



Assessment of the toxicity and biochemical effects of detergent processed cassava on renal function of Wistar rats

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ABSTRACT

Fufu is a major component of food in West Africa. Local cassava farmers use detergent to ferment cassava root tuber during processing into fufu. Studies have established the hazardous health effects of detergent in humans. This study was carried out to determine the median lethal toxicity of detergent-processed cassava, and assessed its effects on the renal function of male Wistar rats. Fufu with different concentrations of detergent (250, 500 and 750 g/l) were given as food twice daily to the rats for the acute toxicity study. The sub-chronic test lasted for four weeks, during which the fufu samples prepared with 10, 20, 30, 40 and 50 g/l of detergent, were separately given to five groups of five rats, respectively. The results of the acute toxicity test showed increase in mortality with corresponding increase in detergent concentration. Plasma and urine creatinine and urea concentrations of all the detergent-treated rats were significantly higher than that of the negative and positive controls. Urinalysis showed significant presence of glucose and ketone bodies, as well as, blood and protein. Renal photomicrographs showed deranged cortical structure with diffuse loss of tubules, inflammatory cells infiltrate in the interstitium. The study concluded that detergent-processed cassava has adverse effects on the structure and function of the kidneys of rats.

1. Introduction

Detergents are cleaning agents that help remove dirt, germs, contaminants [1] and grease from porous (such as fabrics, clothes, non-treated wood) and non-porous surfaces such as metals, plastics, and treated wood [2]. Detergents are made principally of soaps or surfactants [2]. Some typical household detergents are solids except for soap, which are commonly liquids [3]. Surfactants in detergents improve water ability to wet things, spread over surfaces, and seep into dirty clothes fibers [3]. One end of their molecules is attracted to water, while the other end is attracted to dirt and grease [4], so the surfactant molecules help water to get a hold of grease, break it up, and wash it away [5]. There are other chemicals in detergents apart from surfactants. Such include: Linear Alkyl Benzene Sulfonate (LABS), Sodium Carbonate (Na₂CO₃) and Sodium Sulphate (Na₂SO₄) in a typical detergent sachet [6]. Linear Alkylbenzene Sulfonates (LASs) are synthetic anionic surfactants reported by the European Union 1960 [7] as high toxic substances which cause gastric secretion, damage to gastric mucosa and

generation of free radical in the stomach. Consumption of this chemical may cause severe change in acid level of blood (pH balance), that could lead to damage.

Starch is one of the most important plant products to man [8]. Fufu, in form of cassava flour and garri (a processed cassava product), is an essential component of food in West Africa, providing a large proportion of the daily calorific intake [9]. It is also used as a thickener in foods that are not subject to rigorous processing conditions.

Cassava (*Manihot esculenta* Crantz), which belongs to the family of *Euphorbiaceae* (cultivar 419), is one of the most important food crops in the humid tropics [10]. Cassava starch, which is very bland in flavor, is used in the processing of baby foods and bonding agent in confectionary and biscuit industries [11]. More than two-thirds of the total production of cassava is used as food for humans, with lesser amounts used for animal feed [12]. Cassava starch can be converted to maltotriose, maltose, and glucose as well as to other modified sugars and organic acids [13]. Starch from cassava can be used to make fructose syrups [14] and formulate gelatin capsules [15].

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The ingestion of detergent has been reported to have various human health effects which include, breathing difficulty, vomiting, diarrhea, nausea and abdominal distension [16]. Despite the potential health hazard detergent can cause, some local cassava farmers, majorly in West Africa add detergent to cassava root tuber during processing into fufu to whiten, lessen the odour and to preserve it for a long time. It was therefore imperative to evaluate the potential impact of adding detergent to cassava tuber during processing into fufu on vital organs. Thus, this study assessed the effects of detergent processed cassava on renal function of Wistar rats.

2. Materials and methods

2.1. Collection and preparation of detergent processed cassava

Fresh cassava tubers were collected during the month of January from the Obafemi Awolowo University (OAU) Teaching and Research Farm, Ile-Ife, Nigeria with a GPS coordinates of 7° 31' 14.7612'' N and 4° 31' 49.1340'' E. The tubers with its stem and leaves were identified at the Botany Department of the same University and a voucher specimen was deposited in the Herbarium. The same species of cassava was used throughout the study. The tubers were peeled and washed with borehole water whose physicochemical properties were determined. Water mixed with gradient concentrations of detergent (250, 500 and 750 g/l) were used in soaking 15 g of cassava tubers in different bowls. The cassava was allowed to ferment for about five (5) days. After fermentation, the softened cassava was sieved out from the water. The residues were mulched with hand for about 30 min. The mulched product was allowed to settle in form of starch, and the starch was removed and cooked into fufu (indigenous food).

2.2. Animal care and management

Seventy (70) male Wistar rats weighing between 120 and 150 g obtained from the Animal Holding Unit, College of Health Sciences, OAU, Ile-Ife, were used for the experiment. The rats were housed in plastic cages for two weeks under normal laboratory conditions with natural light/dark cycle and they were allowed to have access to water and standard rodent pellet purchased from Ace Feed PLC Ibadan, Nigeria. All experimental protocols employed in this study were in strict accordance with the criteria outlined in the Guide for the Care and Use of Laboratory Animals prepared by the National Institutes of Health's (NIH) *Guide for the Care and Use of Laboratory Animals* (NIH Publications No.8023, revised 1978) [17] and approved by Health Research and Ethics Committee of Institute of Public Health (HREC, IPH) of the Obafemi Awolowo University with reference number IPHOAU/12/1157.

2.3. Acute toxicity test

Twenty-five (25) male Wistar rats were selected for the acute toxicity study. Rat chow, fufu without detergent, and fufu with different concentrations of detergent (250, 500 and 750 g/l) were given as food twice daily to the rats. The group fed with normal rat chow served as the negative control while the group fed with fufu without detergent served as the positive control group. The LD₅₀ was calculated by Finney's method [18] as employed by Sharma et al., [19]. Mortalities were monitored daily and dead rats were removed (Table 1).

2.4. Sub-chronic toxicity test

The remaining 45 rats were used for the sub-chronic toxicity test. The test was based on the LD₅₀ obtained from the acute toxicity test. Rat chow, fufu without detergent and fermented fufu with different concentrations of detergent (10, 20, 30, 40 and 50 g/l) were given as food twice daily to the rats for 4 weeks (Table 2).

Table 1
Acute toxicity test (96 h).

Groups	Number of rats	Treatment or dose regimen
Control 1	5	Rat + feed+ 10 L of water (Negative control)
Control 2	5	Rat + fufu + 10 L of water (Positive control)
Treatment 1	5	Rat + fufu with 250 g of detergent + 10 L of water
Treatment 2	5	Rat + fufu with 500 g of detergent + 10 L of water
Treatment 3	5	Rat + fufu with 750 g of detergent + 10 L of water

The rats were transferred into separate metabolic cages (fabricated by Central Technological Laboratory and Workshops, OAU, Ile-Ife) so as to measure their urine output on weekly basis. 24 h' food consumption was also assessed after 4 weeks of detergent-processed cassava administration. Weekly body weights of the rats were measured with the aid of a digital balance. Thereafter, the rats were sacrificed under ketamine anesthesia and blood was collected via cardiac puncture into separate bottles. The blood was centrifuged at 4000 rpm for 15 min at 4 °C using a cold centrifuge (Model 8881; Centurium Scientific, Centurion Scientific Limited, Stoughton, UK) to separate the plasma for the assay of markers of renal function.

2.4.1. Concentration of detergent in fufu

The actual concentration of detergent absorbed in the detergent processed cassava (fufu) in each group was carried out using reversed-phase high performance liquid chromatography (RP-HPLC) (Hitachi; China). The final liquid chromatography analysis was performed on a Phenomenex Column (250 × 4 mm, 5 µm).

2.4.2. Biochemical assay

Plasma and urine concentrations of creatinine, urea and uric acid were determined by standard colorimetric method using assay kits purchased from Randox Laboratories Ltd (Crumlin, Antrim, UK).

2.4.3. Percentage weight gain and relative kidney weight

The percentage weight gain and relative kidney weight of the rats were calculated using the formulae below:

$$\text{Percentage weight gain} = \frac{\text{final weight} - \text{initial weight}}{\text{initial weight}} \times 100\%$$

$$\text{Relative kidney weight} = \frac{\text{organ weight}}{\text{body weight}} \times 100\%$$

2.4.4. Histological studies

The kidneys of the rats were fixed in 10 % formo-saline, dehydrated in graded alcohol and embedded in paraffin wax. These were cut into

Table 2
Sub- chronic toxicity test (4 weeks).

Groups	Number of rats	Treatment or dose regimen
Negative control (0)	5	Rat + feed + 10 L of water (Negative control)
Positive control	5	Rat + fufu + 10 L of water (Positive control (0))
Negative control detergent	5	Rat + feed +10 g of detergent mixed with 10 L of water
Commercial fufu	5	Rat + Commercial fufu + 10 L of water
Treatment 1	5	Rat + fufu fermented with 10 g of detergent+ 10 L of water
Treatment 2	5	Rat + fufu fermented with 20 g of detergent + 10 L of water
Treatment 3	5	Rat + fufu fermented with 30 g of detergent + 10 L of water
Treatment 4	5	Rat + fufu fermented with 40 g of detergent + 10 L of water
Treatment 5	5	Rat + fufu fermented with 50 g of detergent + 10 L of water

4–5 µm thick sections and stained with haematoxylin–eosin for photomicroscopic assessment using Leica DM 750 (Leica Microsystems, Wetzlar, Germany) at 400x magnification.

2.5. Statistical analysis

The results were expressed as mean ± SEM. Data were analyzed using one-way analysis of variance (ANOVA) followed by Newman-Keuls' multiple comparison test as the post hoc test using (Graph Pad Software Inc., CA, USA) and p-value less than 0.05 was considered statistically significant.

3. Results

3.1. Actual concentration of detergent absorbed in the detergent processed cassava

The results of the actual amount of detergent absorbed in the detergent processed cassava for the acute oral toxicity test (LD₅₀) is shown in Table 3.

The results of the second phase showed the sub-chronic toxicity test of detergent processed cassava, which took place at the space of four (4) weeks (Table 4).

3.2. Effect of detergent processed cassava on food consumption (g) of Wistar rats after 4 weeks of exposure

The food intake of rats in the negative and positive control groups was not significantly different (F = 0.879; p = 0.543) from that of the negative control detergent, 10, 20, 30, 40 and 50 g/l (Fig. 1).

3.3. Effect of detergent processed cassava on body weight gain (%) of Wistar rats after 4 weeks of exposure

The body weight gain of rats in the negative control group (animal feed) was significantly higher (F = 2.949; p = 0.013) when compared with that of the positive control group (fufu without detergent), negative control detergent, 10, 20, 30, 40 and 50 g/l (Fig. 2).

3.4. Effect of detergent processed cassava on relative kidney weight (%) of Wistar rats after 4 weeks of exposure

There was no significant difference in the relative weight of the kidneys among the groups (F = 2.038; p = 0.070) (Fig. 3).

3.5. Effect of detergent processed cassava on plasma creatinine of Wistar rats after 4 weeks of exposure

There was a significant increase in the plasma creatinine concentration of the negative control detergent, commercial fufu, 10, 20, 30, 40

Table 3

Actual concentration of detergent absorbed in the detergent processed cassava for acute oral toxicity test (LD₅₀).

No. of rats	Treatment or dose regimen (g/l)	Mortality (%)	Actual conc. (%) of detergent in fufu
5	Negative control (Animal feed)	00	0.000
5	Positive control (o) (fufu without detergent)	00	0.000
5	Negative control (10 g/l detergent only)	20	292.6791
5	250	60	0.6790
5	500	40	1.0572
5	750	60	528.2004

Table 4

Actual concentration of detergent absorbed in the detergent processed cassava for sub-chronic toxicity study.

No. of rats	Treatment or dose regimen (g/l)	Mortality (%)	Actual conc. of detergent in fufu(%)
5	Negative control (Animal feed)	0	0.000
5	Positive control	0	0.000
5	Negative control (10 g/l detergent only)	0	292.6791
5	Commercial fufu	0	0.36385
5	10	0	0.2464
5	20	0	18.6081
5	30	0	0.23174
5	40	0	0.28425
5	50	0	0.35116

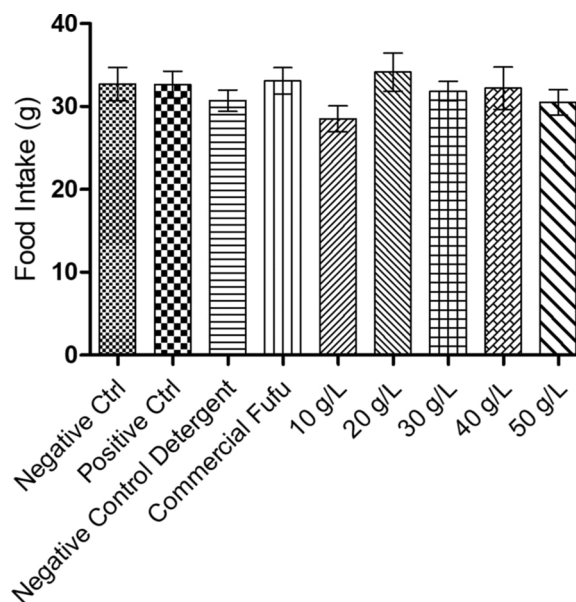


Fig. 1. Effect of detergent-processed cassava on food intake of Wistar rats. Results are presented as mean ± SEM (n = 5).

and 50 g/l when compared with the negative (animal feed) and positive (fufu without detergent) groups. The plasma creatinine concentration of rats in the negative control detergent was significantly lower when compared with commercial fufu and 40 g/l (Fig. 4a).

3.6. Effect of detergent processed cassava on plasma urea of Wistar rats after 4 weeks of exposure

The plasma urea concentration of the negative control (animal feed) was significantly lower when compared with the commercial fufu. The negative control detergent, commercial fufu and groups treated with 10, 30, 40 and 50 g/l had plasma urea concentration that was significantly higher (F = 4.823; p = 0.001) when compared with the positive control (fufu without detergent) (Fig. 4b).

3.7. Effect of detergent processed cassava on plasma uric acid of Wistar rats after 4 weeks of exposure.

The plasma uric acid of rats treated with 40 g/l of detergent was significantly higher when compared with the positive control (fufu without detergent), negative control (animal feed), negative control detergent, commercial fufu, 20 and 30 g/l. Rats treated with 30 g/l of detergent had plasma uric acid concentration that was significantly lower (F = 4.808; p = 0.001) than that of 50 g/l (Fig. 4c).

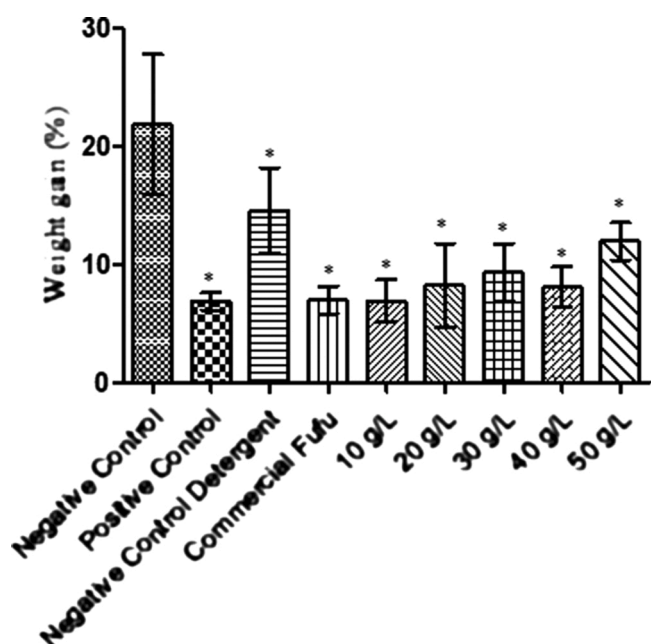


Fig. 2. Effect of detergent-processed cassava on body weight gain (g) of Wistar rats. * = significantly different from the negative control. Result are presented as mean \pm SEM (n = 5).

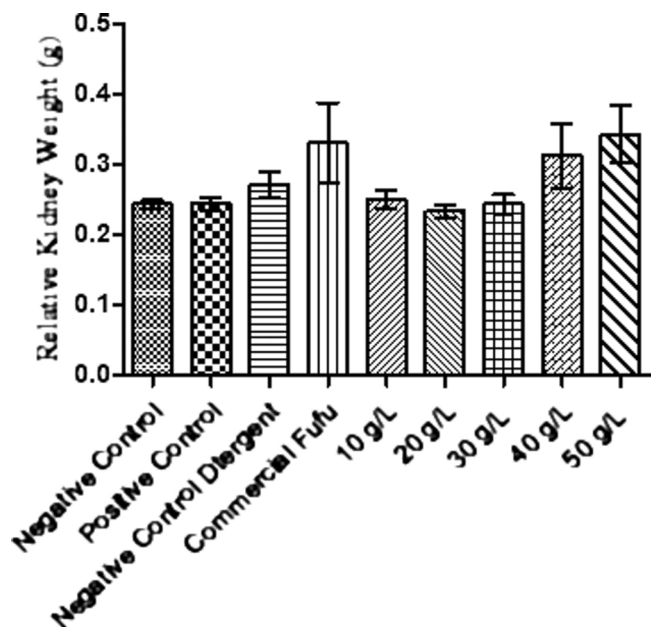


Fig. 3. Effect of detergent-processed cassava on the relative kidney weight of Wistar rats. Result are presented as mean \pm SEM (n = 5).

3.8. Effect of detergent processed cassava on urine creatinine of Wistar rats after 4 weeks of exposure.

A significantly higher urine creatinine concentration was found in the negative control detergent, commercial fufu, 20, 30 and 40 g/l when compared to the positive (fufu without detergent) and negative control (animal feed) groups. However, the urine creatinine of group treated with 40 g/l of detergent was significantly higher ($F = 3.937$; $p = 0.004$) than that of 10 and 50 g/l (Fig. 5a).

3.9. Effect of detergent processed cassava on urine urea of Wistar rats after 4 weeks of exposure

The concentration of urea in the urine of the positive control (fufu without detergent) was significantly lower ($F = 4.321$; $p = 0.023$) than that of the negative control, negative control detergent, 20 and 40 g/l (Fig. 5b).

3.10. Effect of detergent processed cassava on urine uric acid of Wistar rats after 4 weeks of exposure.

The concentration of uric acid in the urine of rats in the negative control detergent group, commercial fufu, 10, 20, 30, 40 and 50 g/l was significantly higher ($F = 4.437$; $p = 0.001$) when compared with the positive (fufu without detergent) and negative controls (animal feed) groups (Fig. 5c).

3.10. Effect of detergent processed cassava on number of leukocyte (cells/ μ l) in urine of Wistar rats after 4 weeks of exposure

The presence of leukocytes was seen in the urine of rats in the negative control detergent, commercial fufu and detergent-treated groups 10, 20, 30, 40 and 50 g/l when compared with positive (fufu without detergent) and negative controls (animal feed) during the four weeks of sub-chronic toxicity study. A significant decrease in leukocyte number was observed in rats treated with 10 and 20 g/l of detergent when compared with the negative control detergent and commercial fufu throughout the four weeks of the study. Also rats treated with 30, 40 and 50 g/l of detergent had significantly higher leukocytes number when compared with rats treated with 10 and 20 g/l (Table 5).

3.11. Effect of detergent processed cassava on number of blood cells (μ l) in urine of Wistar rats after 4 weeks of exposure

The presence of blood cells was found in the urine of the negative control detergent, commercial fufu, 30, 40 and 50 g/l when compared with the positive control, negative control, 10 and 20 g/l. However, the number of blood cells was observed to be significantly lower in the urine of treated groups 30, 40 and 50 g/l when compared with negative control detergent and commercial fufu throughout the study period (Table 6).

3.12. Effect of detergent processed cassava on urine ketone of Wistar rats after 4 weeks of exposure

The urine ketones of rats in the groups treated with 20, 30, 40 and 50 g/l of detergent were significantly higher when compared with the positive and negative controls, negative control detergent and commercial fufu during the first week of the sub-chronic study. However, rats treated with 30 g/l of detergent had urine ketones that were significantly higher ($F = 46.35$; $p < 0.0001$) than that of the 10, 20, 40 and 50 g/l. There was a significant increase in the urine ketone of rats in groups 10, 30 and 40 g/l when compared with the positive control, negative control, negative control detergent, commercial fufu, 20 and 50 g/l during the second week of study ($F = 36.95$; $p < 0.0001$). Rats treated with 10 g/l of detergent had urine ketones that was significantly higher ($F = 703.6$; $p < 0.001$) when compared with the positive control, negative control and other treated groups during the fourth week of study (Table 7).

3.14. Effect of detergent processed cassava on urine protein of Wistar rats after 4 weeks of exposure

There was a significant increase in urine protein in the treated groups 10, 20, 30, 40 and 50 g/l when compared with the positive control, negative control, negative control detergent and commercial fufu during the first week ($F = 6.557$; $p < 0.0001$). The urine protein of rats treated with 10 g/l was significantly lower ($F = 5.311$; $p = 0.0002$) when compared with other experimental groups during the second week. However, there was no significant difference across all groups during the

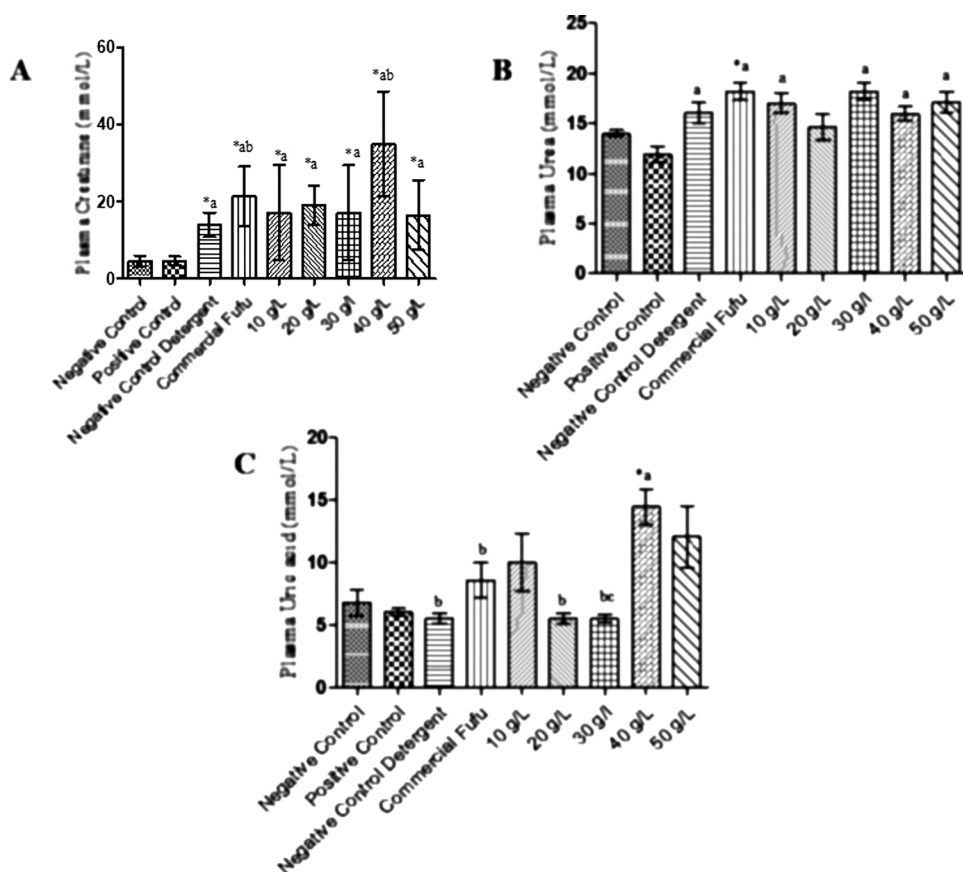


Fig. 4. (a) Effect of detergent-processed cassava on plasma creatinine of Wistar rats. * = Significantly different from negative control group. ^a = Significantly different from the positive control group. ^b = Significantly different from the negative control detergent. Result are presented as mean \pm SEM (n = 5). (b) Effect of detergent-processed cassava on plasma urea of Wistar rats. * = significantly different from the negative control. ^a = significantly different from the positive control. Result are presented as mean \pm SEM (n = 5). (c) Effect of detergent-processed cassava on plasma uric acid of Wistar rats. * = significantly different from the negative control. ^a = significantly different from the positive control. ^b = significantly different from the group treated with 40 g/l. ^c = significantly different from the group treated with 50 g/l. Result are presented as mean \pm SEM (n = 5).

third and fourth week ($F = 1.326$; $p = 0.263$; $F = 0.859$; $p = 0.568$ respectively) (Table 8).

3.15 Effect of detergent processed cassava on histology of the rat kidneys

The photomicrographs of negative and positive controls showed intact morphological pattern of renal corpuscle characterized by capsular covering of simple squamous epithelium, surrounding a fluid containing space within which is a delineable glomerular tufts and capillary lumen. The renal corpuscles and tubules are intact. The negative control detergent, commercial fufu, and rats treated with 20 and 40 g/l of detergent had deranged cortical structure with diffuse loss of tubules, inflammatory cells infiltrate in the interstitium. There are indications of glomerular atrophy in rats treated with 10 g/l of detergent, though the renal corpuscles and tubules appear normal. The photomicrographs of rats treated with 30 and 50 g/l of detergent revealed diffuse regions of tubular congestion/cuboidal epithelial loss and glomerular congestion respectively. Inflammatory cells infiltrate the interstitium of group 50 g/l. Also, rats treated with 40 g/l showed mild glomerular congestion (Fig. 6).

4. Discussion

Cassava starch in form of flour, and garri (a processed product), are essential component of food in West Africa, providing a large proportion of the daily calorific intake [9]. Cassava ranks very high among crops that convert the greatest amount of solar energy into soluble carbohydrates per unit of area. Among the starchy staples, cassava gives a carbohydrate production which is about 40 % higher than rice and 25 % more than maize, with the result that cassava is the cheapest source of calories for both human and animal nutrition.

Local cassava farmers, majorly in the western part of Africa use detergent to ferment cassava root tuber during processing into fufu,

despite its health hazard effects. The knowledge about the potential effects of using detergents to ferment cassava tuber during processing of fufu on the kidney is not yet well documented. The study is therefore aimed at exploring the health impact of detergent-processed cassava on renal function of Wistar rats.

Linear alkylbenzene sulfonate prepared by the sulfonation of straight-chain alkylbenzenes prepared from petroleum distillates is the most widely used surfactant for detergent formulations. The food consumption of rats treated with detergent processed cassava was not significantly different from the control. This is consistent with study of Buehler et al., [20] who reported no significant change in food intake of rats fed for 2 years with LAS at extremely high levels in the diets. They also reported that the body weight gain of the rats did not deviate from the control which is contrast with the result obtained in this study. The significant decrease in body weight gain of the rats in the negative control detergent, 20 and 50 g/l and commercial fufu groups when compare with the negative and positive control groups could not have been due loss of appetite, since their food consumption was not significantly different from the control. However, this is attributed to the presence of Linear Alkylbenzene Sulfonates (LASs) in the detergent. These are synthetic anionic surfactants reported by the European Union 1960 [7] as high toxic substances which cause gastric secretion, damage to gastric mucosa and generation of free radical in the stomach. This is believed to reduce gastric emptying and absorption of food nutrients by the small intestine.

Polysaccharides, including starch can cause some changes in the small and large intestines. Cases of changes in the cecal and colonic enlargement have been reported after use of modified starches. In addition to the structural changes in the intestines which are major sites of nutrient and water absorption, it is possible that the lower amylase activity [21] could contribute to the decrease in their body weight.

Urine undergoes many changes during states of disease or body

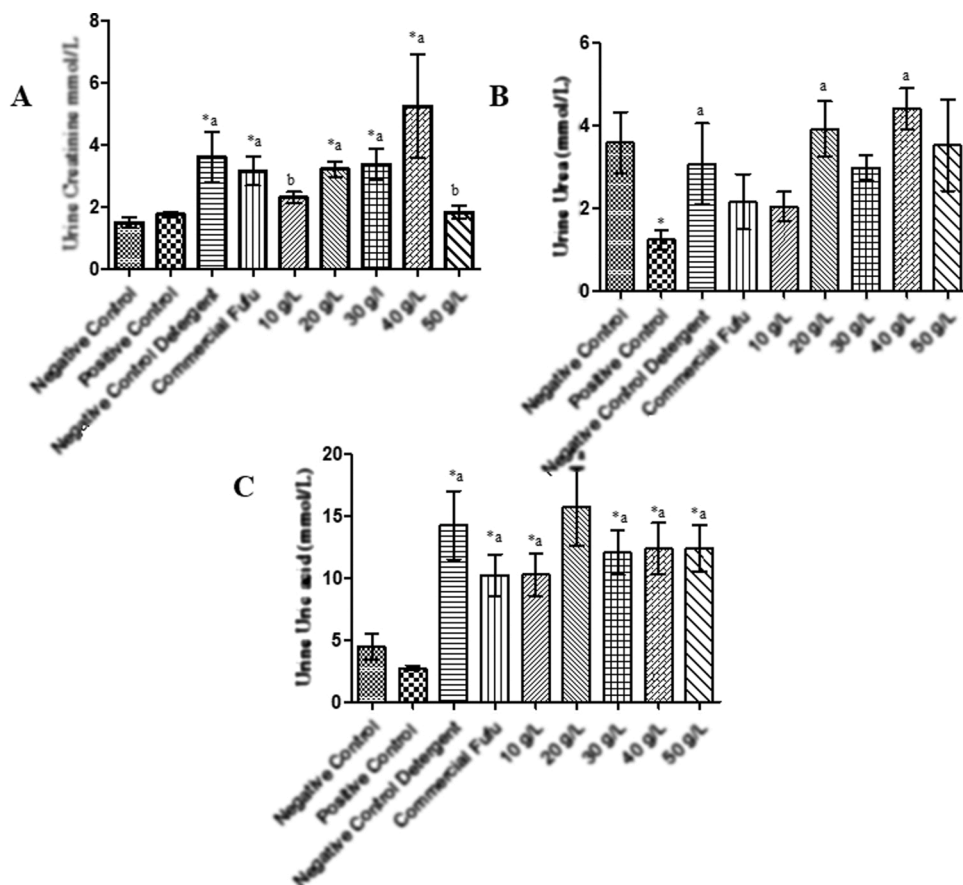


Fig. 5. (a) Effect of detergent-processed cassava on urine creatinine of Wistar rats. * = Significantly different from negative control group. ^a = Significantly different from the positive control group. ^b = Significantly different from group treated with 40 g/l. Result are presented as mean ± SEM (n = 5). (b)Effect of detergent-processed cassava on urine urea of Wistar rats. * = Significantly different from the negative control. ^a = significantly different from the positive control. Result are presented as mean ± SEM (n = 5). (c) Effect of detergent-processed cassava on urine uric acid of Wistar rats. * = significantly different from the negative control group. ^a = significantly different from the positive control group. Result are presented as mean ± SEM (n = 5).

Table 5
Number of leukocytes (cells/μl) in the urine of rats fed with detergent processed cassava.

Weeks	Negative control (o)	Positive control	Negativecontrol (detergent)	Commercial Fufu	10 g/l	20 g/l	30 g/l	40 g/l	50 g/l
1	0.0±0	0.0±0	70.0±0 ^{αβ}	15.0±0 ^{αβ}	15.0±0 ^{αβ§}	15.0±0 ^{αβ§}	48.00±13.47 ^{αβμ}	70.00±0 ^{αβμ}	70.00±0 ^{αβμ}
2	0.0±0	0.0±0	70.0±0 ^{αβ}	70.0±0 ^{αβ}	15.0±0 ^{αβ§¶}	15.0±0 ^{αβ§¶}	70.00±0 ^{αβμ}	59.00±11.0 ^{αβμ}	59.00±0 ^{αβμ}
3	0.0±0	0.0±0	70.0±0 ^{αβ}	70.0±0 ^{αβ}	15.0±0 ^{αβ§¶}	15.0±0 ^{αβ§¶}	48.00±13.47 ^{αβμ}	59.00±0 ^{αβμ}	70.00±0 ^{αβμ}
4	0.0±0	0.0±0	70.0±0 ^{αβ}	59.0±0 ^{αβ}	15.0±0 ^{αβ§¶}	15.0±0 ^{αβ§¶}	70.00±0 ^{αβμ}	70.00±0 ^{αβμ}	70.00±0 ^{αβμ}

Results are presented as mean ± SEM (n = 5). ^α = significantly different from positive control. ^β = significantly different from negative control (o). [§] = significantly different from negative control detergent. [¶] = significantly different from Commercial fufu. ^μ = significantly different from 10 g/l detergent.

Table 6
Number of blood cells (μl) in the urine of rats fed with detergent processed cassava.

Weeks	Negative control (o)	Positive control	Negative control detergent	Commercial fufu	10 g/l	20 g/l	30 g/l	40 g/l	50 g/l
1	0 ± 0	0 ± 0	210 ± 0 ^{αβ}	210 ± 0 ^{αβ}	0 ± 0 ^{§¶}	0 ± 0 ^{§¶}	23 ± 3.39 ^{αβ§¶μ}	27 ± 2.55 ^{αβ§¶μ}	27 ± 2.55 ^{αβ§¶μ}
2	0 ± 0	0 ± 0	210 ± 0 ^{αβ}	210 ± 0 ^{αβ}	0 ± 0 ^{§¶}	0 ± 0 ^{§¶}	28 ± 1.23 ^{αβ§¶μ}	23 ± 6.44 ^{αβ§¶μ}	22 ± 2.80 ^{αβ§¶μ}
3	0 ± 0	0 ± 0	210 ± 0 ^{αβ}	210 ± 0 ^{αβ}	0 ± 0 ^{§¶}	0 ± 0 ^{§¶}	27 ± 3.39 ^{αβ§¶μ}	26 ± 2.92 ^{αβ§¶μ}	28 ± 2.80 ^{αβ§¶μ}
4	0 ± 0	0 ± 0	210 ± 0 ^{αβ}	210 ± 0 ^{αβ}	0 ± 0 ^{§¶}	0 ± 0 ^{§¶}	16 ± 6.96 ^{αβ§¶μ}	28 ± 20.92 ^{αβ§¶μ}	36 ± 3.67 ^{αβ§¶μ}

Results are presented as mean ± SEM (n = 5). ^α = significantly different from positive control. ^β = significantly different from negative control (o). [§] = significantly different from negative control detergent. [¶] = significantly different from Commercial fufu. ^μ = significantly different from 10 g/l detergent.

dysfunction before blood composition is altered to a significant extent. Absence or presence of leukocytes, urobilinogen, bilirubin, blood, nitrite, protein, glucose and ketone bodies in the urine is a useful indicator of health or disease. Routine urinalysis showed significant presence of blood, protein, glucose and ketone bodies in rats treated with detergent processed cassava for 4 weeks.

Urine pH assesses the effectiveness of the kidney in maintaining acid-base homeostasis. A fall in glomerular filtration rate (GFR) reduced the filtered load of HCO₃⁻ in the renal tubules. Large number of H⁺ are secreted into the tubular lumen by the tubular epithelial cells leading to a net loss of hydrogen ion (H⁺) in the urine than bicarbonate, thus removing acid from the blood and lowering the pH [22]. The above

Table 7
Effect of detergent processed cassava on urine ketone (g/l) of Wistar rats.

Weeks	Negative control (o)	Positive control	Negative control detergent	Commercial fufu	10 g/l	20 g/l	30 g/l	40 g/l	50 g/l
1	0.50 ± 0.0	0.70 ± 0.20	0.7 ± 0.20	0.50 ± 0.0	0.50 ± 0.0	2.10 ± 0.0 ^{αβ§∞}	4.10 ± 0.0 ^{αβ§∞}	1.40 ± 0.68 ^{αβ§∞}	2.30 ± 0.0 ^{αβ§∞}
2	0.50 ± 0.0	0.50 ± 0.0	0.5 ± 0.0	0.50 ± 0.0	1.80 ± 0.58 ^{αβ§∞}	0.70 ± 0.0	4.00 ± 0.0 ^{αβ§∞}	1.20 ± 0.70 ^{αβ§∞}	0.50 ± 0.0
3	0.50 ± 0.0	0.50 ± 0.0	0.5 ± 0.0	0.50 ± 0.0	0.70 ± 0.20	0.50 ± 0.0	0.50 ± 0.0	0.50 ± 0.0	0.50 ± 0.0
4	0.50 ± 0.0	0.50 ± 0.0	0.5 ± 0.0	0.50 ± 0.0	1.40 ± 0.67 ^{αβ§∞}	0.50 ± 0.0 [‡]	0.30 ± 0.12 [‡]	0.43 ± 0.7 [‡]	0.50 ± 0.0 [‡]

Results are presented as mean ± SEM (n = 5). ^α = significantly different from positive control. ^β = significantly different from negative control (o). [§] = significantly different from negative control detergent. [‡] = significantly different from Commercial fufu. [∞] = significantly different from 10 g/l detergent. [∞] = significantly different from 30 g/l detergent.

Table 8
Effect of detergent processed cassava on urine protein (g/l) of Wistar rats.

Weeks	Negative control (o)	Positive control	Negative control detergent	Commercial fufu	10g/l	20g/l	30g/l	40g/l	50g/l
1	0.18 ± 0.03	0.15 ± 0.0	0.21 ± 0.04	0.15 ± 0.0	3.06 ± 1.89 ^{αβ§}	4.20 ± 0.80 ^{αβ§}	3.00 ± 0.0 ^{αβ§}	2.60 ± 0.40 ^{αβ§}	2.69 ± 0.40 ^{αβ§}
2	4.03 ± 0.97	3.09 ± 1.17	3.79 ± 1.21	5.00 ± 0.0	0.21 ± 0.04 ^{αβ§}	2.09 ± 1.19	4.6 ± 0.40 [‡]	4.03 ± 0.97 [‡]	4.06 ± 0.94 [‡]
3	4.20 ± 0.49	5.00 ± 0.0	3.79 ± 1.21	5.00 ± 0.0	2.29 ± 1.12	4.03 ± 0.97	4.03 ± 0.97	5.00 ± 0.0	3.06 ± 1.19
4	3.06 ± 1.19	5.00 ± 0.0	3.29 ± 1.15	5.00 ± 0.0	3.63 ± 0.95	3.06 ± 1.19	5.00 ± 0.0	4.03 ± 0.97	4.06 ± 0.94

Results are presented as mean ± SEM (n = 5). ^α = significantly different from positive control. ^β = significantly different from negative control (o). [§] = significantly different from negative control detergent. [‡] = significantly different from Commercial fufu. [‡] = significantly different from 10 g/l detergent.

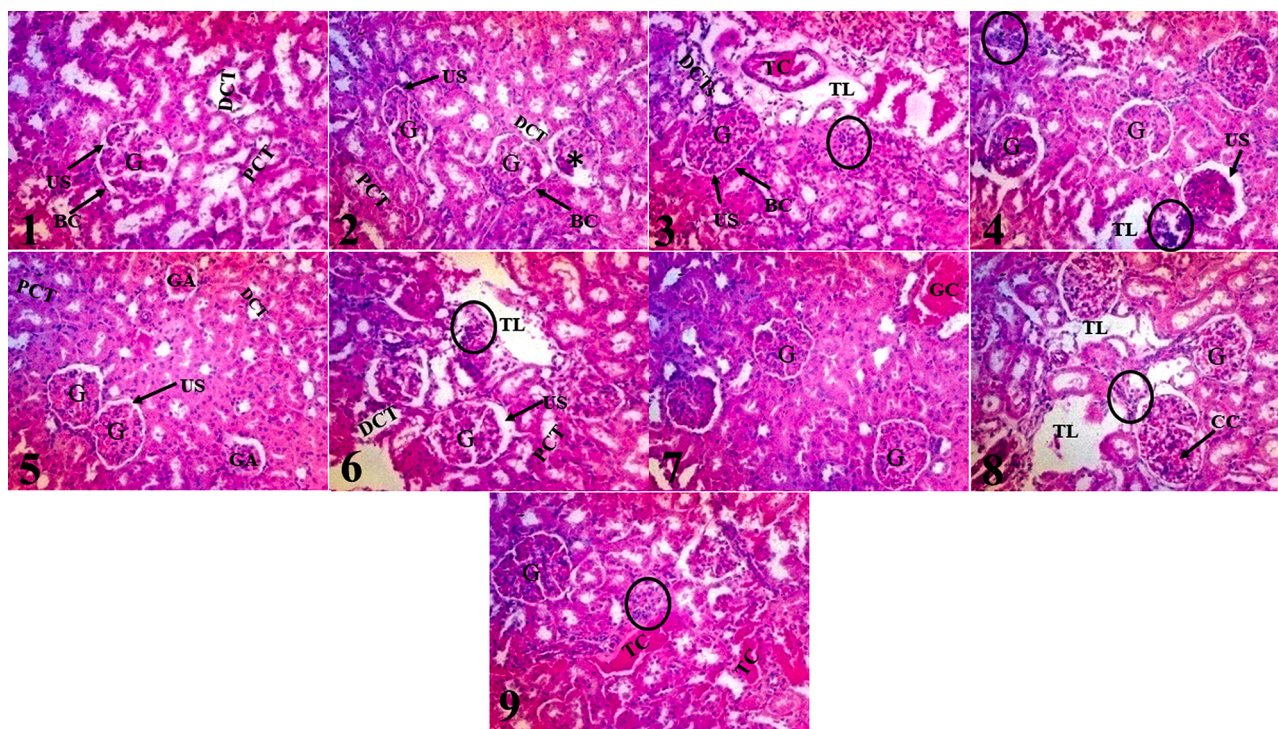


Fig. 6. Effect of detergent processed cassava on histology of rat kidneys. Representative photomicrographs showing the renal cortex of Wistar rats subjected to H&E stain. Observe : (1) intact morphological pattern of renal corpuscle characterized by capsular covering of simple squamous epithelium (BC), surrounding a fluid containing space (BS) within which is a delineable glomerular tufts and capillary lumen (G). Proximal convoluted tubule (PCT), Distal convoluted tubule (DCT), Urinary space (US) are also intact. (2) Renal corpuscles and tubules are intact, with some atrophied glomerulus (*). (3, 4, 6, 8) - show deranged cortical structure with diffuse loss of tubules (TL), inflammatory cells infiltrate in the interstitium (black circles). (5) There are indications of glomerular atrophy (GA) at the upper and lower right sides, though the renal corpuscles and tubules appear normal. [7,9] presents diffuse regions of tubular congestion (TC)/cuboidal epithelial loss and glomerular congestion (GC) respectively. Inflammatory cells infiltrate the interstitium of 9 (black circles). There are some intact glomerulus (G) in these groups. (8) – shows mild glomerular capillaries congestion (CC).

description could explain the significant decrease in urine pH that was seen in detergent treated rats and commercial fufu when compared with the negative control group.

Urine protein analysis is used to assess the normal function of the kidney. However, presence of excess protein in urine (proteinuria) is an indication of glomerular damage [23]. The presence of excess protein in the urine is of clinical significance to man because it represents renal disease. Rats, on the other hand, demonstrate conspicuous proteinuria whose implications are not yet clearly understood [24].

In this study, urine protein in the negative control group and commercial fufu and groups treated with 40 and 50 g/l of detergent was significantly higher when compared to the positive control group. Intratubular nephrosis has been reported in mice treated or fed with hydroxypropyl distarch phosphate, starch acetate, lactose and sodium alginate [25]. This finding suggests that the mechanism by which proteinuria is expressed in the commercial fufu and other treated groups is a function of both the integrity of the glomerular filtration barriers and/or post glomerular reabsorption. Also, the presence of red blood cells (RBC) and leukocytes in the urine of the treated rats may have resulted from glomerular damage [26], which is evident by the photomicrographs of their renal tissue and/or damage to the lower urinary tract e.g. bladder or urethra which causes RBC to leak out of the blood vessels into the urine.

The presence of ketone bodies in the urine is an indication of poor handling of this organic compound by the kidney. Ketones are end-products of fatty acid metabolism [27]. Also Nam-Seok et al. [27] reported that caloric reduction could cause ketonuria. Presence of ketone bodies (ketonuria) is an indication that the only available energy source is fat. In prolonged starvation, fat becomes the predominant body fuel instead of carbohydrates. In this study, urinalysis showed a mild to severe presence of ketone bodies with exposure to detergent. This could imply that prolonged administration of high concentrations of detergent can potentiate further injury to the kidneys.

Kidney injury due to the treatment of detergent processed cassava was assessed by measuring the plasma and urinary markers of renal function. Changes in these biochemical indices are associated with renal impairment. Plasma creatinine and urea are used for assessing renal glomerular function. Renal diseases that caused reduction in GFR would result in elevated plasma concentrations of these products [28]. Thus, the plasma concentrations of urea and creatinine increases as the filtration rate declines. In this study, the plasma creatinine and urea of the treated groups were significantly higher than positive and negative control groups. This agrees with the study of Shama and Wasma. [29], who reported a significant increase in plasma urea in rats fed with methanol extract of cassava for 2 weeks. The urine concentration of these excretory products were significantly higher in the detergent treated rats when compared with the control. The renal photomicrographs of the detergent treated groups showed deranged cortical structure with diffuse loss of tubules, inflammatory cells infiltrate in the interstitium with some intact glomerulus. This suggests that the increased plasma and urine creatinine and urea in the detergent treated groups is indicative of an increased secretion of creatinine and urea by the renal cells and/or increased skeletal muscle metabolism and protein catabolism since creatinine and urea are their byproducts respectively.

Uric acid is the final oxidative product of purine metabolism and is excreted by the kidneys [30]. Therefore, elevated serum uric acid level is associated with reduced glomerular filtration rate (GFR). Previous studies carried out in rats have demonstrated that, in the presence of hyperuricemia, there are structural changes in the renal vasculature. Further, Sánchez-Lozada et al. [31] established that rats with increased serum uric acid levels had renal biopsies demonstrating afferent arteriolar thickening. Thickening of the arterioles decreases renal blood flow. Hence, the reduced levels of uric acid in the urine of the detergent treated rats when compared with the positive and negative control groups is suggestive of decreased GFR resulting from reduced blood flow to the kidneys of the rats.

5. Conclusion

The summary of findings in this study suggests that detergent processed cassava is harmful to the kidneys of Wistar rats as evident by the alterations in the biochemical and histological assessments of the treated rats when compared with the controls. The use of detergent in processing cassava into fufu should be discontinued and agencies for food control should look into this and deploy measures to curtail and end the practice.

CRediT authorship contribution statement

Oghobase G.E: Conceptualization, Writing - original draft. **Aladesanmi O.T:** Methodology, Supervision. **Akomolafe R.O:** Validation, Methodology, Supervision. **Olukiran O.S:** Writing - review & editing, Formal analysis. **Akano P.O:** Methodology, Validation. **Eimunjeze M.H:** Investigation, Formal analysis.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.toxrep.2020.08.007>.

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