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Philemon C Anuforo
Department of Biochemistry, Michael Okpara University of Agriculture, Umudike, Umuahia Abia State, Nigeria

Anthony Cemaluk C Egbuonu
Department of Biochemistry, Michael Okpara University of Agriculture, Umudike, Umuahia Abia State, Nigeria

Elizabeth U Egu
Department of Nutrition and Dietetics, Michael Okpara University of Agriculture, Umudike, Umuahia Abia State, Nigeria

Correspondence:
Philemon C Anuforo
Department of Biochemistry, Michael Okpara University of Agriculture, Umudike, Umuahia Abia State, Nigeria
Email: philanfo@yahoo.com

Terminalia catappa flour extract mitigated monosodium glutamate intoxicated rats' kidney biofunction and histology

Philemon C Anuforo*, Anthony Cemaluk C Egbuonu, Elizabeth U Egu

ABSTRACT

Objective: This study investigated the *Terminalia Catappa* flour mitigated monosodium glutamate intoxicated rats' kidney biofunction and histology. **Materials and Methods:** Twenty-four (24) male albino Wistar rats with mean weight of 120.61±15.15 g were divided into six groups (n=4). Group 1, the normal control group (received distilled water), group II, the negative control (received 8mg MSG/g b.wt), group III, the extract control (received 300 mg extract/kg b.wt), group IV (received 8 mg MSG/g b.wt. + 100 mg extract/kg b.wt.), group V (received 8 mg MSG/g b.wt. + 300 mg kg-1 b.wt. extract) and group VI received 8 mg MSG/g b.wt. + 500 mg extract/kg b.wt). Treatment was administered daily by oral gavage for 14 days. Data were subjected to one-way ANOVA followed by Duncan *post-hoc* test at $p<0.05$ and means were estimated and significant differences noted. **Results:** The significantly ($p<0.05$) higher serum urea concentration of rats exposed to MSG-alone compared to other controls (group I and III), suggested an interference with normal kidney glomerular hyper filtration function. The significantly ($p<0.05$) lower serum urea concentration observed for group III rats administered extract alone implied the possible ameliorative potential of *T.catappa* extract on the functional capacity of the kidney. The serum creatinine concentration of the MSG-alone treated group was non-significantly ($p>0.05$) lower compared to all other groups. There was significant difference ($p<0.05$) between group III and few other groups, as observed for chloride concentration which suggests promising potential for *Terminalia catappa* endocarp flour ethanol extract (TCEFEE) to improving the renal functional capacity of the kidneys of rats exposed to high concentration of MSG. A match of the photomicrographs against the results of the renal biochemical parameters depicts possible correlations. **Conclusion:** Against the backdrop that both urea and creatinine are observed to increase when damage occurs in the kidney, this study could not affirm apparently a dysfunctionality of the kidney since the creatinine concentration rather reduced after exposing the rats to high concentration of MSG. However, the *Terminalia catappa* endocarp flour ethanol extract exhibited mitigated roles, thus improved the renal functional capacity of the kidneys of the rats.

Keywords: *Terminal catappa*, MSG-intoxication, Chinese restaurant syndrome, renal indices, histology.

INTRODUCTION

Flavouring systems are of utmost importance in savoury food manufacturing, playing an important nutritional role, especially in foods that are not very flavourful, thus providing the desired appeal [1]. Among various flavouring agents used in food manufacturing is monosodium glutamate (MSG). Monosodium glutamate is the sodium salt of glutamate, an amino acid widely occurring in nature. Glutamate is also produced in the body and plays an essential role in human metabolism [2]. The flavour enhancing capabilities of MSG has made it a widely consumed food additive, thus resulting to its possible inadvertent abuse [3]. The utilization of MSG as a food additive and the characteristic level of glutamate in foods are not toxicological concerns in humans. However, a prevalent view is that large dosages of MSG can cause headaches and other feelings of uneasiness, known as 'Chinese Restaurant Syndrome [1]. Thus, studies providing the evidence of MSG toxic effects have raised the increasing interest in MSG intake as flavor enhancer.

Studies on experimental animals have confirmed harmful impact of monodium glutamate in various organs, mainly expressed by increased oxidative stress, cytotoxicity, immunosuppression, reproductive toxicity, obesity, asthma and even autism [4]. The kidney has been reported to play critical roles in the excretion of toxins [5]. The kidney damage is marked by increase in both urea and creatinine concentrations [6-7]. The excretion of these biomarkers are made possible by the function of the glomerulus, hence, the glomerular filtration rate (GFR) is responsible for the changes in serum urea and creatinine [8].

Medicinal plants are rapidly growing in demand across the globe for their use as herbal drugs and natural health products [9-10]. The World Health Organization (WHO) has reported that about 80% of the populace in African nations depends on herbal medicine for their primary health care needs [11]. This has made plants not only indispensable in health care, but hope for future medicines [12]. The dependence on medicinal plants as source of medication has gained wide attention particularly among rural dwelling low income earners because of their affordability and availability [13].

Terminalia catappa Linn. (Combretaceae) is a tropical tree as well as a herbal medicinal plant commonly called Indian almond in English. Its tree can grow up to 35 m high and grows mainly in the tropical regions of Africa, Asia, and Australia [14]. It is well-known for its nutritional fruit and varied nutraceutical benefits whilst its leaves, roots and bark have been recommended for the treatment of various disease conditions [3, 15]. There is growing interest in the use of medicinal plants as biosources for bioactive components for ameliorating the adverse health conditions, thus, the need to investigate *Terminalia catappa* flour mitigated monosodium glutamate intoxicated rats' kidney biofunction and histology.

MATERIALS AND METHODS

Plant Materials and Authentication

Fruits of *Terminalia catappa* were harvested from College of Pure and Applied Sciences (COLPAS) of Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria. The plant was identified and authenticated by Mr. N. Ibe of Forestry and Environmental Management, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria.

Preparation of plant materials

The fresh fruits of *Terminali catappa* were washed, peeled (to remove the edible ectocarp), air dried at room temperature (to remove excess moisture), deshelled and the resultant shell (endocarp) milled. The ethanol extract was prepared by soaking 250 g of *Terminalia catappa* endocarp flour in 1 L of 95% ethanol for 72 h at room temperature with rigorous shaking. The mixture was filtered with Whatmann filter paper No. 1. The filtrate was then dried at a temperature of 50 ^{o}C in oven and stored in refrigerator for further use and percentage yield was calculated.

Animal studies

A total of 24 Wistar rats (male) having mean body weight of 120.61±15.15g were used in this experiment. Rats were bought from the animal house of University of Nigeria, Nsukka and housed in animal cage in the animal house of Department of Biochemistry, College of Natural Sciences, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria. After 7 days of acclimatization, they were equally divided into 6 groups of 4 rats each according to their weight in a completely randomized design. This study was carried out in accordance with ethical guidelines for animal welfare as approved by Biochemistry Department, College of Natural Sciences, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria. Rats in Group 1 (the control) were given only distilled water, Rats in group 2 were given only MSG (8mg/g) while rats in group 3 received only the ethanol extract (300mg/kg b.wt.) of *Terminalia catappa* milled endocarp. On the other hand, rats in Groups 4, 5 and 6 were co-treated with MSG (8mg/g b.wt.) and extract (100mg/kg, 300mg/kg and 500mg/kg b.wt.) respectively. The doses were calculated and adjusted based on the WHO recommended daily oral intake for an average person of 70kg. Exposure was per oral and lasted for 14 consecutive days.

Blood collection and preparation

At the end of experiment, the rats were anaesthesized in chloroform chamber and sacrificed and blood sample obtained by cardiac puncture using sterile plain tubes for renal function assays.

Evaluation of biochemical parameters

Determination of serum urea concentration: Urea concentration was determined using Urease Berthelot method as described by [16] based on the principle that urea in serum is hydrolysed to ammonia in the presence of urease and the ammonia measured spectrophotometrically on reacting with hypochlorite and phenol (Berthelot reaction) to form a blue coloured indophenol compound.

Determination of serum creatinine concentration: creatinine concentration was determined using direct endpoint method as described by [17]. The principle of this method is based on reaction of creatinine with picric acid in alkaline conditions to form a colour complex which absorbs at 510 nm. The rate of formation of colour is proportional to the creatinine concentration in the sample. In the endpoint method the difference in absorbance measurements after colour formation yields a creatinine value corrected for interfering substances.

Determination of chloride concentration

Serum chloride ion concentration was determined based on the colorimetric estimation of red colored complex formation from the reaction of the sample (or the standard chloride) and chloride reagent mixed and incubated at 25 ^{o}C for 5 mins, and read at 500 nm.

Determination of potassium ion concentration

Potassium ion concentration was determined using the turbidometric method as described by [17] based on the principle that the extent of turbidity is proportional to the potassium concentration as measured spectrophotometrically at 578 nm.

Histological examination

Kidneys of the sacrificed rats were identified and harvested. They were fixed in 10% buffered formalin for 72 hours. The tissues were then dehydrated in alcohol of graded concentrations and embedded in paraffin. The embedded tissues were cut into sections of 5µm thickness and these were stained with hematoxylin and eosin for photomicroscopic assessment.

Statistical analysis of data

The data were subjected to One-way analysis of variance (ANOVA) test and differences between the control group and extract treatment groups were determined by Duncan post-hoc test and presented as mean ± SEM. Results were considered to be statistically significant at $p<0.05$ at 95% confidence interval.

RESULTS

The results as shown on Table 1 revealed that chloride ion concentration in group III was significantly ($p<0.05$) lower when compared to group I, II and IV. The serum chloride ion concentration in group V was significantly ($p<0.05$) lower when compared to group I, II and IV. Also, serum chloride concentration was significantly ($p<0.05$) lower in group VI when compared to group I, II and IV.

Serum potassium ion concentration of the normal control rats exposed to MSG-alone treated group (group II) showed no significant ($p>0.05$) increase when compared to the normal control, while results of groups III, IV, V and VI showed no significant ($p<0.05$) decrease when compared to the MSG-alone group (group II).

Serum urea concentration of group I rats was significantly ($p<0.05$) lower when compared to group II, IV and VI. Serum urea concentration in group III was significantly ($p<0.05$) lower when compared to II, IV and VI. Also, serum urea concentration was significantly ($p<0.05$) lower compared to group II, IV and VI.

Serum creatinine concentration of rats exposed to MSG alone showed no significant ($p>0.05$) decrease when compared to the normal control group, while the extract group (group III) showed no significant ($p>0.05$) increase when compared to the MSG-alone group (group II). Groups IV, V and VI showed no significant increase in serum creatinine concentration when compared to both the normal control (group I) and MSG-alone treated group (group II).

Table 1: Effect of *Terminalia catappa* endocarp flour ethanol extract (TCEFEE) on renal indices of MSG intoxicated wistar rats.

Group	Chloride (mEq/L)	Potassium (mEq/L)	Urea (mg/dl)	Creatinine (mg/dl)
Group I (Normal Control)	79.68±7.66[a]	4.69±0.16[a]	47.25±3.07[b]	1.80±0.18[a]
Group II (MSG group)	78.61±5.30[a]	4.71±0.20[a]	78.00±1.83[a]	1.75±0.03[a]
Group III (extract group)	36.70±9.43[b]	4.65±0.16[a]	50.00±3.87[b]	1.83±0.13[a]
Group IV (MSG+100mg/kg extr.)	78.67±5.55[a]	4.65±0.09[a]	59.50±1.04[a]	2.00±0.03[a]
Group V (MSG+300mg/kg extr.)	60.39±7.85[b]	4.42±0.26[a]	55.25±5.38[b]	2.00±0.18[a]
Group VI (MSG+500mg/kg extr.)	48.08±12.44[b]	4.56±0.20[a]	65.00±15.11[a]	1.94±0.18[a]

Data are mean±S.E.M. (n=4). Mean in the same column with different superscript letters are significantly different, $p<0.05$ (One-Way ANOVA followed by Duncan post-hoc test).

Key: Group I = Normal Control
Group II = Negative Control (MSG-alone treated rats)
Group III = Extract Control (Extract-alone treated rats)
Group IV = MSG + 100mg/kg TCEFEE
Group V = MSG + 300mg/kg TCEFEE
Group VI = MSG + 500mg/kg TCEFEE

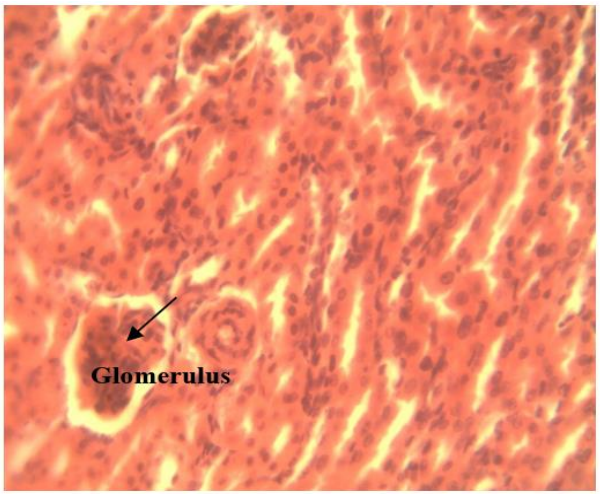

Plate 1: Photomicrograph of kidney section of Group I rats showing normal typical kidney cortex with intact glomerulus and intact tubules (proximal and distal convoluted tubules). H&E. mag. 400X.

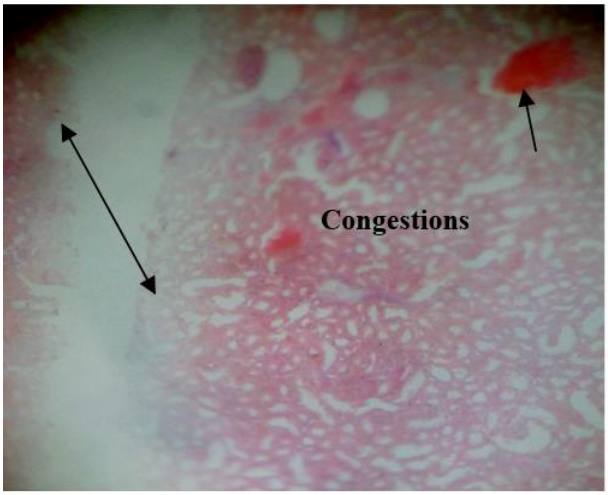

Plate 3: Photomicrograph of kidney section of Group III rats showing (1). minor congestion (2) dilatation of interstitium. (double arrow). H&E. mag. 100X.

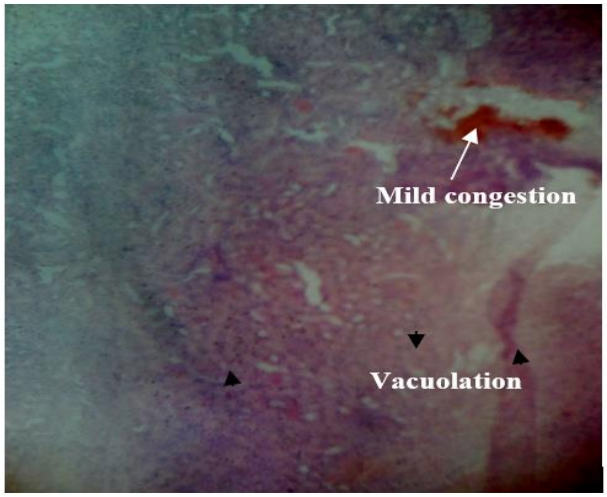

Plate 2: Photomicrograph of kidney section of Group II rats showing (1). Vacuolation (2). Mild congestion. H&E. mag. 100X.

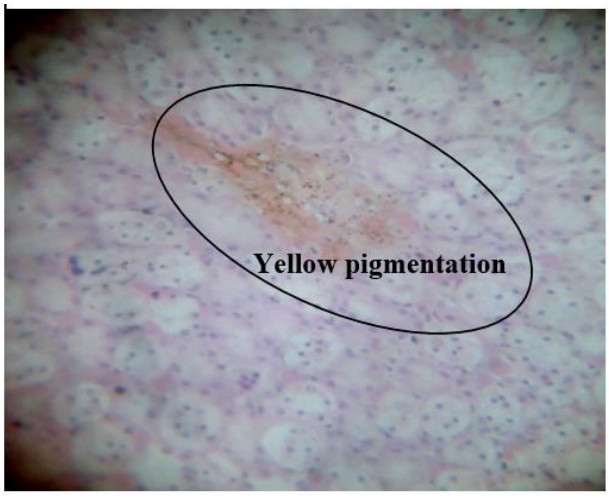

Plate 4: Photomicrograph of kidney section of Group IV rats showing (1). Intact tubules (2). Deposit of yellow pigments. H&E. mag. 400X.

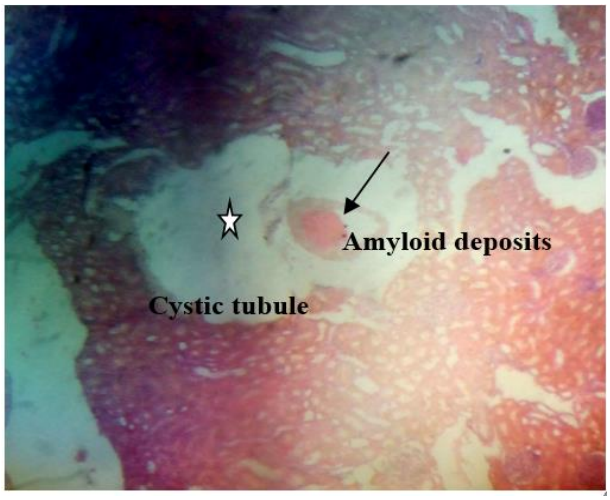

Plate 5: Photomicrograph of kidney section of Group V rats showing (1). Severe ovoid/or circular cystic tubules in the kidney medulla represents marked focal distention with cortical lesions observed in the medulla (star). However, the lumen contains deposits usually amyloid (black arrow). H&E. mag. 400X.

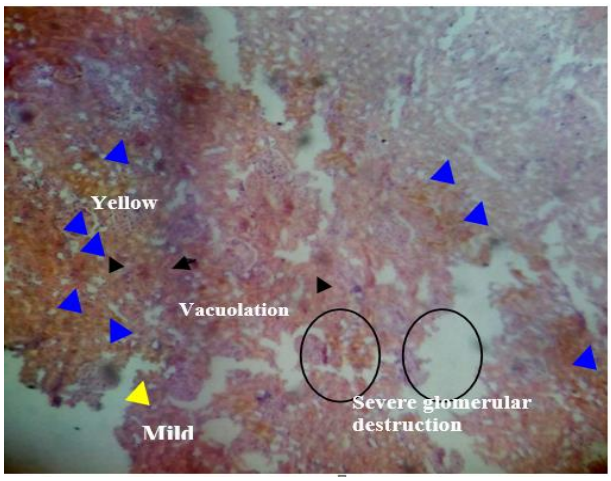

Plate 6: Photomicrograph of kidney section of VI rats showing (1). Severe glomerular destruction with loss of glomerular contents (circles) (2). Severe clear vacuolations suggests hydropic change. However, smaller, more uniform translucent vacuole suggests fat or lipoprotein accumulation (black arrow) (3). Severe intracytoplasmic accumulation of yellow granules pigmentations (blue arrow). (3) Lymphocytic infiltrates proliferations (red arrow) (4) mild tubular mineralization occurred due to replacement of tubule epithelial cells by granular basophilic deposits and is a consequence of tubule cell degeneration (yellow arrow). H&E. mag. 400X.

DISCUSSION

Urea and creatinine are important bio-indicators of renal function. Compromised kidney function suggests severe pathological state [18]. Increased serum urea and creatinine concentrations are considered for examining drug induced nephrotoxicity in animals and man [19].

The significantly ($p<0.05$) higher serum urea concentration of rats exposed to MSG-alone compared to other controls (group I and III), suggested an interference with normal kidney glomerular hyper filtration function. In renal disease, accumulation of serum urea is as a result of serum urea production exceeding the rate of its clearance. Increase in blood urea nitrogen (BUN) may reveal an increased catabolism of protein rather than decreased urinary excretion of urea [20]. Specifically, the MSG-induced effect on the serum urea concentration of the rats was as expected [21] and showed MSG-induced compromised renal function, which could be pathological state in the MSG-intoxicated rats [18]. The significantly ($p<0.05$) lower serum urea concentration observed for group III rats administered extract alone implied a possible ameliorative potential of *T.catappa* extract on the functional capacity of the kidney. This result agrees with previous report of [22] that the oral intake of MSG is linked to renal impairment. The lower serum urea concentration in the MSG-treated rats concurrently administered with variable concentrations of the extracts additionally suggests ameliorative role of the extract on the apparent MSG-induced adverse effect in rats. Urea is an excretion product from protein metabolism [7] and its amount is affected by protein consumption [8]. Actually, urea nitrogen is normally found in blood as waste nitrogen product that comes from food protein breakdown [7]. But, the urea concentration in blood increases beyond normal value due to a marked kidney failure. Formation of urea in body system is influenced by several factors, such as function of kidney, function of liver, protein intake, protein catabolism [23], and hydration status [24].

The serum creatinine concentration showed no increase in rats exposed to MSG alone. This agrees with the report of [25] who proposed that MSG does not alter the creatinine level. However, concomitant increase in both urea and creatinine concentrations was reported by [26] in a similar study where 8000 mg/kg or 8 mg/g b.wt. of monosodium glutamate was also used to intoxicate the rats. The serum creatinine concentration of the MSG-alone treated group was non-significantly ($p>0.05$) lower compared to all other groups. Creatinine increases proportionately with high muscle mass [28]. Also, the level of creatinine concentration depends on tubular secretion of serum creatinine. However, other factors which affect creatinine concentration in blood are age, sex, diet, body habits, and furrow [8].

The significantly ($p<0.05$) lower serum chloride ion concentration in the group exposed to extract alone compared to the normal control, MSG-alone treated, and MSG-extract co-treated groups showed a promising potential for *Terminalia catappa* endocarp flour ethanol extract (TCEFEE) to improving the renal functional capacity of the kidneys of rats exposed to high concentration of MSG. On the other hand, the serum potassium ion concentration of the group treated with extract alone when compared to the normal control and MSG-alone treated groups showed a non-significant ($p>0.05$) reduction which also suggested the ameliorative potential of *Terminalia catappa* endocarp ethanol extract to optimize electrolytes within the body.

Histological examination of the kidney showed vacuolation and congestion of renal blood vessels in the group treated with MSG alone (plate 2) compared to normal control group (plate 1) which showed normal architecture of kidney, while treatment with extract alone (plate 3) showed minor congestion suggesting that the extract had no adverse effect at 300mg/kg body weight of rats. Plate 4 showed intact tubules, suggesting that the co-administration of MSG with extract (100mg/kg body weight) had no adverse effect on the organ but rather showed ameliorative effect of the extract. Plates 5 and 6 showed diffused pigmentation and severe glomerular distribution with loss of glomerular content respectively, suggesting possible toxicity of the extract at the used concentrations (300mg/kg and 500mg/kg body weight of animal). A match of the photomicrographs against the results of the renal biochemical parameters apparently depicted possible correlations.

CONCLUSION

Against the backdrop that both urea and creatinine are observed to increase when damage occurs in the kidney, this study could not affirm apparently a dysfunctionality of the kidney since the creatinine concentration rather reduced after exposing the rats to high concentration of MSG. However, the *Terminalia catappa* endocarp flour ethanol extract exhibited mitigated roles, thus improved the renal functional capacity of the kidneys of the rat.

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