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Evaluation of Liver and Kidney Function Markers of Mature Albino Male Rats Fed with Maize-Plantain Pudding Delicacy Cooked in Metallic Plates and Edible Plant Leaves

***OKPOGHONO, J; OMORIARE, UJ; IGUE, UB; SEIGHA, AA**

Department of Chemical Sciences (Biochemistry Option), College of Natural and Applied Sciences, Novena University, Ogume, Delta State, Nigeria

**Corresponding Author Email: okpoghono@gmail.com; Tel: +2348064618605*
Co-Author Email: classyphina@gmail.com; udobess@gmail.com; seighaanita2001@gmail.com

ABSTRACT: Traditional maize-plantain puddings are usually cooked in diverse containers such as aluminium plates, cast iron plates and all types of edible plant leaves. This paper therefore, evaluates biochemical markers of liver and kidney function of mature albino male rats fed with maize-plantain pudding delicacy cooked in aluminium and cast iron plates and edible *Zingiber officinale* (ginger) and *Musa paradisiaca* (plantain) leaves using appropriate standard methods. Data obtained reveal that the activities of hepatic marker enzymes; alanine transaminase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) in the serum and liver were significantly lower in rats given the pudding extracts when compared with the control. No significant differences were observed in urea and creatinine levels in the serum and kidney of rats given pudding extracts as compared to control. In conclusion, this study has proved that maize-plantain pudding organized using ginger leaves, plantain leaves and cast iron plate are beneficial in the maintenance and improvement of liver and kidney function when compared with aluminium plate. However, in this efficacy maize-plantain pudding cooked using ginger leaves was found to be more effective than the pudding cooked using plantain leaves and cast iron plate.

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Maize (*Zea mays*) is a high carbohydrate containing cereal processed and used in food preparations such as breakfast cereal, weaning foods and other snacks (maize pudding and maize cake) (Anosike *et al.*, 2019). *Zingiber officinale* is believed to have health-promoting effects because of its extensive phytochemistry (Ezzat *et al.*, 2018). Phytochemicals are part of the human diet because they are found in almost all plant derived food items (Andriamadio *et al.*, 2015; George *et al.*, 2015; George *et al.*, 2019; Okpoghono *et al.*, 2018a; 2018b; 2018c). Many chemicals consumed during food preparations may compromise or overwhelmed the liver metabolic process (Okonta *et al.*, 2021). Ukpo oka a popular maize pudding consumed as breakfast or lunch among the low income class in the Niger Delta part of Nigeria is traditionally prepared in diverse containers and food wrapping materials. It is eaten by children, adults and the elderly. The problem of vitamin and mineral malnutrition may be resolved by using the right cooking tools and adding plantains to Ukpo oka may increase the amount of phytochemicals, vitamins, and minerals of the product. Study has showed that plantain is rich in alkaloids, flavonoids and terpenoids

**Corresponding Author Email: okpoghono@gmail.com*

(Uzairu and Kano, 2018). Liver damage has been associated with increased hepatocyte cell death (Jeschke *et al.,* 2001; Okpoghono *et al.,* 2021). Hepatic damage is evidenced by rise in the level of ALP, ALT and AST (Joshi *et al.,* 2008). The kidney (renal) is a major target for induced toxicity due to its function as the major organ of excretion. Kidney is naturally exposed to a greater proportion of circulating chemical compounds and plays an important role as the primary eliminator of exogenous substances (Tiong *et al.,* 2014).

Cooking with different materials such as leaves, glass and metallic utensils has been on-going for several decades in the different localities in Nigeria. Using toxic metallic materials in cooking has disastrous impacts on human health due to leaching of these metals into food. Aluminium is often used as cooking material. The toxicity of aluminium to the liver kidney and brain has been reported (Ekakitie *et al.,* 2021; Kandimalla *et al.,* 2016). Aluminium exposure has been suggested as a risk factor for Alzheimer's disease (Kandimalla *et al.,* 2016). Iron deficiency anemia is mainly due to insufficient iron. Without enough iron, the body can't produce enough of red blood cells. Cast iron materials used in cooking food may provide dietary iron to those with iron shortage (Otuaga *et al.,* 2020a; 2020b). Therefore, this study evaluates the effect of liver and kidney function markers in mature albino male rats fed with maize-plantain pudding delicacy cooked in aluminium and cast iron plates and edible *Zingiber officinale* (ginger) and *Musa paradisiaca* (plantain) leaves.

MATERIALS AND METHODS

Chemicals/materials: Plantain fingers and dried maize (*Zea mays*) were purchased from Amai local market, Delta State, Nigeria. Plantain leaves and ginger leaves were obtained from a local farm in Amai. Aluminium plate and cast iron plate were purchased from Obiaruku market, Delta State. All the chemicals that were used for this study were of analytical grade.

Plantain and maize flour preparation: Unripe plantain fingers were washed using distilled water, then peeled and each finger was cut into halves. All the peeled halves were boiled for 10 minutes. The cooked samples were sliced (1.5 mm) and sun-dried (as it been processed locally). The dried plantain and maize were ground to powder (flour sample) using waring blender. The samples were filtered with sieve that has an aperture size of 425 µm. The plantain and maize flour were blended together 1:1 ratio. The blended sample was used for the preparation of pudding.

Maize-plantain pudding preparation: Ngoddy *et al.* (1986) recipe method was used for maize-plantain pudding formulation as shown in Table 1. The food items were added together in a mixing bowl using a wooden handle to form a smooth paste. The paste was allowed to stand for 3 min and mixed thoroughly. Then 300 ml of the paste was dispensed into aluminum plate, cast iron plate and then wrapped with *M. paradisiaca* and *Z. officinale* leaves. This was allowed to steam for 1 hour using modern cast iron pot. The products were allowed to cool and then dried in electric oven (40 ^{o}C) for further analysis. The cooked maize-plantain pudding samples are shown in Fig. 1.

Table1: Ingredients for maize-plantain pudding formulation.

Ingredient	Amounts
Plantain/maize flour blend	300 g
Tatashe pepper (*Capsicum annum*)	60 g
Onion (*Allium cepa*)	60 g
Salt	20 g
African nutmeg (*Monodora myristica*)	15 g
Hot water (70°C)	900ml
Maggi cube	8 g
Red palm oil from oil palm tree fruit *(Elaeis guineensis*)	140 ml

Fig 1: Materials/Cooked Maize-plantain pudding samples

Preparation of samples: Five gram (5 g) of each dried pudding samples were homogenized in 45 ml aqueous tween 80 (5 % tween 80) using laboratory mortar and pestle to dissolve the oil present in the samples. The samples were filtered into sample bottles with clean muslin cloth and kept in the refrigerator at -4^{0}C for further analysis.

Experimental design: Mature albino male rats (30) weighing 120–126 g were procured from the Delta State University, Abraka, animal house. The testing rats were kept in neat plastic cages and served grower's mash to acclimatize for a week. The rats were weighed and their weights were recorded after the acclimatization period. Weight gain was calculated by subtracting the starting weight from the final weight at the conclusion of the experiment.

Treatment of animals: Thirty rats were divided into six groups per five rats. The rats in each group were allowed free access to clean drinking water and pudding extracts were given to rats at dose of 400 mg/kg (protective dose) three times in a week throughout the experimental period of four weeks. Treatment were as follows; Group A: control (rats were not given pudding aqueous extract and Tween 80). Group B: Tween 80 (rats were administered Tween 80 only). Group C: plantain leaves pudding aqueous extract (rats were administered aqueous extract of maize-plantain pudding prepared using plantain leaves). Group D: ginger leaves pudding aqueous extract (rats were administered aqueous extract of maize-plantain pudding prepared using ginger leaves). Group E: aluminium plate pudding aqueous extract (rats were fed aqueous extract of maize-plantain pudding cooked using aluminium plate). Group F: cast iron plate pudding aqueous extract (rats were given aqueous extract of maize-plantain pudding cooked using cast iron plate)

Blood collection and preparation of tissue homogenate: The rats were sacrificed on the 29th day. The blood was collected by cardiac puncture using hypodermic syringe and needle and then transferred to an anticoagulant free test tube. The clotted blood was centrifuged at 2,500 g for 15 minutes to separate the serum. One gram of various tissues were homogenized in 9 ml of normal saline then centrifuged at 2,500 g for 15 minutes to obtain the supernatant which was stored in the refrigerator, for further biochemical analysis.

Biochemical Analysis: The activities of ALT, AST, ALP, acid phosphatase, albumin and total protein in the serum, and liver were determined colorimetrically according to standard procedures using commercially available diagnostic kits (Randox Laboratories Limited, England). Kidney function markers such as urea and creatinine in the serum and kidney were determined colorimetrically according to standard procedures using commercially available diagnostic kits (Randox Laboratories Limited, England).

Alanine Aminotransferase (EC 2.6.1.2) Alanine aminotransferase activity was assayed by the method of Reitman and Frankel (1957). Into sample and blank test tube were dispensed 250 µl of reagent 1 (100 mmol/L, pH 7.4 phosphate buffer, L-alanine 200mmol/L, and 2.0 mmol/L alpha-oxoglutarate). Into sample test tubes, 50 µl of sample was added and 50 µl of distilled water into blank, then incubated for 60 minutes at 37 °C. 250 µl of reagent 2 (2,4-dinirophenylhydrazine) was added and then incubated at 20 – 25 °C for 20 minutes. Into each test tubes, 2.5 ml of working NaOH reagent was added, mixed well and allowed to stand for 10 minutes. Absorbance was read against reagent blank at a wavelength of 540nm. The activity of ALT was calculated using standard curve supplied by Randox Laboratories.

Aspartate Aminotransferase (EC 2.6.1.1): Aspartate aminotransferase activities were assay by the method of Reitman and Frankel (1957). Into test tubes labelled sample and blank, 250 µl of reagent 1(100 mmol/L, pH 7.4 phosphate buffer, L-alanine 200mmol/L, and 2.0 mmol/L alpha-oxoglutarate) were dispensed. Then, 50 µl of samples were added and mixed. Then, 250 µl of reagent 2 (2,4-Dinitrophenylhydrazine 1.0 mmol/L) was added to tubes after incubation at room temperature for 60 minutes. Thereafter, 2.5 ml of NaOH reagent was added onto each test tube. The absorbance were recorded against reagent blank at 540 nm after ten minutes. The activity of AST was extrapolated using standard curve supplied by Randox Laboratories.

Alkaline phosphatase (EC.3.1.3.1): Alkaline phosphatase activity was assayed for using the method of Kaplan and Righetti (1955). Zero point five millilitres of alkaline phosphatase substrate was added to labelled tubes and then equilibrated to 37 ºC for three minutes. At time interval, 0.05 ml of sample was added to tubes and incubated at 37 ºC for ten minutes. Thereafter, 2.5 ml ALP colour developer was added at time interval. At a wavelength of 580 nm, spectrophotometric absorbance measurements were made against reagent blank comprising distilled water.

Albumin: Doumas *et al.* (1971) method was used in estimating albumin level. The quantification of albumin binding to the indicator 3,3,5,5,-tetrabromo-m-cresol-sulphoepthalein (bromocresol green, BCG) serves as the basis for measurement. Three millilitres (3 ml) of BCG regent was dispensed into test tube

labelled blank, standard and sample. Ten microliters of sample were transferred to tubes. Spectrophotometry absorbance was read at 580 nm after five minutes.

Total Protein: Total protein was determined using the method of Tietz (1976). Protein in the sample forms a blue coloured complex in addition of cupric ions. The formation of blue colour is proportional to the protein concentration. One millilitre (1 ml) of protein regent was transferred to test tubes (test, standard and blank) then 20 µl of sample was pipetted to respective tubes. Samples were read at 546 nm against reagent blank after 30 minutes.

Urea: Urea was determined using the method of Henry (1974). The urea enzyme reagent was reconstituted according to the manufacturer instructions. Urea enzyme reagent (1.5 ml) was pipetted into labelled test tubes and then allowed to equilibrate at room temperature. Precisely, 0.010 ml (10 µl) of sample was transferred to respective tube. Water was used as the sample reagent blank. All tubes were incubated for five minutes at 37 ºC. Thereafter, 1.5 ml of urea colour developer was added and mixed gently and then incubated for five minutes at 37°C. The spectrophotometer was zeroed with the reagent blank at 630nm. The absorbance of samples was read and recorded.

Creatinine: Creatinine was estimated using the method of Henry (1974). Exactly 3 ml of working reagent (Creatinine picric acid reagent and creatinine buffer reagent ratio 1:1) was pipetted into the test tubes and 0.1 ml (100 µl) of sample was transferred to the respective tube and mixed. Distilled water was used as reagent blank. The test tubes were heated in water bath at 37°C for fifteen minutes. The spectrophotometer was then calibrated with the reagent blank and its wavelength was adjusted to 510 nm. The absorbance of all tubes were read and recorded.

Statistical Analysis: All the results were expressed in means ± SD and all data were analysed using Analysis of variance (ANOVA). Significant differences between means were determined at 5% ($p < 0.05$) confidence level using Duncan's Multiple Range Test.

RESULTS AND DISCUSSION

The monitoring of the body weight of experimental rats is important while studying the toxicity and safety of natural product extracts (Mohamed *et al.,* 2011). This may help to hint at the biochemical status of the rats, and gets rid of the investigator from any false observations that may be due to nutritional abnormalities (Mohamed *et al.,* 2011). Table 2 illustrates the weight gain of rats fed maize-plantain pudding aqueous extracts for 28 days. Rats fed with pudding made using aluminium and cast iron plate did not showed significant additional weight gain different from the control. Interestingly, rats fed maize-plantain pudding prepared using ginger leaves had significant increase in weight gain when compared to rats fed pudding prepared using plantain leaves, aluminium plate and cast iron plate.

In this study, none of the experimental groups suffered loss in weight or gained overweight as compared with the control which suggested that the maize-plantain pudding prepared using plantain leaves, ginger leaves, aluminium plate and cast iron plate did not induce significant changes in the appetite and did not exert any deleterious effects on the general health status of the rats. However, significant ($p<0.05$) increase in weight gain was observed in rats fed with pudding prepared using ginger leaves when compared to control.

Table 2: Weight gain of rats administered maize-plantain pudding aqueous extracts for 28 days.

Group	Initial body weight (g)	Final body weight (g)	Weight gain (g)
Group A: Control	120.50± I1. 10^{a}	130.40 ± 20.80^{a,b}	9 .90± 5.20^{d}
Group B: Tween 80 only	125.00 ± I2.40^{a}	134.40±17.70^{b}	9.10 ± 2.50^{d}
Group C: Plantain leaves pudding aqueous extract	126.50 ± 10.90^{a}	140.00± 18.06^{c}	13.50± 2.34^{d}
Group D: Ginger leaves pudding aqueous extract	125.00 ± 21.20^{a}	143.40 ± 23.12^{c}	18.40 ± 6.05^{d,f}
Group E: Aluminium plate pudding aqueous extract	I24.50± 13.00^{a}	135.30 ± 11.15^{b}	10.80± 2.50^{d}
Group F: Cast iron plate pudding aqueous extract	I24.00± 14.00^{a}	135.50 ± 13.00^{b}	11.50 ±5.30^{d}

Values are represented in mean ± SD. n = 5. Different superscript letter of mean values in the same horizontal row differs significantly at p< 0.05

Table 3 presented the changes in AST, ALT and ALP activities in the serum and liver of rats fed maize-plantain pudding aqueous extracts. AST, ALT and ALP activities in the serum and liver of rats fed maize-plantain pudding prepared using plantain leaves, ginger leaves, aluminium plate and cast iron plate (Group C, D, E and F) were significantly lower when compared to control. Likewise, Group D had significantly lower AST, ALT and ALP activities in the serum and liver when compared with Group E and F. In this study, significant decrease in AST, ALT and ALP in the serum and liver after administration of the

extracts of the pudding organised using plantain leaves and ginger leaves could be as results of certain phytochemical compounds in the pudding that might have protective potential on liver tissue. Maize pudding have been shown to have antioxidant protective properties (Ezzat *et al.,* 2018). Traditional medicine and culinary flavouring both incorporate ginger plant leaves (Mohamed *et al.,* 2011). The primary bioactive ingredients in ginger are primarily phenolic compounds (Prasad and Tyagi, 2015). *M. paradisiaca* can serve the dual purpose of dietary/nutritional and therapeutic roles (Abiodun-Solanke and Falade, 2010). *M. paradisiaca* leaves is associated with antioxidant properties due to its bioactive compounds; alkaloids, terpenes and polyphenols (Abiodun-Solanke and Falade, 2010). Study has confirmed that elevated levels of hepatic enzymes (AST, ALT and ALP) in the serum are not a direct link for liver injury but increased levels are responsible to be caused by inflammation, cellular leakage and damage of the cell membrane of cells in the liver (Donkor *et al.,* 2014). The main marked organ for toxicant or bioactive compound is the liver (Donkor *et al.,* 2014).

Table 3: Changes AST, ALT and ALP activities in the serum and liver of rats administered maize-plantain pudding aqueous extracts

Groups	Serum AST *	Liver AST*	Serum ALT*	Liver ALT*	Serum ALP*	Liver ALP*
Group A: Control	107.50 ± 7.15^{a}	115.10 ± 14.12^{a}	96.00 ± 3.53^{a}	102.20 ± 7.08^{a}	430.22 ± 29.13^{a}	481.35 ± 14.33^{a}
Group B: Tween 80 only	106.00 ± 9.40^{a}	114.10 ± 13.80^{a}	94.50 ± 14.10^{a}	101.20 ± 5.50^{a}	430.25 ± 29.24^{a}	480.35 ± 23.25^{a}
Group C: Plantain leaves pudding aqueous extract	85.00 ± 13.97^{b}	92.00 ± 8.12^{b}	78.10 ± 9.00^{b}	78.50 ± 9.00^{b}	369.11 ± 21.46^{b}	447.34 ± 13.28^{b}
Group D: Ginger leaves pudding aqueous extract	80.50 ± 12.25^{b}	91.00 ± 15.00^{b}	66.00 ± 7.10^{c}	70.50 ± 15.05^{c}	360.13 ± 14.73^{c}	440.05 ± 11.47^{c}
Group E: Aluminium plate pudding aqueous extract	99.50 ± 10.87^{c}	106.50 ± 8.10^{c}	78.50 ± 9.11^{d}	87.00 ± 10.57^{d}	380.11 ± 25.14^{d}	467.09 ± 22.53^{d}
Group F: Cast iron plate pudding aqueous extract	90.00 ± 17.87^{d}	99.00 ± 12.10^{d}	70.50 ± 14.10^{c}	80.00 ± 8.09^{e}	376.03 ± 13.15^{e}	450.00 ± 24.42^{e}

Values are represented in mean ± SD. n=5. Values with different superscript letter in same column differ significantly at $p < 0.05$.*U/L

Albumin and total protein level in the serum and liver of rats administered maize-plantain pudding aqueous extracts are shown in Table 4.

The level of albumin and total protein in the serum and liver of rats in Group C, D, E and F showed significant increase compared to Group A and Group B. Also Group D had significantly higher albumin and total protein level in serum and liver when compared with Group E and F. Generally, albumin constitutes the major plasma protein target of oxidant stress (Iroaganachi *et al.,* 2015).

The increased levels of total protein and albumin in the serum and liver (Table 4) might be due to upsurge in protein intake from the intestine as a result of increased protein synthesis in the liver, which may be attributed to the extracts of maize-plantain pudding prepared using plantain leaves and ginger leaves. Unripe plantain and ginger has been shown to exhibit free radical scavenging effect thereby increasing protein level and kidney function marker (Iroaganachi *et al.,* 2015). The most prevalent protein in plasma, albumin (predominant antioxidant in plasma), has exceptional polyphenol binding abilities (Poloni *et al.,* 2019).

The decrease in albumin and total protein level in rats fed pudding cooked using aluminium and iron plate when compared to plantain and ginger leaves may be as results of aluminum and cast iron-forming complex with polyphenol (polyphenol complex formation).

This is in line with previous studies conducted by Speer *et al.,* (2019) and Ugwu *et al.,* (2021) which state that plant polyphenol and flavonoids may binds to metals to form complex, this may reduce their bioavailability.

Study has also showed that aluminum is pro-oxidant and may induce free radicals formation (Mohammadirad and Abdollahi, 2011).

Table 4. Albumin and total protein level in the serum and liver of rats administered maize-plantain pudding aqueous extracts

Groups	Serum Albumin*	Liver Albumin*	Serum Total protein*	Liver Total protein*
Group A: Control	5.38 ± 0.70^{a}	8.31 ± 1.05^{a}	10.31 ± 2.45^{a}	14.39 ± 3.35^{a}
Group B: Tween 80 only	5.60 ± 1.02^{a}	8.21 ± 1.94^{a}	10.34 ± 2.67^{a}	14.16 ± 5.17^{a}
Group C: Plantain leaves pudding aqueous extract	16.60 ± 2.03^{c}	20.28 ± 7.92^{b}	26.34 ± 2.67^{b}	30.16 ± 7.12^{b}
Group D: Ginger leaves pudding aqueous extract	19.23 ± 4.39^{c}	24.12 ± 3.03^{b}	29.17 ± 3.11^{b}	34.15 ± 6.15^{b}
Group E: Aluminium plate pudding aqueous extract	10.24 ± 2.03^{b}	13.44 ± 3.48^{c}	18.14 ± 3.04^{c}	23.28 ± 5.54^{c}
Group F: Cast iron plate pudding aqueous extract	$14.13 \pm 2.41^{b,c}$	$17.43 \pm 3.33^{c,b}$	20.21 ± 3.46^{c}	26.31 ± 4.10^{c}

Values are represented in mean ± SD. n=5. Values with different superscript letter in same column differ significantly at $p < 0.05$. *g/dL.

Table 5: Effect maize-plantain pudding aqueous extracts on urea and creatinine level in the serum and kidney of rats.

Groups	Serum Urea*	Kidney Urea*	Serum Creatinine*	Kidney Creatinine*
Group A: Control	6.51 ± 0.72^{a}	8.45 ± 0.21^{a}	14.76 ± 3.61^{a}	16.34 ± 2.22^{a}
Group B: Tween 80 only	6.99 ± 0.39^{a}	8.33 ± 0.22^{a}	14.14 ± 2.29^{a}	16.29 ± 1.34^{a}
Group C: Plantain leaves pudding aqueous extract	4.51 ± 1.37^{a}	6.14 ± 1.40^{a}	12.85 ± 1.04^{a}	14.33 ± 2.10^{a}
Group D: Ginger leaves pudding aqueous extract	4.01 ± 1.37^{a}	5.63 ± 0.41^{a}	12.05 ± 1.04^{a}	13.33 ± 2.10^{a}
Group E: Aluminium plate pudding aqueous extract	5.27 ± 0.19^{a}	7.21 ± 0.23^{a}	14.23 ± 3.77^{a}	15.31 ± 2.25^{a}
Group F: Cast iron plate pudding aqueous extract	4.74 ± 2.17^{a}	6.10 ± 1.48^{a}	13.35 ± 0.62^{a}	14.38 ± 4.27^{a}

Values are represented in mean ± SD. n=5. Values with different superscript letter in same column differ significantly at $p < 0.05$. *mg/dL.

Urea and creatinine concentrations in the serum of rats are tested as indicators for kidney functions (Iroaganachi *et al.*, 2015). Increased levels of urea and creatinine in blood could be consequences from increased breakdown of tissue or impaired excretion (Iroaganachi *et al.*, 2015). Increased blood urea is correlated with an increased protein catabolism in mammalian body and/or referred to kidney dysfunction (Iroaganachi *et al.*, 2015). Table 5 showed the effect of pudding aqueous extracts on kidney function markers of rats. No significant difference was observed in the entire experimental group. However, the trends of the maize-plantain pudding aqueous extracts in enhancement of kidney function were as follows; ginger leaves > plantain leaves > cast iron plate > aluminium plate. The slight decrease observed in urea and creatinine in rats fed with pudding aqueous extracts as compared with control (Table 5) may be due to the shielding effect of the ingredients used in cooking the maize-plantain pudding, thereby enhancing kidney function. This is in line with the report of Cases *et al.* (2019) who stated that spices and vegetables are abundant in antioxidants that could help improve kidney function to some extent, thus lowering creatinine and urea levels. Spices and vegetables such peppers and onions used in food preparation have been described to contain phytochemicals (Ejueyitsi *et al.*, 2022) and may help to increase kidney filtration rate (Cases *et al.* 2019), thus eliminating wastes from the body in the form of urine thereby preventing high level of creatinine.

Conclusion: This study has showed that maize-plantain pudding prepared using ginger leaves, plantain leaves and cast iron plate are beneficial in the maintenance and improving liver and kidney function. The outcomes could provide basis for developing a valuable new food recipes to enhance clinical nutritional changes linked to liver and kidney ailments primarily in health care. Using edible leaves in the preparation of maize-plantain pudding in rural areas should be encouraged. Furthermore, ginger leaves outperformed plantain leaves and metallic plates in terms of effectiveness.

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