European Institute of Knowledge & Innovation

Annals of Innovation in Medicine (AIM) ISSN: 2977-0335

Research Article

Effects of Administration of Bouillon Cubes on Insulin Resistance, Lipid Profile and Renal Function Parameters in Female Albino Rats

Blessing B. Oodee[1], Ojoye N. Briggs[1*], Adline E. Ben-Chioma[1], Edna O. Nwachuku[1]

[1] Department of Medical Laboratory Science, Rivers State University, Port Harcourt, Rivers State, Nigeria
* Correspondence: Ojoye.briggs@ust.edu.ng

https://doi.org/eiki/10.59652/aim.v2i1.126

Abstract:

Aim: To evaluate the effects of administration of bouillon cubes on insulin resistance, lipid profile and renal function parameters in female albino rats.

Methodology: A total of thirty-five (35) female albino rats, weighing between 120 and 150 grams, were used for the study. The bouillon cubes, Star Maggi and Knorr were administered daily to the rats, using an oral gavage tube for 90days. Fasting plasma glucose (FPG) was determined using the Glucose oxidase method. Fasting plasma insulin (FPI) and Cystatin C levels were quantitatively determined by a rat-specific sandwich-enzyme linked immunosorbent assay (ELISA) method. Insulin resistance was determined using the homeostatic model assessment for insulin resistance (HOMA-IR) method. The electrolytes, sodium (Na+) and potassium (K+) and were determined using ion selective electrode method. Urea was determined using Urease-bertholet method. Creatinine was determined using the Jaffe-Slot method. Total Cholesterol (TC), Triglyceride (TG) and High-Density Lipoprotein Cholesterol (HDL-C) were determined by enzymatic methods. Low Density Lipoprotein Cholesterol (LDL-C) was calculated from the Friedewald's equation. Kidney sections were stained using haematoxylin and eosin (H&E) staining technique. Quantitative analysis of monosodium glutamate (MSG) content of the bouillon cubes was analyzed using ultraviolet (UV) spectroscopy while the sodium content was analyzed using atomic absorption spectrophotometry according to the method of the American Public Health Association.

Results: There were no significant differences (P>.05) in FPG, FPI and HOMA-IR in all the treatment groups. The mean cystatin C value in group E (High Dose Knorr) was significantly higher (P <.05) than the negative control and all other treatment groups. The results also show the mean sodium values in groups D (High Dose Maggi) and E (High Dose Knorr) were significantly lower (P <.05) when compared to the negative control. There were no significant differences (P >.05) in TC and HDL-C levels in the negative control, compared to the treatment groups. There were no significant differences (P >.05) in TG levels, except for group B (Low Dose Maggi) which significantly lower (P <.05) than the negative control. Also, there were no significant differences (P >.05) in LDL-C levels, except for group B (Low Dose Maggi) which significantly higher (P <.05) than the negative control. Histologic analysis of the kidneys of the treated groups showed histological changes in the architecture of the tissues indicating tissue distortion, acute tissue damage, glomerular nephritis and distorted capillaries and degeneration compared to the negative control group which showed no tissue distortion.

Conclusion: Chronic exposure to bouillon cubes did not impact fasting plasma glucose, insulin and insulin resistance in the treated rats. Chronic administration of Knorr cubes impacted the integrity of the kidney as levels of cystatin C and sodium were altered in the albino rats. Histoarchitecture of the kidneys of the treated rats showed histological changes indicating tissue distortion, acute tissue damage, glomerular nephritis and distorted capillaries. Lipid profile/metabolism was relatively not affected by the administration of bouillon cubes.

Received: 08 Jan. 2024

Accepted: 24 Jan. 2023

Published: 25 Jan. 2023

Keywords: Monosodium glutamate; Bouillon cubes; Renal function; Lipid profile; Insulin resistance.

1. INTRODUCTION

Bouillon cubes serve as flavour enhancers, elevating the taste and aroma of food and adding a touch of culinary artistry to the preparation of delicious dishes. Across various African countries, these cubes have become essential components in everyday cooking. Nestlé Company reports that over 100 million bouillon cubes are purchased daily in the West Central Africa region, underscoring their widespread use and popularity [1,2].

Numerous brands of bouillon cubes populate the market, including Star Maggi, Knorr Cubes, Royco, Onga, Aji-no-Moto, and others. Maggi and Knorr bouillon cubes, which are the most common brands, typically consist of a dehydrated broth or mixture condensed into cube form, and these same blends can also be found in powdered form. Despite their lower nutritional content, these cubes have largely replaced many fermented seeds and products, which once contributed diverse flavours and nutritional value to African dishes. Experts liken bouillon cubes to "well-packaged salts," and studies suggest that prolonged exposure to these cubes may have adverse health effects due to their primary ingredients. One of the contents of bouillon cubes which has attracted interest and is of concern is monosodium glutamate (MSG), which has been implicated in toxicity to different organ systems [3, 4].

As health consciousness rises, there is an increased demand for products promoting better well-being. Consequently, bouillon cubes being integral to the food consumed, have come under scrutiny, particularly in our country, Nigeria [5]. This study evaluates the effects of administration of bouillon cubes on insulin resistance, lipid profile and renal function parameters in female albino rats. This research may contribute in understanding the health impact of bouillon cubes, and promote responsible consumption and production by informing decisions related to food safety and regulation, in line with the Sustainable Development Goals (SDGs).

2. MATERIALS AND METHODS

2.1 Experimental Animals

A total of thirty-five (35) female albino rats, weighing between 120 and 150 grams, was used for the study. The rats were kept in standard cages and provided with unrestricted access to feed and water. A period of 14 days was allotted for the animals to acclimatize before the study officially began.

2.2 Treatments

Two (2) commonly used bouillon cubes (Star Maggi and Knorr cubes) purchased from the local market in Port Harcourt were used for the study. They are readily available brands, with a high patronage. Maggi cube is manufactured by Nestle Nigeria PLC, Industrial Avenue 22/24 Ilupeju, Lagos Nigeria. Knorr cube is manufactured by Unilever PLC, RC 113 Agbara Industrial Estate, Agbara, Ogun State, Nigeria

2.3 Acute Toxicity Study

This was done using the fixed dose procedure [6]. Eighteen (18) rats were divided into six (6) groups of three (3) rats each – 3 groups for Maggi and 3 groups for Knorr. 2000mg/kg, 3000mg/kg, and 5000mg/kg of star Maggi cube was administered to the rats in groups 1, 2, and 3 respectively while 2000mg/kg, 3000mg/kg, and 5000 mg/kg of Knorr cube was administered to the rats in groups 4, 5, and 6 respectively. The rats were observed for signs of toxicity for 48 hours. After observation for 48 hours, there were no signs of toxicity, hence the bouillon cubes were considered safe up to a dose of 5000 mg/kg. For the study, 1500 mg/kg and 3000 mg/kg were adopted and used as low and high doses respectively.

2.4 Dose Calculation

2.4.1 Low Dose Maggi (1500 mg/kg)

1500mg/kg of Maggi was administered to the rats daily. That is, a 1kg would take 1500mg of Maggi. Therefore, a 150g rat took 150g /1000g x 1500mg = 225mg of Maggi as daily dose. A 1kg rat would take as vehicle 10 ml of fluid orally. Therefore, a 150g rat took 150g / 1000g x 10 ml = 1.5ml. Hence, 225mg of Maggi was dissolved in 1.5ml of water and administered daily. That is, 150 mg/ml. A stock solution was therefore prepared and administered according to the weight of the rats daily [6, 7].

2.4.2 High Dose Maggi (3000 mg/kg)

3000 mg/kg of Maggi was administered to the rats daily. That is, a 1kg would take 3000mg of Maggi. Therefore, a 150g rat took 150g /1000g x 3000mg = 450mg of Maggi as daily dose. A 1kg rat would take as vehicle 10 ml of fluid orally. Therefore, a 150g rat took 150g / 1000g x 10 ml = 1.5ml. Hence, 450mg of Maggi was dissolved in 1.5ml of water and administered daily. That is, 300mg/ml. A stock solution was therefore prepared and administered according to the weight of the rats daily [6, 7].

2.4.3 Low Dose Knorr (1500 mg/kg)

1500mg/kg of Knorr was administered to the rats daily. That is, a 1kg would take 1500mg of Knorr. Therefore, a 150g rat took 150g /1000g x 1500mg = 225mg of Knorr as daily dose. A 1kg rat would take as vehicle 10 ml of fluid orally. Therefore, a 150g rat took 150g / 1000g x 10 ml = 1.5ml. Hence, 225mg of Knorr was dissolved in 1.5ml of water and administered daily. That is, 150 mg/ml. A stock solution was therefore prepared and administered according to the weight of the rats daily [6, 7].

2.4.4 High Dose Knorr (3000 mg/kg)

3000mg/kg of Knorr was administered to the rats daily. That is, a 1kg would take 3000mg of Knorr. Therefore, a 150g rat took 150g /1000g x 3000mg = 450mg of Knorr as daily dose. A 1kg rat would take as vehicle 10 ml of fluid orally. Therefore, a 150g rat took 150g / 1000g x 10 ml = 1.5ml. Hence, 450mg of Knorr was dissolved in 1.5ml of water and administered daily. That is, 300mg/ml. A stock solution was therefore prepared and administered according to the weight of the rats daily [6, 7].

2.5 Experimental Design

The rats were weighed and divided into five (5) experimental groups (7 rats each) consisting of the negative control group (Group A) and 4 treatment groups (Groups B-E). The bouillon cubes were prepared into suspension form of 150mg/ml of bouillon cubes (Star Maggi and Knorr cubes) respectively for Low Doses and 300mg/ml of bouillon cubes (Star Maggi and Knorr cubes) for High Doses. The suspension was given daily using an oral gavage tube for 90days. Treatments were performed according to the grouping below;

Group A (Negative control group): Received no treatment.

Group B (Low dose Maggi): Received daily oral dose of 1500mg/kg Star Maggi cubes.

Group C (Low dose Knorr): Received daily oral dose of 1500 mg/kg Knorr cubes.

Group D (High dose Maggi): Received daily oral dose of 3000 mg/kg Star Maggi cubes.

Group E (High dose Knorr): Received daily oral dose of 3000 mg/kg Knorr cubes.

On the 91st day, the animals were fasted for 6 hours anaesthetized and later sacrificed. Blood was collected from each rat by means of cardiac puncture. All the animal experiments were conducted according to the ethical norms approved by the Institutional Ethical Committee.

2.6 Reagents and Biochemical Analyses

All reagents were purchased commercially and the standard operating procedures provided by the manufacturers were meticulously adhered to. Quality control (QC) samples were analyzed alongside the biochemical tests. Fasting plasma glucose (FPG) was determined using the Glucose oxidase method [8] as described by Spectrum Diagnostics (Egypt). Fasting plasma insulin (FPI) and Cystatin C levels were quantitatively determined by a rat-specific sandwich-enzyme linked immunosorbent assay (ELISA) method [9] as described by as described by Calbiotech Company limited, China. Insulin resistance (IR) was determined using the homeostatic model assessment for insulin resistance (HOMA-IR) method [10]. The electrolytes, sodium (Na+) and potassium (K^+) and were determined using ion selective electrode (ISE) method [11]. Urea was determined using Urease-bertholet method [12], as modified by Spectrum Diagnostics (Egypt). Creatinine was determined using the Jaffe-Slot method [13], as modified by Spectrum Diagnostics (Egypt). Total Cholesterol (TC) was determined by enzymatic method [14], as modified by Spectrum Diagnostics (Egypt). Triglyceride was determined by enzymatic method [15], as described by Spectrum Diagnostics (Egypt). High Density Lipoprotein Cholesterol (HDL-C) was determined by enzymatic method [16], as modified by Spectrum Diagnostics (Egypt). Low Density Lipoprotein Cholesterol (LDL-C) was calculated from the Friedewald's equation [17]. Quantitative analysis of monosodium glutamate (MSG) content of the bouillon cubes was analyzed using ultraviolet (UV) spectroscopy while the sodium content was analyzed using atomic absorption spectrophotometry according to the method of the American Public Health Association (APHA) [18]. Kidney specimens were harvested and fixed in 10% formal saline for histological analysis using Haematoxylin and Eosin stain, viewed and photomicrographs of the kidney were captured with X40 objective lens using the ScopeTekTM device and software v1.3.

2.7. Statistical Analysis

The data generated was analyzed with GraphPad Prism version 8.0.2. Analysis of variance (ANOVA) and Tukey's post hoc test were performed to compare differences between groups. The results were considered statistically significant at the 95% confidence interval ($p \leq 0.05$). Results are expressed as mean ± SD.

3. RESULTS AND DISCUSSION

Table 1: Quantitative Analysis of MSG and Sodium Content in the Bouillon Cubes

Samples	MSG Conc. (mg/g)	Sodium Conc. (ppm)
Star Maggi	119.636 (11.96%)	38.282 (0.00383 %)
Knorr Cube	111.455 (11.15%)	32.892 (0.00329 %)

Table 1 shows the results of Monosodium Glutamate (MSG) and sodium content of bouillon cubes. It shows that star Maggi had the highest contents of Monosodium Glutamate (MSG) with a concentration of 119.96 mg/g (11.96 %) while that of Beef Knorr cubes is 111.5 mg/g (11.15 %). The results also reveal that star Maggi had the highest sodium content with a concentration of 38 ppm (0.00383 %) while Knorr cube sodium content concentration is 32 ppm (0.00329 %).

These values were compared to the maximum allowable limits set by the National Agency for Food and Drug Administration and Control (NAFDAC), which are 1.5% max for MSG and 12.5% max for sodium in bouillon cubes. From the results above, the sodium content in both the Star Maggi and Beef Knorr cubes falls well within the permissible limit of 12.5%. However, the MSG content in both products exceeds the maximum allowable limit of 1.5% specified by NAFDAC, indicating consumers maybe at risk of toxicity from MSG. Even though the sodium content in the Star Maggi and Beef Knorr cubes is within the NAFDAC limit, it is still crucial to consider the cumulative sodium intake from other food items to stay within the recommended daily limits of less than 2g per day [19]. This is in consonance with the works of Apkanyung [20], and Alonge *et al.* [21], in which they found variable amounts of MSG, sodium, iron and zinc in bouillon cubes produced in Nigeria.

Table 2: Levels of the Fasting Plasma Glucose, Insulin and Insulin Resistance of the Rats after Treatment

Groups (N=7)	FPG (mmol/L)	FPI (mU/L)	HOMA-IR
Group A (Neg. Cont)	5.00 ± 0.85	1.90 ± 0.16	0.42 ± 0.04
Group B (Low Dose Maggi)	5.03 ± 0.65	2.47 ± 0.25	0.55 ± 0.07
Group C (Low Dose Knorr)	4.27 ± 0.38	2.53 ± 0.17	0.48 ± 0.06
Group D (High Dose Maggi)	4.15 ± 0.37	2.22 ± 0.30	0.41 ± 0.06
Group E (High Dose Knorr)	5.10 ± 0.42	2.12 ± 0.20	0.48 ± 0.05
P-value	0.0533	0.2992	0.3229
Summary	NS	NS	NS

N= number of rats, NS= not significant

From Table 2, the results from the study showed no significant differences ($P>.05$) in fasting plasma glucose (FPG), fasting plasma insulin (FPI), and HOMA-IR (Homeostatic model assessment of insulin resistance), in all the treatment groups of rats administered with varying inclusion of bouillon cubes when compared to the negative control group. These results showed that chronic exposure to bouillon cubes did not affect the glucose metabolism and insulin resistance of the rats. Therefore, indicating bouillon cubes (star Maggi and Knorr cubes) may not a predisposing factor to insulin resistance and type 2 diabetes. Insulin resistance is a key factor in the development of type 2 diabetes. It is presented as a suppression in metabolic responses of the liver, muscle, and adipose tissue to insulin action. It leads to impaired regulation of hepatic glucose synthesis, declining beta-cell function, ultimately leading to beta-cell failure [22]. This finding agrees with Tchaou et al. [23], which showed that overnight fasting glucose was not influenced by monosodium glutamate (MSG) and Maggi poulet solution injection. Another study found no effects of MSG administration on postprandial glycaemia and insulinemia [24], however, MSG-obese rats had impaired glucose tolerance and insulin resistance [25].

Table 3: Showing Levels of the Renal Function Markers of the Rats after Treatment

Groups (N=7)	Cystatin C (ng/mL)	Sodium (mmol/L)	Potassium (mmol/L)	Urea (mmol/L)	Creatinine (µmol/L)
Group A (Neg. Cont)	0.35 ± 0.06	146.00 ± 2.45	3.55 ± 0.40	4.82 ± 0.17	89.25 ± 1.26
Group B (Low Dose Maggi)	0.37 ± 0.08 ^{be}	138.3 ± 1.52	3.62 ± 0.20	4.43 ± 0.69	99.33 ± 1.96
Group C (Low Dose Knorr)	0.30 ± 0.06 ^{ce}	138.5 ± 3.41	4.77 ± 0.80	4.80 ± 0.42	116.8 ± 2.66
Group D (High Dose Maggi)	0.50 ± 0.10 ^{de}	128.8 ± 1.65 ^{ad}	3.02 ± 0.28	4.70 ± 0.53	104.8 ± 4.19
Group E (High Dose Knorr)	1.12 ± 0.13 ^{ae}	130.2 ± 2.33 ^{ae}	5.12 ± 0.85	4.72 ± 0.47	97.50 ± 3.07
P-value	0.0004	0.0006	0.1245	0.8424	0.0743
Summary	S	S	NS	NS	NS

N= number of rats, $^{ae, ad}$ =significant vs Neg. control, ^{be} = Group E significant Vs B, ^{ce} = Group E significant Vs C, ^{de} = Group E significant Vs D, NS= not significant.

From Table 3, the results showed the mean cystatin C value (1.12 ± 0.13 ng/mL) in group E (High Dose Knorr) was significantly higher ($P<.05$) than the negative control and all other treatment groups. The results also show the mean sodium values in groups D (High Dose Maggi) and E (High Dose Knorr) were significantly lower ($P<.05$) when compared to the negative control. There were no significant differences ($P>.05$) in Potassium, Urea and Creatinine in the treatment groups, compared to the negative control. The results indicate acute renal damage in the group administered High dose of Knorr cubes, due to the elevated cystatin C, a marker of acute kidney injury. Creatinine and urea are not significantly different, as they may take longer periods to indicate kidney injury. Studies suggest that chronic MSG intake induces kidney damage by oxidative stress with unclear underlying mechanisms. However, excessive renal metabolism of glutamate in chronic MSG intake can be a source of ROS,

which may damage renal tissue and cause renal impairment [26]. In a similar study. MSG had adverse effects on kidney functions as serum urea and serum creatinine were significantly increased. These effects were however, counteracted by the administration of vitamins C and E [27].

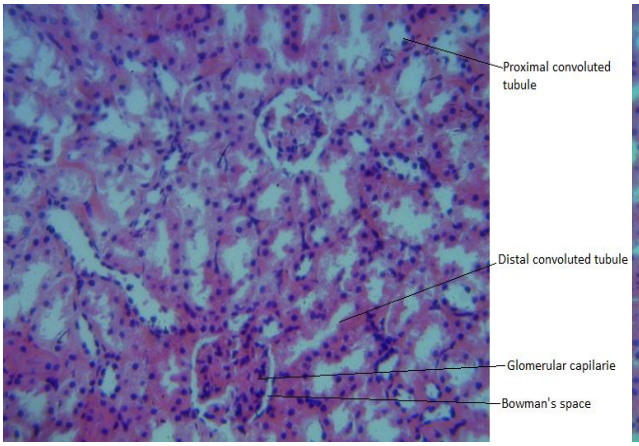
Table 4: Levels of the Lipid Profile Markers of the Rats after Treatments

Groups (N=7)	TC (mmol/L)	TG (mmol/L)	HDL-C (mmol/L)	LDL-C (mmol/L)
Group A (Neg. Cont)	2.55 ± 0.31	1.38 ± 0.30	1.62 ± 0.07	0.30 ± 0.05
Group B (Low Dose Maggi)	2.40 ± 0.15	0.72 ± 0.06 [ab]	1.50 ± 0.04	0.57 ± 0.04 [ab]
Group C (Low Dose Knorr)	2.20 ± 0.18	1.02 ± 0.04	1.40 ± 0.07	0.34 ± 0.06 [bc]
Group D (High Dose Maggi)	2.50 ± 0.18	1.06 ± 0.06	1.60 ± 0.07	0.42 ± 0.03
Group E (High Dose Knorr)	2.45 ± 0.30	1.22 ± 0.08 [be]	1.45 ± 0.13	0.44 ± 0.03
P-value	0.1484	0.0004	0.2047	0.0036
Summary	NS	S	NS	S

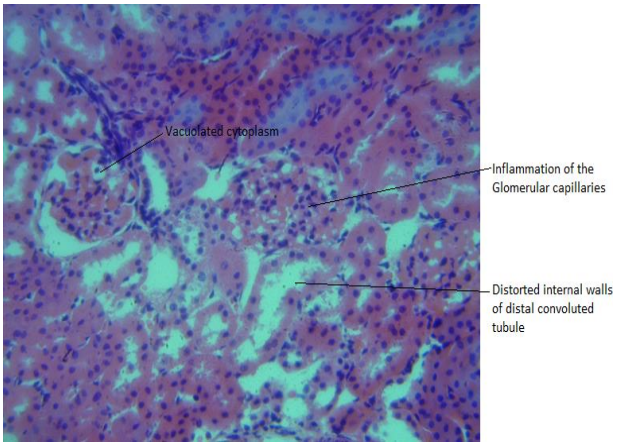
N= number of rats, [ab] =significant vs Neg. control, [be] = E significantly Vs B, [bc] = C significantly Vs B, NS= not significant

Table 4 shows results of total cholesterol (TC), triglycerides (TG), high density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C) in the rats after treatment. The results show there were no significant differences ($P > .05$) in total cholesterol and HDL-C levels in the negative control, compared to the treatment groups. There were no significant differences ($P > .05$) in triglyceride levels, except for group B (Low Dose Maggi) which significantly lower ($P < .05$) than the negative control. Also, there were no significant differences ($P > .05$) in low density lipoprotein cholesterol levels, except for group B (Low Dose Maggi) which significantly higher ($P < .05$) than the negative control. The results indicate lipid metabolism was relatively not affected by the administration of bouillon cubes. In similar studies, MSG exposed albino rats had significantly increased serum triglycerides, total and LDL cholesterol levels [26,28.29]. in many of these studies however, MSG was administered directly, not as seasoning cubes.

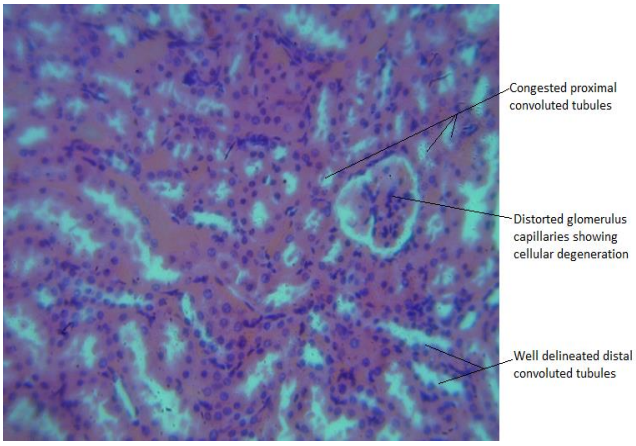
Histologic analysis of the kidney of the treated groups showed histological changes in the architecture of the tissues indicating tissue distortion, acute tissue damage, glomerular nephritis and distorted capillaries and degeneration compared to the negative control group which showed no tissue distortion. Group B (Low Dose Maggi) showed dilated glomerular capillaries indicating cellular hyperplasia and congested renal vessels. The tissues show hypertrophy of luminal epithelia of distal convoluted tubules. Group C (Low Dose Knorr) showed tissue damage. The glomeruli capillary cells show degeneration and cell death. Several vacuoles observed within the glomeruli. The High Dose Maggi group showed shrinkage of the glomerular capillaries with bowman's space. The glomerular cells appear pyknotic. Proximal and distal convoluted tubules appear normal. Glomerular nephritis is indicated. The High dose Knorr group showed distorted glomerular capillaries and cellular degeneration. The distal convoluted tubules show no distortion but mild congestions are observed in the proximal convoluted tubules. In our previous study, we reported changes in the architecture of the hepatocytes of the rats, indicating moderate dilation of the central vein, moderate disruption of the hepatic lobules, and mild sinusoidal dilation, after chronic administration of bouillon cubes [30]. In similar studies, Paul et al. [26] reported morphological changes in liver (central venous congestion, diffuse degeneration, necrosis of hepatocytes) and cortical tubular degeneration in the kidney of albino rats. In another study, MSG treated rats showed disorganized renal structure; shrunken glomerular tufts with dilatation of the capsular space, vacuolated cytoplasm of tubular cells with periglomerular fibrosis and interstitial nephritis. [31,32].



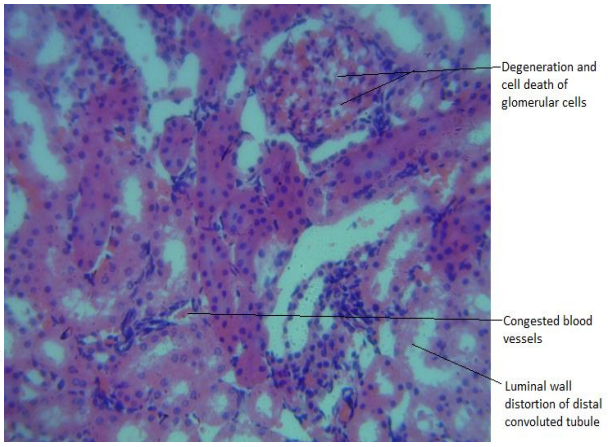
Group A (a)



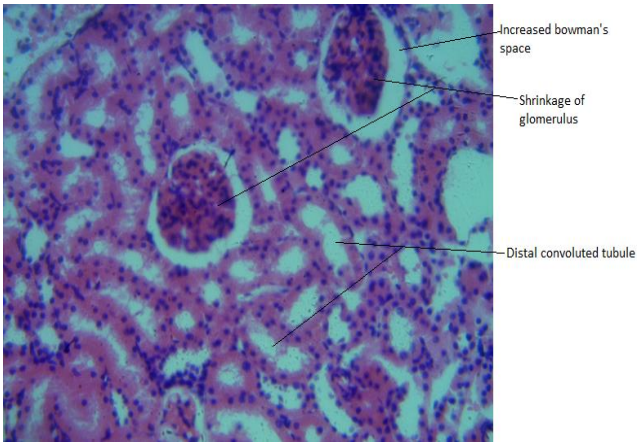
Group B (b)



Group C (c)



Group D (d)



Group E (e)

Figure 1(a), (b), (c), (d) and (e). Shows photomicrograph (X 400) of H&E-stained histologic sections of the Kidney of the rats. The negative control (a) shows normal histoarchitecture of the glomerular capillaries, Bowman's capsule and distal convoluted tubule. Kidney tissue showed no distortion. The Low Dose Maggi group (b) showed dilated glomerular capillaries indicating cellular hyperplasia and congested renal vessels. Tissues show hypertrophy of luminal epithelia of distal convoluted tubules. Tissue distortion (arrows). The Low Dose Knorr group (c) showed tissue damage. The glomeruli

capillaries cells show degeneration and cell death. Several vacuoles observed within the glomeruli. Acute tissue damage (arrows). The High Dose Maggi group (d) showed shrinkage of the glomerular capillaries with bowman's space. The glomerular cells appear pyknotic. Proximal and distal convoluted tubules appear normal. Glomerular nephritis is indicated. The High Dose Knorr group (e) showed distorted glomerular capillaries and cellular degeneration. The distal convoluted tubules show no distortion but mild congestions are observed in the proximal convoluted tubules.

4. CONCLUSION

Chronic exposure to bouillon cubes did not impact fasting plasma glucose, insulin and insulin resistance in the treated rats. Chronic administration of Knorr cubes impacted the integrity of the kidney as levels of cystatin C and sodium were altered in the albino rats. Histoarchitecture of the kidneys showed histological changes indicating tissue distortion, acute tissue damage, glomerular nephritis and distorted capillaries. Lipid profile/metabolism was relatively not affected by the administration of bouillon cubes. The usage of bouillon cubes should be moderated, considering their potential impact on long-term health and well-being. Local authorities need to verify the components of bouillon cubes to guarantee consumer safety.

Competing Interests

The authors have affirmed the absence of any competing interests.

Authors' Contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Limitations of the Study

This study was initially designed as a human study, however with issues surrounding the actual dose of the bouillon cubes consumed, and follow-up of human subjects in a resource limited setting, the animal study was adopted. Hence, human studies are encouraged to carried on the cubes. Although initial toxicity in animals is a better choice as it allows use of higher dosages, but follow-up human studies are needed to confirm the findings.

REFERENCES

1. Nwajei, J. C., Onuoha, S. C., & Essien, E. B. (2015). Effects of oral administration of selected food seasonings consumed in Nigeria on some sex hormones of Wistar albino rats. *IOSR Journal of Biotechnology and Biochemistry (IOSR-JBB), 1*(5), 15-21.
2. Nestle Nigeria. (2011). *Annual Report of Nestle Nigeria, 2011*. Retrieved from https://www.nestle-cwa.com/sites/g/files/pydnoa346/files/asset-library/documents/nestle_2011_annual_report_.pdf
3. Inuwa, H. M., Aina, V. O., Gabi, B., Aimola, I., & Ja'afuru, L. (2011). Determination of nephrotoxicity and hepatotoxicity of monosodium glutamate (MSG) consumption. *British Journal of Pharmacology and Toxicology, 2*(3), 148-153.
4. Umedum, N. L., Udeozo, I. P., Muoneme, O., Okoye, N., & Iloamaka, I. (2013). Proximate analysis and mineral content of three commonly used seasonings in Nigeria. *Journal of Environmental Science, Toxicology and Food Technology, 5*, 11-14.
5. Tandel, K. R. (2011). Sugar substitutes: Health controversy over perceived benefits. *Journal of Pharmacological Pharmacotherapy, 2*(4), 236-243.
6. Organisation for Economic Co-operation and Development. (2001). *Guidance document on acute oral toxicity testing: Environmental health and safety monograph series on testing and assessment No. 24*. Retrieved from https://ntp.niehs.nih.gov/iccvam/suppdocs/feddocs/oecd/oecd-gd24.pdf
7. Erhirhie, E. O., Ekene, N. E., & Ajaghaku, D. L. (2014). Guidelines on dosage calculation and stock solution preparation in experimental animals' studies. *Journal of Natural Sciences Research, 4*(18), 100–105.
8. Barham, D., & Trinder, P. (1972). An improved colour reagent for the determination of blood glucose by the oxidase system. *Analyst, 97*(151), 142-145.
9. Engvall, E., & Perlmann, P. (1972). Enzyme-linked immunosorbent assay, ELISA. *The Journal of Immunology, 109*(1), 129-135.
10. Matthews, D. R., Hosker, J. P., Rudenski, A. S., Naylor, B. A., Treacher, D. F., & Turner, R. C. (1985). Homeostasis model assessment: Insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia, 28*(7), 412-419.
11. Buck, R., & Lindner, E. (1994). Recommendations for nomenclature of ion selective electrodes (IUPAC Recommendations 1994). *Pure and Applied Chemistry, 66*(12), 2527-2536. Retrieved from https://doi.org/10.1351/pac199466122527
12. Bretaudiere, J. P., Phung, H. T., & Bailly, M. (1976). Direct enzymatic determination of urea in plasma and urine with a centrifugal analyzer. *Clinical Chemistry, 22*(10), 1614-1617.
13. Slot, C. (1965). Plasma creatinine determination. A new and specific Jaffe reaction method. *Scandinavian Journal of Clinical and Laboratory Investigation, 17*(4), 381-387. DOI: 10.3109/00365516509077065

14. Allain, C. C., Pooon, L. S., Cicely, S. G. C., Richmond, W., & Fu, P. C. (1974). Enzymatic determinants of total serum cholesterol. *Journal of Clinical Chemistry, 20*(4), 470–475.
15. Tietz, N. W. (1990). *A Clinical Guide to Laboratory Tests (2nd Ed.)*. Philadelphia: WB. Sanders.
16. Lopes-Virella, M. F., Stone, P., & Colwell, J. (1977). Cholesterol determination in high-density lipoproteins separated by three different methods. *Clinical Chemistry, 28*, 882–884.
17. Friedewald, W. T., Levy, R. I., & Fredickson, D. S. (1972). Estimation of the concentration of LDL cholesterol in plasma without the use of the preparative ultra-centrifugation. *Journal of Clinical Chemistry, 18*, 499–502.
18. American Public Health Association. (1995). *Standard methods for the determination of sodium using Agilent FS240AA Atomic Absorption Spectrophotometer. American Journal of Public Health*, 9th edition. Byrd Prepess Springfield, Washington DC.
19. World Health Organization (WHO). (2013). *Global Action Plan for the Prevention and control of Noncommunicable Diseases 2013-2020*.
20. Apkanyung, E. O. (2005). Proximate and mineral composition of bouillon cubes produced in Nigeria. *Pakistan Journal of Nutrition, 4*(5), 327-329.
21. Alonge, P. O., Idemudia, O. S., & Odokuma-Alonge, O. (2019). Direct assay of monosodium glutamate in multi-sourced bouillon cubes by first derivative potentiometric titration. *Journal of Applied Sciences and Environmental Management, 23*(2), 299-304.
22. Briggs, O. N., Elechi-Amadi, K. N., Ohaka, J. C., Nwachuku, E. O., & Bartimaeus, E. S. (2021). Effects of metformin in combination with a herbal capsule (Glucoblock) on insulin resistance and oxidative stress index in type 2 diabetic rats. *Journal of Complementary and Alternative Medical Research, 14*(3), 18-25.
23. Tchaou, M. N., Lamboni, C., Eklu-Gadegbeku, K., Abalokoka, E., & Aklikokou, K. A. (2013). Effects of Food Flavour Enhancer (Monosodium Glutamate and Maggi Poulet) Supplementation on Glucose Tolerance in Sprague Dawley Rat. *International Journal of Biological and Chemical Science, 7*(1), 161 – 171.
24. Husarova, V., & Ostatnikova, D. (2013). Monosodium glutamate toxic effects and their implications for human intake: a review. *The Journal of Medical Research, 1–12*. doi: 10.5171/2013.608765.
25. Zanfirescu, A., Ungurianu, A., Tsatsakis, A. M., Niţulescu, G. M., Kouretas, D., Veskoukis, A., Tsoukalas, D., Engin, A. B., Aschner, M., & Margină, D. (2019). A review of the alleged health hazards of monosodium glutamate. *Comprehensive Reviews in Food Science and Food Safety, 18*(4), 1111-1134. doi:10.1111/1541-4337.12448.
26. Paul, M. V., Abhilash, M., Varghese, M. V., Alex, M., & Nair, R. H. (2012). Protective effects of alpha-tocopherol against oxidative stress related to nephrotoxicity by monosodium glutamate in rats. *Toxicology Mechanisms and Methods, 22*(8), 625-630.
27. Tawfik, M. S., & Al-Badr, N. (2012). Adverse effects of monosodium glutamate on liver and kidney functions in adult rats and potential protective effect of vitamins C and E. *Food and Nutrition Sciences, 3*(5), 651-659.
28. Baky, N. A., Mohamed, A. M., & Faddah, L. M. (2009). Protective effect of N-acetyl cysteine and/or pro vitamin A against monosodium glutamate-induced cardiopathy in rats. *Journal of Pharmacology and Toxicology, 4*(5), 178–193.
29. Nahed, A. H., Amani, A. A., & Mamdouh, M. E. (2017). Effect of sesame on liver enzymes and lipid profile in rats exposed to oxidative stress induced by monosodium glutamate. *Journal of American Science, 13*(1), 71-78. doi:10.7537/marsjas130117.10.
30. Oodee, B. B., Briggs, O. N., Ben-Chioma, A. E., & Nwachuku, E. O. (2023). Effects of chronic administration of some bouillon cubes on oxidative stress and liver function markers in female albino rats. *World Journal of Biology Pharmacy and Health Sciences, 16*(1), 52-59.
31. Slima, S. R., & Ragab, R. (2023). Protective effect of curcumin against monosodium glutamate-induced oxidative renal damage: biochemical and histopathological study. *Ain Shams Journal of Forensic Medicine and Clinical Toxicology, 40*, 68-75.
32. Nnadozie, J. O., Chijioke, U. O., Okafor, O. C., Olusina, B. D., Oli, A. N., Nwonu, P. C., Mbagwu, H. O., & Chijioke, C. P. (2019). Chronic toxicity of low dose monosodium glutamate in albino Wistar rats. *BMC Research Notes, 12*(593), 1-7. https://doi.org/10.1186/s13104-019-4611-7