Pulverized Mangifera Indica (mango) Seed kernel Mitigated Monosodium Glutamate-Intoxicated rats’ Kidney Histology and Bio-functions

Anthony Cemaluk C Egbuonu* and Sandra O Oriji

Department of Biochemistry, College of Natural Sciences, Michael Okpara University of Agriculture Umudike, Abia State, Nigeria

Abstract

Monosodium Glutamate (MSG), used as a flavor enhancing food additive, may not be indicated on the label which could result to its inadvertent use and abuse with possible adverse effects in especially MSG sensitive individuals. The study evaluated some minerals and antioxidant vitamins in pulverized Mangifera indica seed kernel, and the effects of the crude ethanol extract of the pulverized M. indica seed kernel on monosodium glutamate (MSG)-intoxicated rats’ kidney using standard protocols. Sodium (390.96 mg/100g) followed by vitamin C (377.66 mg/100g) were higher (p<0.05) than the other determined minerals and antioxidant vitamins, respectively, in the M. indica seed kernel. Apart from the MSG group, the serum concentration of urea, albumin and creatinine were higher (p<0.05) in the extract group than in the control and MSG + high extract groups. Changes in the kidney histology of rats in the extract group were milder than in the MSG group and were comparable with those in the control. The study confirmed a definite MSG-intoxication of the kidney whereas M. indica seed kernel containing high sodium and vitamin C dose dependent mitigated the MSG-intoxication, on the rats’ kidney. Further studies are warranted to determine the interactive role(s) of sodium and vitamin C in the mango seed kernel on MSG-intoxicated rats’ kidney. Harnessing such studies could result to beneficial use of mango seed kernel in diet and drugs thereby reducing the hitherto waste status.

Keywords: Mangifera Indica Seed Kernel; MSG-Intoxication; Kidney Histology; Antioxidant Vitamins; Sodium

Introduction

Monosodium Glutamate (MSG) is used the world over as a unique flavor enhancing food additive [1] but may not be indicated on the label [2,3] which could result to inadvertent use and abuse with possible adverse effects in especially MSG sensitive individuals. The manufacturers of MSG maintained that it is safe for human consumption [4]. On the contrary, various research reports indicated monosodium glutamate-induced toxic influences in animals [5-8]. The mechanism of MSG intoxication is not fully known. However, since MSG is catabolyzed to glutamate, sodium and water [9], any or a combination of these catabolic products could be responsible for MSG intoxication in animals.

Mango is a very common tropical fruit-bearing plant that belongs to the genus Mangifera [10] and family Anacardiaceae [11]. Mango has varied health benefits associated with its various parts [11] and attributable to its varied phyto-constituent. For instance, mangiferin a phytoconstituent of mango had anti-diabetic activity in experimental animals [12]. Hence mango is of immense economic importance [10] and in particular, the mango fruit is extensively exploited for food, juice, flavor, fragrance and color [13]. As a consequence of the varied uses of mango fruit, mango seed that constitutes approximately 50 % of the total weight of mango fruit is generated as waste [14]. The disposal of such waste could contribute to environmental problems. However, mango seed could offer nutritional benefits because of its high carbohydrate and oil contents [13] as well as minerals and vitamins [15, 16]. In addition, mango seed could be useful as a therapeutic agent or as a drug. Dried and pulverized M. indica seed has been used in ethnomedicinal practices for managing diarrhea [17]. Essentially, M. indica seed is oblong-shaped and is made up of a stony endocarp that covers the soft kernel. The M. indica kernel may constitute up to 75 % of the seed and 20 % of the fruit [14]. The M. indica seed kernel, usually discarded along with the seed after consuming the fleshy part of the fruit, could serve dietary and pharmacologic purposes, warranting this study.

The kidneys are one of the major organs that function to remove toxic metabolites and waste products from the blood and to maintain fluid and electrolytes balance in the body [6]. Thus, the kidneys play a vital role in the elimination of many metabolic waste products resulting from many xenobiotics, including MSG. And, any damage to the kidney could adversely affect the normal functioning of the body. It is worthwhile investigating the influence of M. indica seed kernel on MSG-induced toxicity on the kidney. Thus, this study investigated some minerals and antioxidant vitamins in Mangifera indica (mango) seed kernel and the effects of the ethanol extract of the seed kernel on the kidney...
histology and some bio-functions of normal and monosodium glutamate (MSG)-challenged rats.

**Experimental**

**Plant material and extract**

This study was conducted between June and August, 2016. Fresh mango fruits collected from a particular mango tree were purchased in June, 2016 at Orije ugba, a fruit and foodstuff market in Umuahia, Abia state. The mango fruits were identified and authenticated as Mangifera indica (German variety) by a taxonomist in the department of Plant Science and Biotechnology, Michael Okpara University of Agriculture Umudike, Nigeria.

The German variety of mango fruits was thoroughly washed with tap water. The fleshy part of each fruit was removed to obtain the seed stones which were sun-dried for three days. The sun-dried seed stones were carefully cut with clean table knife to remove the stony seed coat and obtain the seed kernels. The kernels thus obtained were chopped using home choice knife into bits and sun-dried for one week (seven days). The dried mango seed kernels were pulverized using Arthur Thomas Laboratory Mill, Crypto Model, USA. Two hundred grams (200 g) of the pulverized mango seed kernel was extracted with 800 ml of 98 % ethanol as solvent as described earlier [18]. The resulting extract was centrifuged and filtered through Whatman number one filter paper to obtain the filtrate which was allowed to evaporate to dryness using a rotary evaporator maintained at 40 °C. An approximate value of 34 g was obtained and was stored in a refrigerator at 4 °C until used.

**Chemicals and reagents**

A commercially available brand of MSG (99.9 % purity) used in this study was procured from Ubani market, a daily food condiments market in Umuahia, south east Nigeria. Ethanol, formalin, picric acid, Bouyer's reagent, chloroform, ammonia, sodium hypochlorite, phenol, sodium nitroprusside, sodium hydroxide, urea, sodium borate, oxalic acid, trichloroacetic acid, ferric chloride, tetraoxo sulphate VI acid, perchloric acid and all chemicals were procured from the chemical store of the department of Biochemistry, Michael Okpara University of Agriculture as certified analytical grade and were used without further purification.

**Animals**

Twenty adult male albino rats (weight range, 104 – 170 g) used in this study were procured from the animal house of the Faculty of Biological Sciences, University of Nigeria, Nsukka. The rats were acclimatized for 2 weeks and randomized (based on weight) to five groups with sample size of four rats. The animals housed in clean and dry iron cages at room temperature 25°C, a normal daylight/dark cycle and humid tropical conditions. The animals as sought and approved by the Ethical Committees of the Department of Biochemistry and College of Natural Sciences, Michael Okpara University of Agriculture Umudike, Nigeria.

After 2 weeks exposure, the rats were sacrificed the next day after overnight fast by cardiac puncture technique [19-21] and the blood sample of the respective rats was collected individually into clean polystyrene tubes. The blood samples thus collected were respectively centrifuged at 3000 rpm for 10 minutes. The resultant sera were respectively collected into polystyrene tubes and stored in deep freezer for the determination of serum creatinine, urea and albumin concentrations.

**Determination of the antioxidant vitamins (A, C and E)**

Vitamin A was determined by the spectrophotometric method described earlier [22, 23], whereas vitamin C (ascorbic acid) was determined by the method described by Okwu and Josiah [24]. Vitamin E in the M. indica seed kernel was determined by the method of Pearson [25]. Triplicate determinations were carried out and the mean calculated.

**Determination of minerals (zinc, iron, sodium)**

Sodium (Na), zinc (Zn) and iron (Fe) in the M. indica seed kernel were variously determined according to the methods described by the Association of Analytical Chemists [26]. Triplicate determinations were carried out and the mean calculated.

**Determination of serum urea concentration**

Urea concentration was determined using urease Berthelot according to the method of Fawcett and Scott [27]. This method was based on the principle that ammonia (from the urease catalyzed hydrolysis of urea to ammonia and carbon dioxide) is converted to coloured indophenols blue in the presence of sodium nitrofenryanide-phenol and hypochlorite reagents. The absorbance of the indophenols blue was read with a spectrophotometer (Jenway Digital Spectrophotometer, Model 6320D, manufactured by Jenway Equipment Company, France) set at 625 nm.

**Determination of serum creatinine concentration**

Creatinine concentration in the rats’ serum was determined by the Direct Endpoint method according to Henry [28]. This was based on the principle that creatinine reacts with picric acid in


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alkaline conditions to form a coloured complex which absorbs at 510 nm. The intensity of the colour formed is proportional to the creatinine concentration in the sample and is measured with a spectrophotometer.

**Determination of serum albumin concentration**

Albumin concentration in the rats' serum was determined using Bromocresol Green (BCG) according to the method of Doumas et al. [29]. This was based on the principle that serum albumin binds to the indicator 3,3',5,5'-tetrabromo-m-cresolsulphonephthelain (bromocresol green, BCG) to form the albumin-BCG-complex that absorbs maximally at 578 nm. The absorbance as measured with a spectrophotometer is directly proportional to the concentration of albumin in the sample.

**Histopathological examination of the kidney sections**

On sacrifice, sections of the rats' kidneys collected for histopathological examination were fixed in 10 % phosphate buffered formalin for 48 hours, trimmed, dehydrated in 4 grades of alcohol (70 %, 80 %, 90 % and 100 % or absolute alcohol), cleared in 3 grades of xylene and embedded in molten wax. On solidifying, the blocks were sectioned into 5 μm thickness with a rotary microtome, floated in water bath and incubated at 60 °C for 30 minutes. The 5 μm thick kidney sections were subsequently cleared in 3 grades of xylene and rehydrated in 3 grades of alcohol (90 %, 80 % and 70 %). The sections were then stained with Hematoxylin for 15 minutes and blued (stained blue) with ammonium chloride. Differentiation was done with 1 % acid alcohol before counterstaining with Eosin. Permanent mounts were made on degreased glass slides using a permanent DPX mountant (Model 44581, Sigma-Aldrich, United Kingdom).

The prepared slides were examined with a Motic™ compound light microscope at various magnifications (x4, x10 and x40) of the objective lenses. The photomicrographs were taken using a Motic™ 9.0 megapixels microscope camera at x100 and x 400 magnifications.

**Statistical analysis**

All numerical data collected were analyzed by one way analysis of variance (ANOVA) using the statistical package for Social Science (SPSS version 17; SPSS Inc., Chicago, IL, USA). Result were presented as means ± standard error of the mean (Mean ± SEM at 95 % significance level (p < 0.05).

**Results**

The result of some minerals and antioxidant vitamins (mg/100 g) in the pulverized *M. indica* seed kernel was shown on Table 1 and revealed that sodium (390.96 mg/100 g) was the highest followed by vitamin C, zinc, iron and vitamin A while the least was vitamin E.

As shown on Table 2, the urea concentration in the rats exposed to MSG alone was higher (p<0.05) than that in the control and in the other groups while the urea concentration in the rats exposed to the extract alone was higher (p<0.05) than that in the control and in the rats exposed to MSG together with higher extract concentration. The observation relative to the control was highest (70.74 %) in the rats exposed to MSG alone and least (18.02 %) in the rats exposed to MSG together with highest concentration of the extract. The observed change relative to the MSG group was highest in rats in the control group (41.43 %) followed by those in the MSG + highest extract group (30.88 %).

The result as shown on Table 3 revealed that the serum albumin concentration in the rats exposed to MSG alone was higher (p<0.05) than that in the control and in the other groups while the albumin concentration in the rats exposed to the extract alone was higher (p<0.05) than that in the control and in the rats exposed to MSG together with higher extract concentration. The observation relative to the control was highest (45.56 %) in the rats exposed to MSG alone and least (10.03 %) but reversed by 12.03 % in the rats exposed to MSG together with low and highest concentration of the extract, respectively. The observed change.
relative to the MSG group was highest in rats in the MSG + highest extract group (39.57 %) followed by those in the control group (31.29 %).

As shown on Table 4, the serum creatinine concentration in the rats exposed to MSG alone was higher (p<0.05) than that in the control and in the other groups while the creatinine concentration in the rats exposed to the extract alone was higher (p<0.05) than that in the control but lower than that in the rats exposed to MSG together with either low or high extract concentration. The observation relative to the control was highest (66.67 %) in the rats exposed to MSG alone and least (25.76 %) in the rats exposed to MSG together with highest concentration of the extract. The observed change relative to the MSG group was highest in rats in the control (40.00 %) followed by those in the MSG + highest extract group (25.55 %).

Effect of M. indica (mango) seed kernel extract on the kidney histology of normal and monosodium glutamate-intoxicated rats

Photomicrographs from sections of the kidney collected from the animals in the control group showed the normal renal histo-architecture for laboratory rats. The sections showed normal Glomeruli (G) in their Bowman’s capsules embedded in a framework of normal renal tubules (proximal convoluted tubules, distal convoluted tubules, pars recta and collecting ducts) and normal renal interstitium, renal tubules (T) (Plate 1). Sections of the kidney collected from the rats exposed to MSG (8 g/kg b.w) showed a mild to moderate multifocal degeneration of the renal tubular epithelial cells (arrow) in the cortex and inner medulla. Affected tubular epithelial cells have numerous clear cytoplasmic vacuoles. Also, multifocal areas of intense infiltration of the interstitium by inflammatory mononuclear leucocytes were observed (Plate 2).

Sections of the kidney collected from the rats in the extract (300 mg/kg b.w) group showed the normal renal histo-architecture for laboratory rats. The sections showed normal Glomeruli (G) in their Bowman’s capsules embedded in a framework of normal renal tubules (proximal convoluted tubules, distal convoluted tubules, pars recta and collecting ducts) and normal renal interstitium, renal tubules (T) (Plate 3). Sections of the kidney collected from the rats in the MSG (8 g/kg b.w) plus extract (200 mg/kg b.w) group showed a mild to moderate multifocal degeneration of the renal tubular epithelial cells (Black arrow) in the cortex and inner medulla. Compare with the normal tubules (white arrow), Glomerulus (G); Affected renal tubules (T) (Plate 4).

Sections of the kidney collected from the rats in the MSG (8 g/kg b.w) plus extract (400 mg/kg b.w) group, just as observed above, showed a mild to moderate multifocal degeneration of the renal tubular epithelial cells (Black arrow) in the cortex and inner medulla. Affected cells are swollen and show multiple cytoplasmic vacuoles. Compare with normal tubules (white arrow), Glomerulus (G); Affected renal tubules (T) (Plate 5).

**Discussion**

The M. indica (mango) seed kernel, usually discarded along with the seed after consuming the fleshy part of the fruit, could contain dietary minerals and vitamins and could affect food condiment-induced adverse effects in animals. Thus, this study investigated some minerals and antioxidant vitamins in Mangifera indica (mango) seed kernel and the effects of the ethanol extract of the seed kernel on kidney histology and some kidney bio-functions of normal and monosodium glutamate (MSG) challenged rats. The study revealed the preponderance of the determined minerals and antioxidant vitamins although sodium (390.96 mg/100 g) was higher than the other determined minerals and antioxidant vitamins in the M. indica seed kernel followed by vitamin C (377.66 mg/100 g). The result suggests that M. indica seed kernel could be a good dietary source for these minerals and antioxidant vitamins which are of physiologic importance [30, 31].

The iron content (90.66 mg/100 g) in the Mangifera indica seed kernel was higher than, hence not comparable with, that (11.90 mg/100 g) reported by Fowomola [15] but for mango (M.}

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**Table 3:** Effect of M. indica (mango) seed kernel extract on the serum albumin concentration (mmol/l) of normal and monosodium glutamate-intoxicated rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Albumin (mmol/l)</th>
<th>Change relative to the control (%)</th>
<th>Change relative to MSG group (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (distilled water 2 ml/kg b.w)</td>
<td>3.49±0.37</td>
<td>0</td>
<td>−31.29</td>
</tr>
<tr>
<td>MSG (8 g/kg b.w)</td>
<td>5.08±0.11</td>
<td>45.56</td>
<td>0</td>
</tr>
<tr>
<td>Extract (300 mg/kg b.w)</td>
<td>3.68±0.17</td>
<td>5.44</td>
<td>−27.56</td>
</tr>
<tr>
<td>MSG (8 g/kg b.w + Extract 200 mg/kg b.w)</td>
<td>3.84±0.09</td>
<td>10.03</td>
<td>−24.41</td>
</tr>
<tr>
<td>MSG (8 g/kg b.w + Extract 400 mg/kg b.w)</td>
<td>3.07±0.12</td>
<td>−12.03</td>
<td>−39.57</td>
</tr>
</tbody>
</table>

Value presented as mean ± SEM of sample size, n = 4 rats. + denotes higher by; − denotes lower by. Significant difference at p<0.05

**Table 4:** Effect of M. indica (mango) seed kernel extract on the serum creatinine concentration (mmol/l) of normal and monosodium glutamate-intoxicated rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Creatinine (mmol/l)</th>
<th>Change relative to the control (%)</th>
<th>Change relative to MSG group (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (distilled water 2 ml/kg b.w)</td>
<td>1.32±0.17</td>
<td>0</td>
<td>−40.00</td>
</tr>
<tr>
<td>MSG (8 g/kg b.w)</td>
<td>2.20±0.16</td>
<td>66.67</td>
<td>0</td>
</tr>
<tr>
<td>Extract (300 mg/kg b.w)</td>
<td>1.75±0.12</td>
<td>32.58</td>
<td>−20.45</td>
</tr>
<tr>
<td>MSG (8 g/kg b.w + Extract 200 mg/kg b.w)</td>
<td>1.78±0.19</td>
<td>34.85</td>
<td>−19.09</td>
</tr>
<tr>
<td>MSG (8 g/kg b.w + Extract 400 mg/kg b.w)</td>
<td>1.66±0.33</td>
<td>25.76</td>
<td>−25.55</td>
</tr>
</tbody>
</table>

Value presented as mean ± SEM of sample size, n = 4 rats. + denotes higher by; − denotes lower by. Significant difference at p<0.05
Indica) whole seed. Generally, minerals reported here (sodium, zinc and iron) were higher than the corresponding value reported by Yatnatti et al. [14] for Totapuri variety of Mangifera indica. These may be related to the mango variety used and/or whether the whole seed was used. Mango seed comprises the outer hard part and the inner tender part (kernel). The present study used the German variety and only the kernel part of mango seed. In particular, aside difference in variety, such variation in the minerals of Mangifera indica seed kernels could be due to soil type and prevailing environmental conditions [14].

Zinc and iron contents (Table 1) were quite higher than the range 1.25 – 10.13 mg/100 g and 4.63 – 7.08 mg/100 g respectively recorded in Citrullus lanatus rind and seed [32]. Sodium in the sample (Table 1) was higher than the range (0.33 - 19.44 mg/100 g) for seeds and peels [33], (4.10 – 7.73 mg/100 g) for stem, leaf and fruit of tomato and garden egg [34 Citrus sinesis] and (7.01 – 7.74 mg/100 g) for Amaranthus hybridus and Telfairia occidentalis leaves [35]. Vitamin C in the sample was higher than that reported for Citrus sinesis peels and seeds [33] and the range (2.23 – 12.97) obtained for stem, leaf and fruit of tomato and garden egg [34]. Vitamin A content in the study sample ((18.94 mg/100g) was below the range (56.00 – 267.33 mg/100 g) recorded for stem, leaf and fruit of tomato and garden egg [34] and the value (85.71±0.63 IU) in Citrus sinensis peels [33], but compared with that in the seeds (22.51 IU) of Citrus

Plate 1: Photomicrograph of sections of kidney of the rats in the control group showing normal renal histo-architecture for normal rats. H&Ex400.

Plate 2: Photomicrograph of sections of kidney of the rats in the MSG (8 g/kg b.w) group showing a mild to moderate multifocal degeneration of the renal tubular epithelial cells (arrow) in the cortex and inner medulla. H&Ex400.

Plate 3: Photomicrograph of sections of kidney of the rats in the Extract (300 mg/kg b.w) group showing the normal Glomeruli (G) in their Bowman’s capsules and normal renal tubules (T) indicative of normal renal histo-architecture for laboratory rats. H&Ex400.

Plate 4: Photomicrograph of sections of kidney of the rats in the MSG (8 g/kg b.w) plus extract (200 mg/kg b.w) group showing mild to moderate multifocal degeneration of the renal tubular epithelial cells (Black arrow) in the cortex and inner medulla. Affected cells are swollen and show multiple cytoplasmic vacuoles (T). H&Ex400.

Plate 5: Photomicrograph of sections of kidney of the rats in the MSG (8 g/kg b.w) plus extract (400 mg/kg b.w) group showing mild to moderate multifocal degeneration of the renal tubular epithelial cells (Black arrow) in the cortex and inner medulla. Affected cells are swollen and show multiple cytoplasmic vacuoles (T). H&Ex400.
Possible nephroprotective effect of MSG at low concentration was reported earlier [37]. However, several other studies reported adverse effects of higher concentration of MSG on the kidney [5-7]. In the present study, MSG at 8 g/kg body weight compromised the rats’ kidneys in support of earlier study [8] and as indicated by the lesions on the kidney and higher (p<0.05) urea, albumin and creatinine concentrations in the MSG group compared to the control and the other groups. The urea concentration in the rats exposed to the extract alone was higher (p<0.05) than that in the control and in the rats exposed to MSG together with higher extract concentration. A similar trend was recorded for albumin and creatinine concentrations in the rats. Furthermore, the observation on these kidney function parameters (serum urea, albumin and creatinine concentrations) relative to the control was highest in the rats exposed to MSG alone and either least or even reversed in the rats exposed to MSG together with highest concentration of the extract. On the other hand, the observed change relative to the MSG group was either highest in rats in the control group followed by those in the MSG + highest extract group or vice versa. These results while paradoxical confirmed MSG-induced intoxication of the rats’ kidneys, suggested apparent extract-induced adverse influence on the rats’ kidneys and indicated that the extract at higher dose apparently interacted with MSG to reduce the respective MSG and extract-induced effects on the rats’ serum urea, creatinine and albumin concentrations. Higher serum urea and creatinine concentrations suggested impaired kidney functions, depletion of intracellular arginine concentration and inhibition of protein synthesis [38], and impaired functional capacity of the kidneys to filter fluids [39].

The rats’ kidney histology was assessed to collaborate the serum chemistry results [40-42]. The kidney histology of rats fed M. indica seed kernel extract at 300 mg/kg body weight was comparable to that of the control group, a pointer that the observed higher serum urea, albumin, creatinine concentrations in the rats fed the extract alone at the tested concentration did not elicit definitive adverse effects on the rats’ kidneys histology. As expected, the lesions in the kidney histology of rats in the MSG group on comparison with those in the control, extract and MSG + extract groups confirmed a definite MSG-related intoxication of the rats’ kidneys. However, the lesions observed in the MSG plus extract co-treated groups were milder than that in the MSG group indicating extract-related mitigation of MSG-induced adverse effects on the rats’ kidney histology. This is supported by the dose dependent effects of the extract on MSG-intoxication as shown in the serum chemistry results, confirming that at a higher concentration the extract sufficiently protected the rats’ kidney from MSG-intoxication.

We speculated that interactive mechanisms probably involving sodium and the antioxidant vitamins in the mango seed kernel could explain the paradoxical responses of mango seed kernel. For instance, the high sodium content in the M. indica seed may explain its similar effect on the serum kidney function parameters as the MSG group, suggesting that like MSG its intoxication among other possible mechanisms may be via the release of free sodium ion. Further to this, the high vitamin C content, a potent antioxidant, in the M. indica seed may explain the apparent capacity of M. indica seed kernel to mitigate the MSG-intoxication in the rats’ kidney. Manal and Nawal [6] reported that vitamins C and E ameliorated MSG-induced adverse effects on rats’ kidney functions. Also, vitamin C given simultaneously with MSG for 14 days protected against MSG-induced toxic effects in male albino rats [43]. Further studies are thus warranted as the results and the discussion thereto point to the apparent fundamentality of sodium in MSG-intoxication, an antioxidant vitamins-related modulation and protective potential of the higher doses of mango seed kernel extract against MSG-intoxication on the rats’ kidney histology and bio-functions.

In conclusion, M. indica seed kernel containing high sodium and vitamin C elicited non-definite adverse effects, but dose dependently mitigated the MSG-intoxication on the rats’ kidney histology and some bio-functions. Further studies are warranted to determine the interactive role(s) of sodium and vitamin C in (particularly at higher concentration) of the mango seed kernel on MSG-intoxicated rats’ kidney. Harnessing such studies could result to beneficial use of mango seed kernel in diet and drugs thereby reducing the hitherto waste status.

**Ethical consideration**

This study on the evaluation of biochemical effects of mango seed kernel extract on normal and monosodium glutamate-intoxicated experimental models considered and adhered to the standard ethical use of experimental animals approved by the Department of Biochemistry, Michael Okpara University of Agriculture Umudike, Nigeria.

**References**


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