

Pulverized *Mangifera indica* (Mango) Seed-kernel Modulated Serum Lipid Profile in Monosodium Glutamate-challenged Rats

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Abstract

Mangifera indica seed kernel was assessed for antinutrient content and effects on serum lipid profile of monosodium glutamate-challenged rats. Group A rats received 8000 mg/kg body weight, b.w of monosodium glutamate (MSG). Group B rats received the sample extract (300 mg/kg b.w). Group C rats were sham-administered distilled water. Groups D and E rats received 8000 mg/kg b.w of MSG in addition to 200 and 400 mg/kg b.w, respectively of sample extract. Tannin (29.21 ± 5.39 mg/100 g) was highest ($p < 0.05$) while phytate (0.38 ± 0.02 mg/100g) was least. After fourteen days daily oral exposure, MSG-exposed rats had higher ($p < 0.05$) total cholesterol (CHOL), triacylglycerol (TAG) and low density lipoprotein (LDL) but lower ($p < 0.05$) high density lipoprotein (HDL) concentration compared to rats in the other groups. MSG-intoxicated rats co-treated respectively with 200 and 400 mg/kg b.w of the extract had dose dependent reduction ($p < 0.05$) compared to the corresponding effects in non-extract-co-treated MSG-intoxicated rats. Compared to others, rats in the Extract group had higher ($p < 0.05$) CHOL: TAG but lower ($p < 0.05$) TAG:CHOL ratios while rats in the MSG-group had higher ($p < 0.05$) HDL:LDL but lower ($p < 0.05$) LDL:HDL ratios. Results demonstrated the preponderance of tannin in, and significant serum lipid lowering potential in normal and MSG-challenged rats of, the sample. Studies using

antinutrient-process-reduced sample in hyperlipidemia-related health-challenged models and collaborating the suggested implication of lipid-profile-component-ratios are warranted and recommended.

Keywords: Tannins, Phytates, Trypsin inhibitor, Cholesterol-triacylglycerol ratio, High density lipoprotein-low density lipoprotein ratio

1. Introduction

Monosodium glutamate (MSG), a flavor enhancing food additive (Husarova & Ostatnikova, 2013; Veni et al., 2010) which could be abused owing to its non-indication on the labels of most packaged foods (Egbonu, 2012; Egbonu et al., 2009a) was reportedly toxic in animals (Manal & Nawal, 2012; Egbonu et al., 2010b,c,d; Mariyamma et al., 2009). Thus, its safety has generated much controversy. Monosodium glutamate, as the name suggests, has a proton or hydrogen moiety at the carboxylic part of glutamic acid substituted by a sodium ion. And, in physiological condition MSG is expected to dissociate into free glutamate and sodium ion. This implies that the reported adverse effects of MSG could be exerted by MSG or any of its dissociation products. For instance, glutamate, an amino acid but an excitotoxin, is implicated in both physiological and pathological processes (Mattson 2008) mediated *via* glutamate receptors (Collison et al., 2012).

Lipid profile including total cholesterol, LDL-cholesterol, HDL-cholesterol and triacylglycerol are important diagnostic tools, and effects of MSG ingestion on lipid profile have been reported. Recently, Okediran et al. (2014) reported a significant increase in the plasma total cholesterol and LDL-cholesterol but a non-significant decrease in TAG and HDL-cholesterol in MSG-treated rats compared to control. This was supported by earlier report suggesting attendant dysfunctions related to impaired lipid metabolism including diabetes and cardiovascular diseases (Egbonu et al., 2010d; Schummer et al., 2008). The increasing need for herbs and foods apparently heightened by the additional health-burden caused by food additives, notably monosodium glutamate (Egbonu & Osakwe, 2011; Egbonu et al., 2010a,b,c; Egbonu et al., 2009a) necessitate increasing search for plants and plant parts with nutraceutical potentials (Egbonu, 2015a; Egbonu & Nwankwo, 2012).

Mango, a common and an economically important tropical fruit-bearing plant belongs to the genus *Mangifera* (Zahedi et al., 2016) and family *Anacardiaceae* (Shah *et al.*, 2010). Mango plant parts have health benefits associated with varied phyto-nutrients including mangiferin (Shah et al., 2010; Li et al., 2010; Ajila & Prasada-Rao, 2008). Reports of pharmacological activities of *M. indica* plant parts abound and include hypolipidemic (Jagetia et al., 2005), anti-microbial (Shabani & Sayadi, 2014; Vega-Vega et al., 2013), anti-diabetic (Bhowmik et al., 2009), anti-proliferative (Timsina et al., 2015; Ali et al., 2012; Noratto et al., 2010) and nephroprotective (Amien et al., 2015) activities. Therefore, *M. indica* (mango) fruit is extensively exploited for food and drugs resulting to the generation of mango seed as waste (Yatnatti et al., 2014). In a recent study, *M. indica* seed kernel usually discarded along with the mango seed mitigated monosodium glutamate-intoxicated rats' kidney histology and bio-functions (Egbonu & Oriji, 2017).

The pharmacologic utilization of *M. indica* seed kernel, as other plant-sourced foods, could be limited by diverse natural compounds (Shanthakumari et al., 2008) including antinutrients .

For instance, tannins and phytates are known to limit the nutrient value of plant foods by chelating (binding and precipitating) many organic compounds including amino acids, proteins, irons and alkaloids (Gemede & Ratta, 2014; Soetan & Oyewole, 2009). Thus, this study aimed at determining some antinutrients in the mango seed kernel and the effects of the mango seed kernel extract on the serum lipid profile of normal and monosodium glutamate-challenged rats. The objectives of the study set to achieve the aim as stated were to determine the tannins, phytate and trypsin inhibitor contents of *Mangifera indica* seed kernel and to evaluate the effects of the extract of *Mangifera indica* seed kernel on the serum lipid profile (CHOL, TAG, LDL and HDL concentration as well as CHOL: TAG, TAG: CHOL, HDL: LDL and LDL: HDL ratios) of normal and monosodium glutamate-challenged rats.

2. Materials and Methods

2.1 Sample Procurement and Identification

A commercially available brand of MSG (99% purity) was procured from *Ubani* market, a daily food condiments market in Umuahia, south east Nigeria. Ethanol, sodium carbonate, tannic acid, hydrochloric acid, ammonium thiocyanate, iron chloride, 4-aminoantipyrine, sodium cholate p-hydroxybenzenesulfonate and other chemicals used were certified analytical grade and products of reputable companies. They were purchased from reputable chemical dealers and were used without further purification.

This study was conducted between June and August, 2016. Fresh mango fruits collected from a particular mango tree were purchased in June, 2016 at *Orie 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.

2.2 Sample Preparation and Extraction

The mango fruits (German variety) were thoroughly washed using 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 with 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. The pulverized mango seed-kernel was extracted with ethanol (98%) as described earlier (Egbuonu, 2015b) and stored in a refrigerator at 4 °C until used.

2.3 Animal Study

2.3.1 Animal Procurement and Exposure Groups

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 animals were acclimatized for 2 weeks and then randomized (based on weight) to five experimentation groups comprising four rats each.

Rats in the control group were sham-dosed distilled water (without either the extract or MSG) while rats in the MSG group were fed intoxicating dose (8000 mg/kg body weight) of MSG according to Mariyamma *et al.* (2009). Rats in the extract group were fed mango seed kernel

extract (300 mg/kg body weight while rats) in the MSG + low extract group were concomitantly fed the mango seed kernel extract (200 mg/kg body weight) and intoxicating dose of MSG (8000 mg/kg body weight) whereas rats in the MSG + high extract group were co-administered 400 mg/kg body weight of the mango seed kernel extract and intoxicating dose of MSG (8000 mg/kg body weight). The exposure was *per* oral and daily for 14 days.

2.3.2 Sacrifice, Blood Sample Collection and Preparation

After 2 weeks (14 days) exposure, the rats were sacrificed the next day after overnight fast by cardiac puncture technique (Lucas et al., 2004) and the blood sample of the respective rats was collected individually into clean polystyrene tubes, allowed to clot and respectively centrifuged at 3000 rpm for 10 minutes. The resultant serum was respectively collected into polystyrene tubes and stored in deep freezer for the determination of serum total cholesterol (CHOL), triacylglycerol (TAG), high density lipoprotein-cholesterol (HDL) and low density lipoprotein-cholesterol (LDL) concentrations. Important diagnostic ratios including CHOL:TAG, TAG:CHOL, LDL:HDL and HDL:LDL ratios were calculated from the corresponding value as obtained in this study.

2.3.3 Ethical Consideration

This study which is a continuation of our line of studies on the evaluation of biochemical effects of mango seed-kernel extract on normal and monosodium glutamate-challenged experimental models considered and adhered to the standard ethical use of experimental animals. Throughout out the experimentation (acclimatization and exposure periods), all rats were housed at 25 °C in stainless steel cages under normal daylight/dark cycle and humid tropical conditions. The rats were allowed free access to rat's feed (Vital feed, Jos Nigeria) and tap water, and generally received humane care in accordance with the guidelines of the National institute of Health, USA for ethical treatment of laboratory animals as approved by the various (departmental and college) ethical committees of Michael Okpara University of Agriculture Umudike, Nigeria.

2.4 Determination of Tannin, Phytate and Trypsin Inhibitor Contents

The concentration of tannin in the mango seed-kernel was determined by the method of Jaffe et al. (2000). One gram of the sample was dissolved in 10 ml distilled water, agitated and left to stand for 30 minutes at room temperature. Each sample was centrifuged and the extract recovered. Then, 2.5 ml of the supernatant was dispersed into 50 ml volumetric flask. Similarly, 2.5 ml of standard tannic acid solution was dispersed into a separate 50 ml flask. A 10 ml Folin-Dennis reagent was measured in each flask followed by 2.5 ml of saturated sodium carbonate (Na₂CO₃) solution. The mixture was diluted to 50 ml in the flask and incubated for 90 min at room temperature. The absorbance of each sample and standard was measured at 250 nm with the reagent blank set at zero. The corresponding concentration of tannin (mg/100 g) in the sample was calculated in relation with that of the standard.

The phytate content in the mango seed-kernel was determined as phytic acid using the procedure described by Lucas and Markaka (1975). This entailed weighing 2 g of the sample into 250 ml conical flask. The mango seed kernel sample was soaked in 100 ml of 2% concentrated hydrochloric acid (HCL) in a conical flask for 3 hours and then filtered through a double layered filter paper. Then, 50 ml of the filtrate was placed in a 250 ml beaker and

107 ml of distilled water added to give proper acidity. This was followed by the addition of 10 ml of 0.3% ammonium thiocyanate solution to each sample solution as indicator before titrating with standard iron chloride solution (0.00195 g iron/ml) to the end point shown by brownish-yellow coloration that persisted for 5 minutes. The corresponding phytic acid concentration (mg/100 g) in the sample was calculated in relation with that of the standard.

The concentration of trypsin inhibitor in the sample was determined according to the method of Prokopet and Unlenbruck (2002). One gram of the sample was dispersed in 50 ml of 0.5 M sodium chloride (NaCl) solution. The mixture was stirred for 30 minutes at room temperature and centrifuged at 1500 rpm for 5 minutes. The supernatants were filtered and the filtrate used for the assay. This assay involved the addition of 2 ml of the standard trypsin solution to 10 ml of the filtrate as the substrate of the sample. The absorbance of the mixture was taken at 410 nm using 10 ml of the same substrate as blank. The corresponding trypsin inhibitor concentration ($\mu\text{l}/\text{mg}$) in the sample was calculated in relation with that of the standard.

2.5 Determination of Serum CHOL Concentration

Serum CHOL concentration was determined by the slightly modified colorimetric method of Zlatkis et al. (1953). Five ml of ethanol was added to 0.1 ml of serum. The content was shaken and centrifuged at $3000 \times g$ for 5 min and the resultant supernatant was aspirated into another tube. Then, 2 ml of chromogen was added to the tube and the sample was allowed to stand for 40 min. after which the absorbance was measured at 550 nm.

2.6 Determination of Serum Triacylglycerol (TAG) Concentration

Serum TAG concentration was determined by the method of Calson (1963). This was based on the principle that glycerol obtained after alkaline hydrolysis of the glycerides could be measured colorimetrically at 570 nm. To 0.1ml of the sample pipetted into a labelled clean tube, 1.0ml of trichloroacetic acid (TCA) was added, mixed and then centrifuged at 250 rpm for 10 minutes. The content was allowed to stand for 20 minutes at room temperature and the absorbance of the sample and standard read against the blank at 570 nm.

2.7 Determination of Serum High Density Lipoprotein-Cholesterol Concentration

Serum HDL-cholesterol concentration was determined using the method of Albers *et al.* (1978) as contained in QCA commercial kits. The was based on the principle that low density lipoprotein (LDL) and very low density lipoproteins (VLDL) cholesterols are precipitated from serum by the action of polysaccharide in the presence of divalent cations to allow for spectrophotometric determination or measurement of high density lipoprotein-cholesterol (HDL) present in the supernatant.

2.8 Calculation of Some Lipid Profile Component Ratios

Some lipid profile component ratios, including cholesterol-triacylglycerol (CHOL:TAG), triacylglycerol-cholesterol (TAG:CHOL), low density lipoprotein-high density lipoprotein (LDL: HDL) and high density lipoprotein-low density lipoprotein (HDL:LDL) ratios were calculated from the corresponding results of this study as in earlier studies (Egbonu & Osakwe, 2011; Egbonu & Ezeanyika, 2012).

2.9 Statistical Analysis

Antinutrient data from triplicate determinations and the lipid profile data from sample size, n , of four rats were evaluated using one-way analysis of variance (ANOVA) as described

(Egbuonu et al., 2009b). Results were given as mean \pm standard error of mean (SEM). The difference in mean was considered significance at $p < 0.05$.

3. Results

3.1 Antinutrient Content

The results of the antinutrients content of mango seed were as shown on Table 1 above. Tannin content (29.21 ± 5.39 mg/100 g) was higher than the other determined antinutrients while the least was phytate (0.38 ± 0.02) mg/100g.

Table 1. Some antinutrient contents in the pulverized *M. indica* seed kernel

	Tannin (mg/100 g)	Trypsin inhibitor (μ l/mg)	Phytate (mg/100 g)
Concentration	29.21 ± 5.39	9.53 ± 0.02	0.38 ± 0.02

Value presented as mean \pm SEM of triplicate determinations. Significant difference at $p < 0.05$

3.2 Lipid Profile

The result as shown on Table 2 revealed that after fourteen day daily oral exposure, rats in the MSG group had higher ($p < 0.05$) serum total cholesterol concentration compared to rats in the other groups. However, MSG-intoxicated rats co-treated respectively with 200 mg/kg and 400 mg/kg body weight of the sample extract showed a significant ($p < 0.05$) dose dependent reduction in the serum total cholesterol concentration compared to the corresponding effect observed in rats exposed to MSG alone. The observation relative to the control was highest (10.59%) in the rats exposed to MSG alone and least (0.59%) 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 (9.57%) followed by rats in the MSG + highest extract group (9.04%).

As depicted on Table 3, rats in the MSG Group had higher ($p < 0.05$) serum triacylglycerol concentration compared to rats in the other groups. However, MSG-intoxicated rats co-treated respectively with 200 mg/kg and 400 mg/kg body weight of the sample extract showed a significant ($p < 0.05$) dose dependent reduction in the serum triacylglycerol concentration compared to the corresponding effect observed in rats exposed to MSG alone. The observation relative to the control was highest (16.03%) in the rats exposed to the extract alone followed by (15.38%) in the rats exposed to MSG together with highest concentration of the extract. Similarly, the observed reduction relative to the MSG group was highest in rats in the extract group (10.27%) followed by that in the MSG + highest extract group (9.59%).

Rats in the MSG Group had lower ($p < 0.05$) high density lipoprotein concentration compared to rats in the other groups. However, MSG-intoxicated rats co-treated respectively with 200 mg/kg and 400 mg/kg body weight of the sample extract showed a lower ($p < 0.05$) high density lipoprotein concentration compared to that of rats in the control and in the extract groups. The observation relative to the control was equal (20.73%) but opposite in the MSG and Extract groups while the observed change relative to the MSG group was highest

(52.31%) in rats in the Extract group and least (20.77%) in the MSG + highest extract group of rats (Table 4).

As shown on Table 5, after fourteen day daily oral exposure, rats in the MSG Group had higher ($p < 0.05$) low density lipoprotein cholesterol concentration compared to rats in the other groups. However, MSG-intoxicated rats co-treated respectively with 200 mg/kg and 400 mg/kg body weight of the sample extract showed a significant ($p < 0.05$) dose dependent reduction in low density lipoprotein cholesterol concentration compared to that in rats exposed to MSG alone. The observation relative to the control was highest (155.25%) in the rats exposed to MSG alone and least (45.88%) in the rats exposed to the extract alone. The observed reduction relative to the MSG group was highest in rats in the control (60.83%) followed by those in the extract group (42.86%) but least in the MSG + least extract group (25.81%).

Table 2. Effect of *M. indica* (mango) seed kernel extract on the serum CHOL concentration ($\text{mg } 100\text{ml}^{-1}$) of normal and monosodium glutamate-intoxicated rats

Groups	CHOL ($\text{mg } 100\text{ml}^{-1}$)	Change relative to the Control (%)	Change relative to MSG group (%)
Control (distilled water 2 ml/kg b.w)	3.40±0.07*	0.00	-9.57
MSG (8000 mg/kg b.w)	3.76±0.09	+10.59	0.00
Extract (300 mg/kg b.w)	3.48±0.10	+2.35	-7.18
MSG (8000 mg/kg b.w) + Extract 200 mg/kg b.w)	3.47±0.11*	+2.06	-7.71
MSG (8000 mg/kg b.w) + Extract (400 mg/kg b.w)	3.42±0.10*	+0.59	-9.04

Value presented as mean \pm SEM of sample size, $n = 4$ rats. + denotes higher by; - denotes lower by. Significant difference at $p < 0.05$. *significantly different from MSG ($P < 0.05$) (one way ANOVA followed by post-hoc LSD).

Table 3. Effect of *M. indica* (mango) seed kernel extract on the serum TAG concentration ($\text{mg } 100\text{ml}^{-1}$) of normal and monosodium glutamate-intoxicated rats

Groups	TAG ($\text{mg } 100\text{ml}^{-1}$)	Change relative to the control (%)	Change relative to MSG group (%)
Control (distilled water 2 ml/kg b.w)	1.56±0.04*	0.00	+6.84
MSG (8000 mg/kg b.w)	1.46±0.03	-6.41	0.00
Extract (300 mg/kg b.w)	1.31±0.01*	-16.03	-10.27

MSG (8000 mg/kg b.w + Extract 200 mg/kg b.w)	1.41±0.02	-9.62	-3.42
MSG (8000 mg/kg b.w) + Extract (400 mg/kg b.w)	1.32±0.01*	-15.38	-9.59

Value presented as mean ± SEM of sample size, n = 4 rats. + denotes higher by; - denotes lower by. Significant difference at p<0.05. *significantly different from MSG (P<0.05) (one way ANOVA followed by post-hoc LSD).

Table 4. Effect of *M. indica* (mango) seed kernel extract on the serum HDL-CHOL concentration (mg 100ml⁻¹) of normal and monosodium glutamate-intoxicated rats

Groups	HDL-CHOL (mg 100ml ⁻¹)	Change relative to the control (%)	Change relative to MSG group (%)
Control (distilled water 2 ml/kg b.w)	1.64±0.07*	0.00	+26.15
MSG (8000 mg/kg b.w)	1.30±0.03	-20.73	0.00
Extract (300 mg/kg b.w)	1.98±0.05*	+20.73	+52.31
MSG (8000 mg/kg b.w + Extract 200 mg/kg b.w)	1.58±0.07*	-3.66	+21.54
MSG (8000 mg/kg b.w + Extract (400 mg/kg b.w)	1.57±0.06*	-4.27	+20.77

Value presented as mean ± SEM of sample size, n = 4 rats. + denotes higher by; - denotes lower by. Significant difference at p<0.05. *significantly different from MSG (P<0.05) (one way ANOVA followed by post-hoc LSD).

Table 5. Effect of *M. indica* (mango) seed kernel extract on the serum LDL-CHOL concentration (mg 100ml⁻¹) of normal and monosodium glutamate-intoxicated rats

Groups	LDL-CHOL (mg 100ml ⁻¹)	Change relative to the control (%)	Change relative to MSG group (%)
Control (distilled water 2 ml/kg b.w)	0.85±0.05*	0.00	-60.83
MSG (8000 mg/kg b.w)	2.17±0.11*	+155.29	0.00
Extract (300 mg/kg b.w)	1.24±0.13*	+45.88	-42.86
MSG (8000 mg/kg b.w + Extract 200 mg/kg b.w)	1.61±0.13*	+89.41	-25.81

MSG (8000 mg/kg b.w + Extract (400 mg/kg b.w)	1.58±0.10*	+85.88	-27.19
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Value presented as mean ± SEM of sample size, n = 4 rats. + denotes higher by; - denotes lower by. Significant difference at p<0.05. *significantly different from MSG (P<0.05) (one way ANOVA followed by post-hoc LSD).

3.3 Some Lipid Profile Ratios

As depicted on Table 6, after fourteen day daily oral exposure, rats in the Extract group had higher (p<0.05) CHOL:TAG ratio but lower (p<0.05) TAG:CHOL ratio compared to rats in the other groups. Similarly, the observation relative to the control was highest (22.02% and 19.57%, respectively for CHOL:TAG and TAG:CHOL ratios) in the rats exposed to Extract alone. The observed increase in CHOL:TAG ratio relative to the MSG group was highest (3.10%) in rats in the Extract group while the observed decrease in TAG:CHOL ratio relative to the MSG group was highest (5.13%) also in rats in the Extract group.

As depicted on Table 7, after fourteen day daily oral exposure, rats in the MSG group had lower (p<0.05) HDL:LDL ratio but higher (p<0.05) LDL:HDL ratio compared to rats in the other groups. Similarly, the observations relative to the control was highest (225.69.27% and 225.49 %, respectively for HDL: LDL and LDL:HDL ratios) in the rats exposed to MSG alone. However, the observed increase in HDL:LDL ratio relative to the MSG group was highest (225.42%) in rats in the Control group while the observed decrease in LDL:HDL ratio relative to the MSG group was highest (69.27%) also in rats in the Control group.

Table 6. Effect of *M. indica* (mango) seed kernel extract on the serum CHOL: TAG ratio of normal and monosodium glutamate-intoxicated rats

Groups	CHOL:TAG ratio (TAG:CHOL ratio)	Change relative to the control (%)	Change relative to MSG group (%)
Control (distilled water 2 ml/kg b.w)	2.18(0.46)	0.00	-15.50(+17.95)
MSG (8000 mg/kg b.w)	2.58(0.39)	+18.35(-15.22)	0.00(0.00)
Extract (300 mg/kg b.w)	2.66(0.37)	+22.02(-19.57)	+3.10(-5.13)
MSG (8000 mg/kg b.w + Extract 200 mg/kg b.w)	2.46(0.41)	+12.84(-10.87)	-4.65(+5.13)
MSG (8000 mg/kg b.w + Extract (400 mg/kg b.w)	2.59(0.38)	+18.81(-17.39)	+0.39(-2.56)

Value presented as mean ± SEM of sample size, n = 4 rats. + denotes higher by; - denotes lower by. Significant difference at p<0.05. *significantly different from MSG (P<0.05) (one

way ANOVA followed by post-hoc LSD).

Table 7. Effect of *M. indica* (mango) seed kernel extract on the serum HDL: LDL ratio of normal and monosodium glutamate-intoxicated rats

Groups	HDL:LDL ratio (LDL:HDL ratio)	Change relative to the control (%)	Change relative to MSG group (%)
Control (distilled water 2 ml/kg b.w)	1.92(0.51)	0.00	+225.42(-69.27)
MSG (8000 mg/kg b.w)	0.59(1.66)	-69.27(+225.49)	0.00(0.00)
Extract (300 mg/kg b.w)	1.59(0.62)	-17.19(+21.57)	+169.49(-62.65)
MSG (8000 mg/kg b.w + Extract 200 mg/kg b.w)	0.98(1.01)	-48.96(+98.03)	+66.10(-39.16)
MSG (8000 mg/kg b.w + Extract (400 mg/kg b.w)	0.99(1.00)	-48.44(+96.08)	+67.80(-39.76)

Value presented as mean \pm SEM of sample size, n = 4 rats. + denotes higher by; - denotes lower by. Significant difference at $p < 0.05$. *significantly different from MSG ($P < 0.05$) (one way ANOVA followed by post-hoc LSD).

4. Discussion

This study determined some antinutrients in *Mangifera indica* (mango) seed-kernel and the effects of ethanol extract of the seed-kernel on serum lipid profile of normal and monosodium glutamate-challenged male albino rats (weight range, 104-107 g; 5 groups; n = 4). The result revealed the preponderance of the determined antinutrients in the *Mangifera indica* seed kernel although tannin content (29.21 ± 5.39 mg/100 g) was highest while phytate content (0.38 ± 0.02) mg/100g) was the least. The content of these antinutrients in *M. indica* (german variety) seed kernel, notably tannin could not compare with that report by Fowomola (2010) also in *M. indica* seed kernel. However, Fowomola (2010) did neither indicate the *M. indica* variety nor clarify whether the whole seed or the seed kernel was used. Phytochemical and nutrient contents of mango vary across mango cultivars (Rocha-Ribeiro, et al., 2007) and varieties (Abdalla et al., 2007), implying possible variation in their effects in, when used by, animals. Nevertheless, the comparatively high content of notably tannin reported in this study suggests that the German variety of mango seed kernel may be toxic when consumed. Antinutrients and proximate content of plant-sourced foods are easily removed by simple processing methods including boiling and roasting (Nzewi & Egbuonu, 2011a; Nzewi & Egbuonu, 2011b). Thus these and other methods for reducing antinutrients should be applied hence recommended to reduce or eliminate totally the antinutrients contained in the seed

kernel so as to enable proper application of the seed kernel in both animal feeds and in industries.

To the knowledge of the authors, this is the first study that investigated the effect of mango seed kernel on lipid profile of MSG-intoxicated animal model. After fourteen days daily oral exposure, rats in the MSG Group had higher ($p < 0.05$) total cholesterol, triacylglycerol and low density lipoprotein but lower ($p < 0.05$) high density lipoprotein concentration compared to rats in the other groups. Notably, the rats intoxicated with MSG also showed a significantly higher serum level of CHOL and TAG. This confirmed the reported hyperlipidemia in rats exposed to 8 g/kg bw of MSG (Mariyamma *et al.*, 2009) and concurred with other findings (Okediran *et al.*, 2014), indicating altered cholesterol and triacylglycerol metabolism and the attendant risk of cardiovascular diseases in the rats (Egbuonu *et al.*, 2010d). Generally, elevated serum CHOL and TAG concentrations indicate hyperlipidemia. However, treating rats with *M. indica* seed kernel extract led to significant elevation of their serum HDL-cholesterol concentration. Also, MSG-intoxicated rats co-treated respectively with 200 and 400 mg/kg b.w of the sample extract showed a significant ($p < 0.05$) dose dependent reduction compared to the corresponding effects observed in rats exposed to MSG alone. This suggested modulation of MSG-induced effects on the rats lipid profile irrespective of co-treated sample dose, and possible promising protective role of *M. indica* seed kernel against the adverse effects related to MSG-induced responses in the rats' serum lipid profile. The health implication of this modulation is noteworthy. The association between a low level of HDL-cholesterol and an increased risk of CVD was established (Assmann & Gotto, 2004). Low level of HDL-cholesterol plays a direct role in the atherogenic process and therapeutic intervention to raise HDL-cholesterol is widely encouraged. This study showed that *M. indica* seed kernel extract exerted a significant dose dependent decrease in TAG, CHOL and LDL and a significant dose dependent increase in HDL, hence did significantly counter the attendant hyperlipidemic-related adverse effects elicited by MSG intoxication. Further studies are warranted to determine the exact component in *M. indica* responsible.

The corresponding ratios of the components of lipid profile are not frequently considered though they could be diagnostic. Millan *et al.* (2009) reported that lipid profile component ratios have greater risk factors predictive value than the use of isolated parameters. In this study, some ratios were computed. Compared to other groups, rats in the Extract group had higher ($p < 0.05$) CHOL:TAG ratio but lower ($p < 0.05$) TAG:CHOL ratio while rats in the MSG group had higher ($p < 0.05$) HDL:LDL ratio but lower ($p < 0.05$) LDL:HDL. The biochemical implications of this lipid profile component ratios are hitherto not fully known. We therefore speculated that higher CHOL:TAG or lower TAG:CHOL ratios could be indicative of beneficial lipid profile mix in the extract-treated rats whereas higher HDL:LDL or lower LDL:HDL ratios could be a pointer to deleterious lipid profile mix in the MSG-intoxicated rats. MSG-intoxication produced adverse influence on the lipid profile status of rats (Okediran *et al.*, 2014; Mariyamma *et al.*, 2009; Egbuonu *et al.*, 2010d) while the result of this study suggested beneficial response on the lipid profile status of rats following exposure to the *M. indica* seed kernel. In support of the speculation, increased LDL:HDL ratio predicted cardiovascular risk due to an increase in the atherogenic potential in the numerator but a decrease in the anti-atherosclerotic potential of the denominator

(Millan *et al.*, 2009).

5. Conclusion

In conclusion, the study demonstrated the preponderance of tannin in, and significant serum lipid lowering potential in normal and MSG-challenged rats of, the sample. Further studies aimed at using antinutrient-process-reduced sample in hyperlipidemia-related health-challenged models and collaborating the suggested implication of the studied lipid-profile-component-ratios are warranted and recommended.

References

- Abdalla, E. M., Darwish, S. M., Ayad, E. H. E., & El-Hamahmy, R.M. (2007). Egyptian mango by-product 1. Compositional quality of mango seed kernel. *Food Chemistry*, *103*, 1134-1140. <https://doi.org/10.1016/j.foodchem.2006.10.026>
- Ajila, C. M., & Prasada-Rao, U. J. (2008). Protection against hydrogen peroxide induced oxidative damage in rat erythrocytes by *Mangifera indica* leaf peel extract. *Food Chemical Toxicology*, *46*(1), 303-309. <https://doi.org/10.1016/j.fct.2007.08.024>
- Albers, J. J., Warmick, G. R., & Cheng, M. C. (1978). Determination of high density lipoprotein (HDL)-cholesterol. *Lipids*, *13*, 925-932. <https://doi.org/10.1007/BF02533852>
- Ali, M. R., Yong, M. J., Gyawali, R., Mosaddik, A., Ryu, Y. C., & Cho, S. K. (2012). Mango (*Mangifera indica* L.) Peel extracts inhibit proliferation of HeLa Human Cervical Carcinoma Cell via induction of apoptosis. *Journal of Korean Social Applied Biological Chemistry*, *55*, 397-405. <https://doi.org/10.1007/s13765-012-1024-x>
- Amien, A. I., Fahmy, S. R., Abd-Elgleel, F. M., & Elaskalany, S. M. (2015). Reno protective effect of *Mangifera indica* polysaccharides and silymarin against cyclophosphamide toxicity in rats. *The Journal of Basic and Applied Zoology*, *72*, 154-162. <https://doi.org/10.1016/j.jobaz.2015.09.006>
- Assmann, G., & Gotto, A. M. (2004). HDL cholesterol and protective factors in arteriosclerosis. *Circulation*, *109*, 8-14. <https://doi.org/10.1161/01.CIR.0000131512.50667.46>
- Bhowmik, A., Khan, L. A., Akhter, M., & Rokeya, B. (2009). Studies on the antidiabetic effects of *Mangifera indica* stem-barks and leaves on nondiabetic type-1 and type-2 diabetic model rats. *Bangladesh Journal of Pharmacology*, *4*, 110-114. <https://doi.org/10.3329/bjp.v4i2.2488>
- Carlson, L. A. (1963). Colorimetric determination of triglycerides in serum. *Journal Athero. Res.*, *3*, 334-339. [https://doi.org/10.1016/S0368-1319\(63\)80012-5](https://doi.org/10.1016/S0368-1319(63)80012-5)
- Collison, K. S., Makhoul, N. J., Zaidi, M. Z., Al-Rabiah, R., Inglis, A., Andres, B. L., Ubungen, R., Shoukri, M., & Al-Mohanna, F. A. (2012). Interactive effects of neonatal exposure to monosodium glutamate and aspartame on glucose homeostasis. *Nutrition & Metabolism*, (Lond), *9*(1), 58. <https://doi.org/10.1186/1743-7075-9-58>
- Egbonu, A. C. C. (2015a). Comparative investigation of the antibacterial and antifungal potentials of the extracts of watermelon (*Citrullus lanatus*) rind and seed. *European Journal of Medicinal Plants*, *9*(4), 1-7. <https://doi.org/10.9734/EJMP/2015/18142>
- Egbonu, A. C. C. (2015b). Comparative investigation of the proximate and functional

- properties of watermelon (*Citrullus lanatus*) rind and seed. *Research Journal of Environmental Toxicology*, 9(3), 160-167. <https://doi.org/10.3923/rjet.2015.160.167>
- Egbonu, A. C. C. (2012). Sub-chronic concomitant ingestion of L-arginine and monosodium glutamate improves feed efficiency, lipid metabolism and antioxidant capacity in male Wistar rats. *Pakistan Journal of Biological Sciences*, 15(6), 301-305. <https://doi.org/10.3923/pjbs.2012.301.305>
- Egbonu, A. C. C., Ejikeme, P. M., & Obasi, N. L. (2010b). Monosodium glutamate: Potentials at inducing prostate pathologies in male Wistar rats. *African Journal of Biotechnology*, 9(36), 5950-5954.
- Egbonu, A. C. C., Ejikeme, P. M., & Obasi, N. L. (2010c). Influence of sub-chronic oral exposure to high monosodium glutamate on some serum markers of the renal functions in male Wistar rats. *African Journal of Biochemistry Research*, 4(9), 225-228.
- Egbonu, A. C. C., Ezeanyika, L. U. S., Ejikeme, P. M., & Obidoa O. (2010a). Histomorphologic alterations in the liver of male Wistar rats treated with l-arginine glutamate and monosodium glutamate. *Research Journal of Environmental Toxicology*, 4(4), 205-213. <https://doi.org/10.3923/rjet.2010.205.213>
- Egbonu, A. C. C., & Ezeanyika, L. U. S. (2012). Effect of L-arginine on markers of metabolic syndrome related to abdominal obesity and disorder of lipid metabolism in female Wistar albino rats. *American Journal of Biochemistry*, 2(2), 7-13. <https://doi.org/10.5923/j.ajb.20120202.02>
- Egbonu, A. C. C., & Nwankwo, N. E. (2012). Phytochemical properties of some solvent fractions of petroleum ether extract of the African mistletoe (*Loranthus micranthus* Linn) leaves and their antimicrobial activity. *African Journal of Biotechnology*, 11, 12595-12599.
- Egbonu, A. C. C., Obidoa, O., Ezeokonkwo, C. A., Ezeanyika, L. U. S., & Ejikeme, P. M. (2009b). Low dose oral administration of monosodium glutamate in male albino rats may be nephroprotective. *Bio-Research*, 7(1), 470-473. <https://doi.org/10.4314/br.v7i1.45475>
- Egbonu, A. C. C., Obidoa, O., Ezeokonkwo, C. A., Ejikeme, P. M., & Ezeanyika, L. U. S. (2010d). Some biochemical effects of sub-acute oral administration of L-arginine on monosodium glutamate-fed Wistar albino rats 1: Body weight changes, serum cholesterol, creatinine, and sodium ion concentrations. *Toxicol. and Environ. Chem*, 92(7), 1331-1337. <https://doi.org/10.1080/02772240903450645>
- Egbonu, A. C. C., Obidoa, O., Ezeokonkwo, C. A., Ezeanyika, L. U. S., & Ejikeme, P. M. (2009a). Hepatotoxic effects of low dose oral administration of monosodium glutamate in male albino rats. *African Journal of Biotechnology*, 8(13), 3031-3035.
- Egbonu, A. C. C., & Oriji, S. O. (2017). Pulverized *Mangifera indica* (mango) seed kernel mitigated monosodium glutamate-intoxicated rats' kidney histology and bio-functions. *Journal of Nutritional Health and Food Science*, 5(1), 1-7. <https://doi.org/10.15226/jnhfs.2017.00193>
- Egbonu, A. C. C., & Osakwe, O. N. (2011). Effects of high monosodium glutamate on some serum markers of lipid status in male Wistar rats. *Journal of Medicine & Medical Science*, 2(1), 653-656.

- Fowomola, M. A. (2010). Some nutrients and antinutrients content of mango (*Mangifera indica*) seed. *African Journal of food Sciences*, 4(8), 472-476.
- Gemedo, H. F., & Negussie, R. (2014). Antinutritional Factors in Plant Foods: Potential Health Benefits and Adverse Effects. *International Journal of Nutrition and Food Sciences*, 3(4), 284-289. <https://doi.org/10.11648/j.ijnfs.20140304.18>
- Husarova, V., & Ostatnikova, D. (2013). Monosodium glutamate, toxic effects and their implications for human intake: A review. *JMED Research*, 1-12. <https://doi.org/10.5171/2013.608765>
- Jaffe, A. S., Ravkilde, J., Roberts, R., Naslund, U., Apple, F. S., Galvani, M., & Katus, H. (2000). It's time for a change to a troponin standard. *Circulation*, 102(11), 1216-1220. <https://doi.org/10.1161/01.CIR.102.11.1216>
- Jagetia, G. C., & Baliga, S. (2005). Radioprotection by mangiferin in DBAxC57BL mice: A preliminary study. *Phytomedicine*, 12(3), 209-215. <https://doi.org/10.1016/j.phymed.2003.08.003>
- Li, X., Cui, X., Sun, X., Li, X., Zhu, Q., & Li, W. (2010). Mangiferin prevents diabetic nephropathy progression in streptozotocin-induced diabetic rats. *Phytotherapy Research*, 24, 893-899.
- Lucas, G. M., & Markakas, P. (1975). Phytic acid and other phosphorus compounds of bean (*Phaseolus vulgaris*), *J. Agric. Ed Chem.*, 23, 13-15. <https://doi.org/10.1021/jf60197a016>
- Lucas, R. L., Lentz, K. D., & Hale, A. S. Collection and preparation of blood products. *Clinical Techniques in Small Animal Practice*, 19(2), 55-62. <https://doi.org/10.1053/j.ctsap.2004.01.007>
- Manal, S. T., & Nawal, A. (2012). Adverse effects of monosodium glutamate on liver and kidney function in adult rats and potential protective effect of vitamins C and E. *Food and Nutrition Sciences*, 3(5), 651-659. <https://doi.org/10.4236/fns.2012.35089>
- Mariyamma, T., Sujatha, K. S., & Sisilamma, G. (2009). Protective effect of *Piper longum* Linn. on monosodium glutamate induced oxidative stress in rats. *Indian Journal of Experimental Biology*, 47, 186-192.
- Mattson, M. P. (2008). "Glutamate and Neurotrophic Factors in Neuronal Plasticity and Disease," *Annals of the New York Academy of Sciences*, 1144, 97-112. <https://doi.org/10.1196/annals.1418.005>
- Millan, J., Pinto, X., Munoz, A., Zunig, M., Rubies-Prat, J., Pallardo, L.F., Masana, L., Mangas, A., Hernandez-Mijares, A., Gonzalez-Santos, P., Ascaso, J., & Pedro-Botet, J. (2009). Lipoprotein ratios: Physiological significance and clinical usefulness in cardiovascular prevention. *Vasc Health Risk Manag.*, 5, 757-765.
- Noratto, G. D., Bertoldi, M. C., Krenek, K., Talcott, S. T., Stringheta, P. C., & Mertens-Talcott, S. U. (2010). Anticarcinogenic effects of polyphenolics from mango (*Mangifera indica*) varieties. *Journal of Agricultural and Food Chemistry*, 58(7), 4104-4112. <https://doi.org/10.1021/jf903161g>
- Nzewi, D. C., & Egbunu, A. C. C. (2011a). Effect of boiling and roasting on some anti-nutrient factors of asparagus bean (*Vigna sesquipedalis*) flour. *African Journal of Food*

Science and Technology, 2(3), 075-078.

Nzewi, D. & Egbunu, A.C.C. (2011b). Effect of boiling and roasting on the proximate properties of asparagus bean (*Vigna sesquipedalis*). *African Journal of Biotechnology*, 10(54), 11239-11244. <https://doi.org/10.5897/AJB11.452>

Okediran, B. S., Olurotimi, A. E., Rahman, S. A., Michael, O. G., & Olukunle, J. O. (2014). Alteration in the lipid profile and liver enzymes of rats treated with monosodium glutamate. *Sokoto Journal of Veterinary Sciences*, 12(3), 42-46. <https://doi.org/10.4314/sokjvs.v12i3.8>

Prokopet, G., & Unlenbruck, W. (2002). Protectine eine nenkalsse anti-kowperahlich verbindungen dish. *Ges. Heit.*, 23, 318.

Rocha-Ribeiro, S. M., Queiroz, J. H., Lopes-Ribeiro de Queiroz, M. E., Campos, F. M., Pinheiro-Sant'ana, H. M. (2007). Antioxidant in mango (*Mangifera indica* L.) pulp. *Plant Foods and Human Nutrition*, 62(1), 13-17. <https://doi.org/10.1007/s11130-006-0035-3>

Schummer, C. M., Werner, U., Tennagels, N., Schmol, D., Haschke, G., Juretschke, H., Patel, M. S., Gerl, M., Kramer, W., & Herling, A. W. (2008). Dysregulated pyruvate dehydrogenase complex in Zucker diabetic fatty rats. *Am. J. Physiol. Endocrinol. Metab.*, 294, E88-E96. <https://doi.org/10.1152/ajpendo.00178.2007>

Shabani, Z., & Sayadi, A. (2014). The Antimicrobial in Vitro Effects of Different Concentrations of Some Plant Extracts Including Tamarisk, March, Acetone and Mango. *Journal of Applied Pharmaceutical Science*, (5), 75-79.

Shah, K. A., Patel, M. B., Patel, R. J., & Parmer, P. K. (2010). *Mangifera indica* (Mango). *Pharmacognosy Review*, 4(7), 42-48. <https://doi.org/10.4103/0973-7847.65325>

Shanthakumari, S., Mohan, V., & Britto, J. (2008). Nutritional evaluation and elimination of toxic principles in wild yam (*Dioscorea* spp.). *Tropical and Subtropical Agroecosystems*, 8, 319-225.

Soetan, K., & Oyewole, O. (2009). The need for adequate processing to reduce the antinutritional factors in plants used as human foods and animal feeds: A review. *African Journal of Food Science*, 3(9), 223-232.

Timsina, B., & Kilingar, N. (2015). Mango seeds: A potential source for the isolation of bioactive compounds with anti-cancer activity. *International Journal of Pharmacy and Pharmaceutical Science*, 7(3), 89-95.

Vega-Vega, V., Silva-Espinoza, B. A., Cruz-Valenzuela, M. R., Bernal-Mercado, A. T., Gonzalez-Aguilar, G. A., & Ruiz-Cruz, S. (2013). Antimicrobial and antioxidant properties of byproduct extracts of mango fruit. *Journal of Applied Botany and Food Quality*, 86, 205-211.

Veni, N. K., Karthika, D., Devi, M. S., Rubini, M. F., Vishalini, M., & Pradeepa, Y. J. (2010). Analysis of monosodium l-glutamate in food products by high-performance thin layer chromatography. *Journal of Young Pharmacology*, 2, 297-300. <https://doi.org/10.4103/0975-1483.66795>

Yatnatti, S., Vijayalakshmi, D., Chandru, R. (2014). Processing and nutritive value of mango seed kernel flour. *Current Research in Nutrition and Food Science*, 2(3), 1-6. <https://doi.org/10.12944/CRNFSJ.2.3.10>

Zahedi, S. M., Asgarian, Z. S., Ersi, F. K., & Seydi, A. (2016). Evaluating marketability of

ten selected cultivars of mango. *Open Journal of Ecology*, 6, 219-224.
<https://doi.org/10.4236/oje.2016.65022>

Zlatkis, A., Zak, B., & Boyler, J. (1953). Colorimetric determination of cholesterol in serum. *J. Lab. Clin. Med.*, 41, p.43c.

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