted tubule with normal nuclei arrangement but the Kidney section of the rat fed with the flour from disc milling plates showed loss of nuclei in the convoluted tubule and acinic differentiation of the glomerulus. This goes to suggest that the disc milling plate has the highest potential to cause nephrotoxicity.

5.0. Conclusion.

The results of this study indicated that both disc and hammer milling plates contain high Fe and Ni content and maize processing into flour has very high potential of metals contamination. Disc milling plates have higher potential than hammer milling plates for metals contamination. The Kidney section of the rat fed with the flour of day 1 of the disc milling plates showed loss of nuclei in the convoluted tubule and acinic differentiation of the glomerulus. This goes to suggest that the disc milling plate has the highest potential to cause nephrotoxicity.

5.1 Recommendation

i. Individuals should make use of hammer milling machines for grinding of grains, as it is less harmful than disc milling machine.

ii. The grinding machine should have a compartment that will remove Fe filings introduced into the food sample ground by magnetization.

iii. There should be an improvement in the hardness and metal contents of milling plates to prevent the high contamination of metals in milled products. iv. The manufacturers of grinding plates should be well monitored to ensure that their product meets the required quality.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. 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.

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