

## Effect of Pulverized *Mangifera indica* (Mango) Seed Kernel on Monosodium Glutamate-Intoxicated Rats' Serum Antioxidant Capacity, Brain Function and Histology

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### Abstract

Monosodium glutamate, a common food-flavoring additive, affects the brain and induces oxidative stress while the underutilized *Mangifera indica* L seed kernel (MSK) has phyto-medicinal values. This study assessed the effect of pulverized MSK on monosodium glutamate-intoxicated rats' serum antioxidant capacity, brain function and histology *via in vitro* (1,1-diphenyl-2-picryl hydrazyl radical scavenging activity (DPPH), ferric reducing antioxidant power (FRAP) and total antioxidant capacity (TAC)), and *in vivo* (catalase (CAT), glutathione peroxidase (GPx), superoxide dismutase (SOD) malondialdehyde (MDA), reduced glutathione (GSH), uric acid (UA)) determinations as well as rats' brain histology examination. *In vitro*, MSK increased ( $p < 0.05$ ) the DPPH, FRAP and TAC dose-dependently compared to the respective standards. *In vivo*, lower ( $p < 0.05$ ) CAT and SOD activity in MSG-exposed rats as compared to rats in control and MSK-groups dose-dependently increased ( $p < 0.05$ ) in rats concomitantly exposed to MSG and MSK extract. Higher ( $p < 0.05$ ) MDA, GPx and GSH in MSG-exposed rats as compared to rats in control and MSK-groups dose-dependently reduced ( $p < 0.05$ ) in rats concomitantly exposed to MSG and MSK extract. However, higher ( $p < 0.05$ ) UA concentration in MSG-exposed rats as compared to rats in control was lower ( $p < 0.05$ ) in MSK-treated rats and in rats concomitantly exposed to MSG and MSK extract. Hypothalamic neuronal degeneration and necrosis in the brain histology of MSG-exposed rats contrasted those of MSK- and control-rats. The study confirmed a definite MSG-induction of oxidative stress in, probably *via* enhanced oxidant generation that distorted brain histology of, rats. It further indicated *in vitro* antioxidant potential of MSK and demonstrated the antioxidant capacity of MSK in improving and modulating the brain histology and serum antioxidant capacity of normal and MSG-intoxicated rats, respectively probably by way of enhanced oxidant scavenging activity. Further studies are warranted to explore and harness MSK antioxidant potential in managing, especially MSG-induced, health-dysfunctions presented with oxidative stress in animals.

**Keywords:** *Mangifera indica*; Seed Kernel; Monosodium Glutamate; Intoxicated Rats

### Abbreviations

MSK: *Mangifera indica* L Seed Kernel; DPPH: 1,1-Diphenyl-2-Picryl Hydrazyl Radical Scavenging Activity; FRAP: Ferric Reducing Antioxidant Power; TAC: Total Antioxidant Capacity; CAT: Catalase; GPx: Glutathione Peroxidase; SOD: Superoxide Dismutase; MDA: Malondialdehyde; GSH: Reduced Glutathione; UA: Uric Acid; MSG: Monosodium Glutamate; TBARS: Thiobarbituric Acid Reacting Substances; TBA: Thiobarbituric Acid; bw: Body Weight; AAE/g: Acid Equivalent Per Gram; IU/L: International Unit Per Liter; ANOVA: Analysis of Variance; SPSS: Statistical Package for Social Science; SEM: Standard Error of the Mean; GAE/g: Gallic Acid Equivalent Per Gram; AAE/g: Ascorbic Acid Equivalent Per Gram

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## **Introduction**

The increasing health burden contribution from food additives, including monosodium glutamate has warranted increasing search for alternative food and drug sources [1-7]. Mango, genus *Mangifera* [8] and family *Anacardiaceae* [9] parts, has potential applications in nutrition and medicine owing to the phytoconstituents [9-16] resulting in extensive utilization of mango fruit and the attendant generation of mango seed as waste [17]. 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 [18]. In particular, the soft seed kernel in the oblong-shaped stony seed endocarp constituting up to 75% of the seed exhibited anti-microbial, anti-malarial [19] and hepato-protective effects [20] hence could have antioxidant potential required in combating oxidative stress that is fundamental in many diseased conditions, necessitating this study.

Monosodium glutamate (MSG), a common food-flavoring additive [21], could affect the brain and could induce oxidative stress. Sadly, MSG could be abused owing to its non-indication on most packaged foods [5]. Reported diverse MSG-induced adverse effects in animals [2,3,22] generated controversy on its safety. The biochemical basis of MSG-intoxication is not fully understood but may involve either MSG or its metabolic precursors and products, notably glutamate and sodium. Generally, the anabolism of MSG involves glutamate deprotonation and subsequent substitution with sodium that could partly involve at least transient oxidation-reduction reactions and in particular, Ajibade, *et al.* [23] reported MSG-induced oxidative stress in rats. And, oxidative stress has been implicated in the etiology of many diseases, including cardiovascular diseases, cancer, neurological disorders and even ageing [24,25].

Generally, the brain is central to endocrine expression and neuronal transmission involved in the initiation, regulation and even termination of biochemical reactions that could lead to oxidative stress. Thus, brain damage and dysfunction as deciphered from changes in seric chemistry results and brain histology could suggest or explain enhanced sequence of events leading to oxidative stress. Manfredini, *et al.* [26] noted very high extracellular concentration of glutamate (a major component and possible metabolite of MSG which is toxic on the brain) following diminished concentration of uric acid that prevents glutamate-induced cell damage. These warranted this study aimed at assessing the effect of pulverized underutilized mango (*Mangifera indica* L) seed kernel (MSK) on monosodium glutamate-intoxicated rats' serum antioxidant capacity, brain function and histology. *In vitro* antioxidant potential of MSK was assessed by measuring the ferric reducing antioxidant power (FRAP), DPPH radical scavenging activity and total antioxidant capacity (TAC). *In vivo* antioxidant potential of MSK was assessed in rats serum by enzymatic determination of the activity of catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GPx) as well as non-enzymatic determination of the concentration of uric acid, malondialdehyde, and reduced glutathione (GSH) in relation to histological changes in the rats brain tissue. Uric acid concentration is unique in serving as an indicator of oxidative stress and also of brain function.

## **Materials and Methods**

### **Sample procurement, identification, preparation and extraction**

A commercially available brand of MSG (99% purity) used in this study was procured from *Ubani* market, a daily food condiments market in Umuahia, south east Nigeria. Chemicals and solvents used in this study were products of reputable companies procured 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, Nigeria. 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 mango fruits (German variety) were thoroughly washed with tap water. The fleshy part of each of the fruits 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 [27] and stored in a refrigerator at 4°C until used.

### **Animal procurement and exposure groups**

Twenty adult male albino rats (weight range, 104 - 170g) 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 with sample size of four rats.

Rats in the control group were sham-dosed with 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* [22]. Rats in the extract group were fed mango seed kernel extract at 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.

### **Sacrifice, blood sample collection, preparation and ethical issues**

After 2 weeks (14 days) exposure, the rats were sacrificed the next day after overnight fast by cardiac puncture technique [28] 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 serum was respectively collected into polystyrene tubes and stored in deep freezer for the determination of serum catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GPx) activity as well as serum malondialdehyde (MDA), reduced glutathione (GSH) and uric acid (UA) concentration.

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 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.

### **Determination of ferric reducing antioxidant power (FRAP), 1,1, DPPH radical scavenging ability and total antioxidant capacity (TAC)**

The FRAP of MSK was determined based on the principle that in an acidic medium, an antioxidant reduces  $Fe^{3+}$  to  $Fe^{2+}$  as described in Egbuonu [29] with slight modification. A 2.0 ml of MSK was mixed with 2.0 ml of 0.2 M phosphate buffer (pH 6.6) and 2.0 ml of 10 mg/l potassium ferricyanide (0.1% w/v) solution. The mixture was incubated in a water bath at 50°C for 20 minutes and 2.0 ml of 100 mg/l trichloroacetic acid solution (10% w/v) was added. Then, 2.0 ml of the resultant mixture was mixed with 2.0 ml of distilled water and 0.4 ml of 0.1% (w/v) ferric chloride ( $FeCl_3 \cdot 6H_2O$ ) solution. The absorbance of the reaction mixture was measured at 700 nm after 10 minutes of the reaction. The ferric reducing antioxidant power of extract was however expressed as gallic acid equivalent/gram (GAE/g).

Scavenging activity on DPPH free radicals by the extract was determined using the method of Gyamfi, *et al.* [30] with slight modification. A 2-fold in 80% methanol dilution of 1ml of the extract at different concentration was mixed with 0.5 ml of 0.076 mM DPPH in methanol. The mixture was vigorously shaken and allowed to stand at room temperature in the dark for 25 minutes. The negative control

was 1 ml of 0.076 mM DPPH in methanol. L-ascorbic acid was used as the positive control. Thereafter, the absorbance of the assay mixture was measured at 517 nm. Lower absorbance of the reaction mixture indicated higher radical scavenging activity. DPPH radical scavenging activity was calculated as % inhibition or % reduction of DPPH radical activity.

The total antioxidant capacity (TAC) of MSK was determined by the phosphor molybdenum assay using the method described by Ritu., *et al* [31]. MSK extract at different concentrations (15.63.-1000 mg/ml) were mixed with 1ml of reagent solution (0.6M sulphuric acid, 28 mM sodium phosphate and 4mM ammonium molybdate) in capped tubes. The capped tubes were incubated in thermal block at 95°C for 90 minutes. After cooling to room temperature, the absorbance of the aqueous solution of each was measured at 695 nm against blank. Total antioxidant capacity, TAC, of the sample was expressed as ascorbic acid equivalent per gram (AAE/g).

#### **Determination of serum activity of catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (Gpx)**

The catalase activity was assayed by the method of Sinha [32]. To 0.9 ml of phosphate, 0.1 ml of serum and 0.4 ml of H<sub>2</sub>O<sub>2</sub> was added. The reaction was initiated by adding 2 ml of dichromate acetic acid mixture. The tubes were kept in a boiling water bath for 10 minutes, cooled and the colour developed was read at 530 nm at intervals of 30 minutes for 2 hrs. Standards in the concentration range of 20 - 100 micromoles were processed for the test. The catalase activity was calculated as micromoles of H<sub>2</sub>O<sub>2</sub> utilised/second expressed as IU/L.

Superoxide dismutase activity was assayed by the method of Arthur and Boyne [33] as contained in Randox kit. To a 0.05 ml diluted sample in a test tube was added 1.7 ml mixed substrate solution and mixed. Xanthine oxidase (0.25 ml) was added and the initial absorbance taken after 30 seconds. The final absorbance was taken after 3 minutes and the SOD activity per gram haemoglobin was extrapolated from a standard curve and expressed as IU/L.

Glutathione peroxidase was determined according to the method of Paglia and Valentine [34]. A known volume, 0.05 ml of serum was diluted with 2 ml of diluting reagent. To 50 µl of either the diluted sample or blank was mixed with 1 ml of reagent 1 (glutathione+ glutathione reductase+ NADPH) and reagent 2 (cumene hydroperoxide) respectively. The initial absorbance of the test or the blank was respectively read after 1 min and the timer started simultaneously. Absorbances were read again after 1 and 2 minutes intervals at 340 nm.

Glutathione Peroxidase activity was calculated from the formulae below.

$$GPx (IU/L) = 8412 \times \Delta A_{340} \text{ nm/minute.}$$

#### **Determination of serum concentration of reduced glutathione (GSH), uric acid and malondialdehyde**

The reduced glutathione (GSH) concentration was determined based on the principle as stated in Mbah and Egbuonu [35].

The uric acid concentration was determined according to the method of Trinder [36] as contained in Biotrust assay kit. Test tubes were set up for sample and standard respectively. Uric acid reagent, 1 ml was dispensed into all tubes, 25 µl of sample and standard were added to appropriate tubes, mixed and incubated at room temperature for 10 mins. Absorbances were taken at 520 nm using spectrophotometer. Concentration of magnesium in sample was calculated by dividing absorbance of sample tube with absorbance of standard tube multiplied by the concentration of the standard and expressed as Mg/100 ml.

Malondialdehyde, one of the thiobarbituric acid reacting substances (TBARS) that measures the extent of lipid peroxidation, was determined by spectrophotometric estimation of as described by Wallin., *et al* [37]. To 0.1 ml of the serum was mixed with 0.9 ml of H<sub>2</sub>O in a test tube. A volume, 0.5 ml of 25% TCA (trichloroacetic acid) and 0.5 ml of 1% TBA (thiobarbituric acid) in 0.3% NaOH were also added to the mixture. The mixture was boiled for 40 minutes in water-bath and then cooled in cold water. Then 0.1 ml of 20% sodium dodecyl sulfate (SDS) was added to the cooled solution and mixed properly. The absorbance was taken at wavelength 532 nm and 600 nm against a blank.

$$\% \text{TBARS} = \frac{A532 - A600 \times 100}{0.5271 \times 0.1}$$

### Histopathological examination of the brain sections

On sacrifice, sections of the rats' brain 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 bathe 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 blue (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 (×4, ×10 and ×40) 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). Results were presented as means ± standard error of the mean (Mean ± SEM) at 95% significance level (p < 0.05).

## Results and Discussion

### Results

From table 1 above, mango seed kernel had a dose dependent and higher (p < 0.05) DPPH radical scavenging activity or % inhibition compared to the standard antioxidant, vitamin C which peaked at 125 (µg/ml) but declined from 250 (µg/ml).

Concentration (µg/ml)	MSK (DPPH scavenging activity or % inhibition)	Standard antioxidant, Vitamin C (DPPH scavenging activity or % inhibition)
15.63	90.43 ± 0.02	78.25 ± 0.13
31.25	92.6 ± 0.04	73.92 ± 0.22
62.5	93.62 ± 0.12	78.87 ± 0.21
125	94.05 ± 0.02	82.7 ± 0.34
250	95.21 ± 0.23	77.42 ± 0.21
500	95.36 ± 0.04	75.36 ± 0.11

**Table 1:** 1,1-diphenyl-2-picryl hydrazyl (DPPH) radical scavenging activity (% DPPH inhibition) of pulverized mango (*M. indica*) seed kernel, MSK, as compared to standard antioxidant, vitamin C.

Value presented as mean ± SEM of triplicate determinations. Significant difference at p < 0.05

From table 2, MSK extract exhibited a dose dependent increase in the ferric reducing antioxidant ability as expressed as Gallic acid equivalent per gram. It also, exhibited a dose dependent increase in total antioxidant capacity expressed as Ascorbic acid equivalent per gram, AAE/ g).

Concentration (µg/ml)	FRAP ability (Gallic Acid Equivalent per gram, GAE/g)	Total antioxidant capacity, TAC (Ascorbic acid equivalent per gram, AAE/ g)
15.63	0.160 ± 0.03	0.068 ± 0.00
31.25	0.190 ± 0.31	0.757 ± 0.02
62.5	0.218 ± 0.02	1.53 ± 0.01
125	0.223 ± 0.00	2.64 ± 0.01
250	0.262 ± 0.02	3.8 ± 0.01
500	0.291 ± 0.02	5.78 ± 0.01

**Table 2:** In vitro antioxidant capacity of pulverized mango (*M. indica*) seed kernel, MSK, via ferric reducing antioxidant power (FRAP) measured as Gallic acid equivalent per gram, GAE/g and total antioxidant capacity, TAC, measured as ascorbic acid equivalent per gram, AAE/g

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

The result as shown on table 3 revealed that lower ( $p < 0.05$ ) CAT activity in MSG-exposed rats as compared to rats in control and MSK-groups dose-dependently increased ( $p < 0.05$ ) in rats concomitantly exposed to MSG and MSK extract. The observed increase relative to the control and MSG groups was highest (134.00% and 680.00%, respectively) in the MSG + high dose extract group.

Groups	CAT activity (IU/L)	Change relative to the Control (%)	Change relative to MSG group (%)
Control (distilled water 2 ml/kg b.w)	2.00 ± 0.26	0.00	233.33
MSG 8000 mg/kg b.w)	0.60 ± 0.01	-70.00	0.00
Extract 300 mg/kg b.w)	4.61 ± 0.02	+130.50	+668.33
MSG 8000 mg/kg b.w) + low Extract 200 mg/kg b.w)	3.59 ± 0.08	+79.50	+498.30
MSG 8000 mg/kg b.w) + high Extract 400 mg/kg b.w)	4.68 ± 0.09	+134.00	+680.00

**Table 3:** Effect of pulverized mango (*M. indica*) seed kernel extract, MSK, on catalase, CAT, activity (IU/L) of normal and monosodium glutamate-challenged rats' serum

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

As shown in table 4, results revealed that lower ( $p < 0.05$ ) SOD activity in MSG-exposed rats as compared to rats in control and MSK-groups dose-dependently increased ( $p < 0.05$ ) in rats concomitantly exposed to MSG and MSK extract. The observed increase relative to the control and MSG groups was highest (3.08% and 12.47%, respectively) in the rats exposed to MSG and high dose of the extract.

Groups	SOD activity (IU/L)	Change relative to the Control (%)	Change relative to MSG group (%)
Control (distilled water 2 ml/kg b.w)	+11.35 ± 0.01	0.00	+16.05
MSG 8000 mg/kg b.w)	+9.78 ± 0.13	-13.83	0.00
Extract 300 mg/kg b.w)	+10.76 ± 0.21	-5.20	+10.02
MSG 8000 mg/kg b.w) + low Extract 200 mg/kg b.w)	+10.22 ± 0.19	-9.96	+4.50
MSG 8000 mg/kg b.w) + high Extract 400 mg/kg b.w)	+11.00 ± 0.03	-3.08	+12.47

**Table 4:** Effect of pulverized mango (*M. indica*) seed kernel extract, MSK, on superoxide dismutase, SOD, activity (IU/L) of normal and monosodium glutamate-challenged rats' serum.

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

As shown in table 5, results revealed that higher ( $p < 0.05$ ) GPx activity in MSG-exposed rats as compared to rats in control and MSK-groups dose-dependently reduced ( $p < 0.05$ ) in rats concomitantly exposed to MSG and MSK extract. The observed reduction relative to the control and MSG groups was highest (-26.31% and 32.79%, respectively) in the rats exposed to MSG and high dose of the extract.

Groups	GPx activity (IU/L)	Change relative to the Control (%)	Change relative to MSG group (%)
Control (distilled water 2 ml/kg b.w)	+35.69 ± 0.62	0.00	-8.79
MSG 8000 mg/kg b.w)	+39.13 ± 0.97	+9.64	0.00
Extract 300 mg/kg b.w)	+22.43 ± 1.03	-37.15	-42.68
MSG 8000 mg/kg b.w) + low Extract 200 mg/kg b.w)	+31.95 ± 1.10	-10.48	-18.35
MSG 8000 mg/kg b.w) + high Extract 400 mg/kg b.w)	+26.30 ± 0.38	-26.31	-32.79

**Table 5:** Effect of of pulverized mango (*M. indica*) seed kernel extract, MSK, on glutathione peroxidase, GPx, activity, (IU/L) of normal and monosodium glutamate-challenged rats' serum

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

As shown in table 6, results revealed that higher ( $p < 0.05$ ) GSH activity in MSG-exposed rats as compared to rats in control and MSK-groups dose-dependently reduced ( $p < 0.05$ ) in rats concomitantly exposed to MSG and MSK extract. The observed reduction relative to the control and MSG groups was respectively highest in the MSK extract group (-119.33%) and in the rats exposed to MSG and high dose of the extract.

Groups	GSH concentration (Mg/100 ml)	Change relative to the Control (%)	Change relative to MSG group (%)
Control (distilled water 2 ml/kg b.w)	+3.26 ± 0.01	0.00	-28.35
MSG 8000 mg/kg b.w)	+4.55 ± 0.17	+39.57	0.00
Extract 300 mg/kg b.w)	+2.63 ± 0.08	-19.33	-42.20
MSG 8000 mg/kg b.w) + low Extract 200 mg/kg b.w)	+3.41 ± 0.05	+4.60	-25.05
MSG 8000 mg/kg b.w) + high Extract 400 mg/kg b.w)	+3.97 ± 0.04	+21.78	-12.75

**Table 6:** Effect of pulverized mango (*M. indica*) seed kernel extract, MSK, on reduced glutathione (GSH) concentration (Mg/100 ml) of normal and monosodium glutamate-challenged rats' serum.

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

As shown in table 7, results revealed that higher ( $p < 0.05$ ) MDA concentration in MSG-exposed rats as compared to rats in control and MSK-groups dose-dependently reduced ( $p < 0.05$ ) in rats concomitantly exposed to MSG and MSK extract. The observed reduction relative to the control and MSG groups was highest (18.36% and -14.68%, respectively) in the rats exposed to MSK extract only.

Groups	MDA concentration (% TBARS)	Change relative to the Control (%)	Change relative to MSG group (%)
Control (distilled water 2 ml/kg b.w)	+5.11 ± 0.11	0.00	+27.72
MSG 8000 mg/kg b.w)	+7.07 ± 0.11	+38.36	0.00
Extract 300 mg/kg b.w)	+6.04 ± 0.02	+18.20	-14.68
MSG 8000 mg/kg b.w) + low Extract 200 mg/kg b.w)	+6.92 ± 0.02	+35.42	-2.12
MSG 8000 mg/kg b.w) + high Extract 400 mg/kg b.w)	+6.42 ± 0.06	+25.64	-9.19

**Table 7:** Effect of pulverized mango (*M. indica*) seed kernel extract, MSK, on Malondialdehyde, MDA, concentration (% TBARS) of normal and monosodium glutamate-challenged rats' serum

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

As shown in table 8, results revealed that higher (p < 0.05) uric acid concentration in MSG-exposed rats as compared to rats in control increased (p < 0.05) in MSK-treated rats and not lowered in rats concomitantly exposed to MSG and MSK extract. The observation relative to the control and MSG groups was highest (457.66% and 33.52%, respectively) in the rats exposed to MSK extract alone.

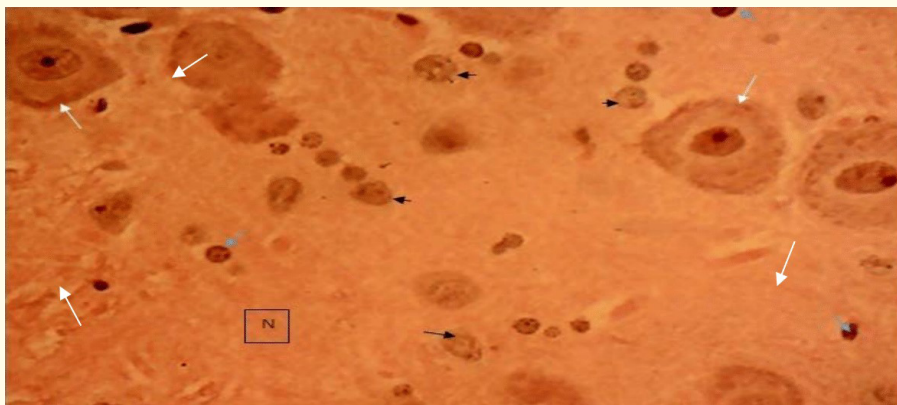
Groups	UA concentration (Mg/100 ml)	Change relative to the Control (%)	Change relative to MSG group (%)
Control (distilled water 2 ml/kg b.w)	+6.85 ± 0.45	0.00	-0.76
MSG 8000 mg/kg b.w)	+28.61 ± 0.27	+317.66	0.00
Extract 300 mg/kg b.w)	+38.20 ± 0.54	+457.66	+33.52
MSG 8000 mg/kg b.w) + low Extract 200 mg/kg b.w)	+28.43 ± 0.65	+315.04	-0.63
MSG 8000 mg/kg b.w) + high Extract 400 mg/kg b.w)	+34.25 ± 0.62	+400.00	+19.71

**Table 8:** Effect of pulverized mango (*M. indica*) seed kernel extract, MSK, on uric acid, UA, concentration (Mg/100 ml) of normal and monosodium glutamate-challenged rats' serum

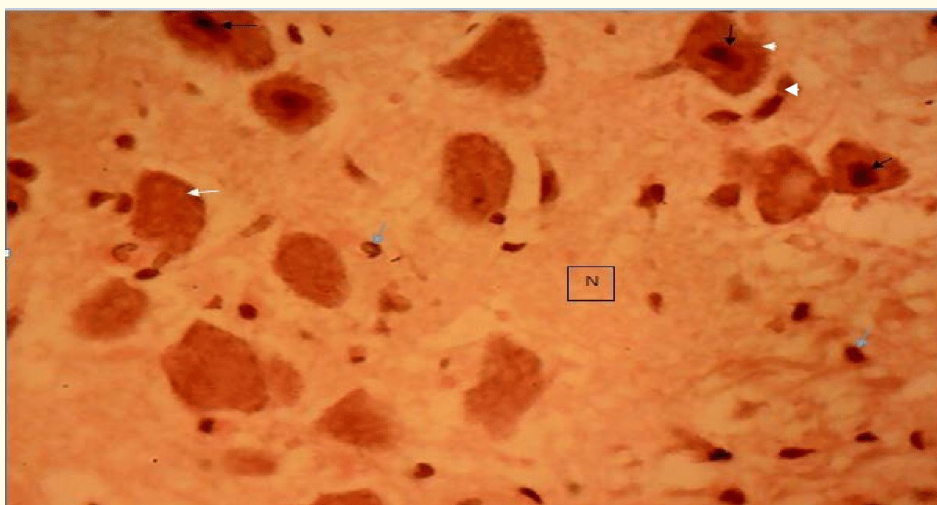
Value presented as mean ± SEM of sample size, n = 4 rats. + denotes higher by; - denotes lower by. Significant difference at p < 0.05.

The effect of pulverized mango (*M. indica*) seed kernel extract, MSK, on the brain histology of normal and monosodium glutamate-challenged rats was shown on the plates (Plate 1-5). Generally, hypothalamic neuronal degeneration and necrosis in the brain histology of MSG-exposed rats contrasted those of MSK- and control- rats. In particular, photomicrographs from sections of the brain collected from the rats in the normal group showed the normal histo-architecture of the mammalian brain (Plate 1). The sections show brain parenchyma composed of neurons with cell bodies which vary in size and in shape, large amount of pale blue cytoplasm and centrally located vesicular nuclei with nucleolus. The neurons are embedded in a framework of glial cells, capillaries and neuropil with normal cell bodies of neurons (white arrow), centrally located nucleus, prominent nucleoli, astrocytes (black arrow), oligodendrocytes (blue arrow) and Neuropil (N). Sections of the brain collected from the MSG-only animals showed hypothalamic neuronal degeneration and necrosis. The affected neuron appeared shrunken (necrotic) or swollen (degenerative), with shrunken to pyknotic nuclei (black arrow) and darker than normal cytoplasm (white arrow). Numerous dark neurons were also observed in the grey matter (artefacts), neuropil (N) and oligodendrocytes (blue arrow).

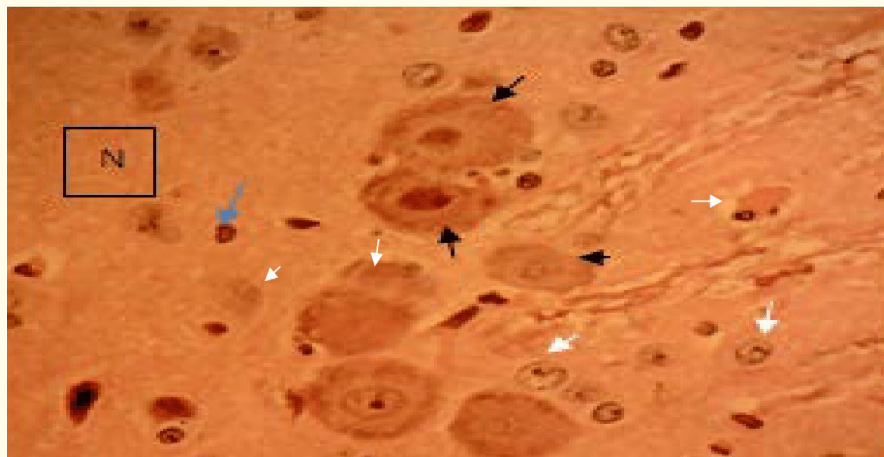




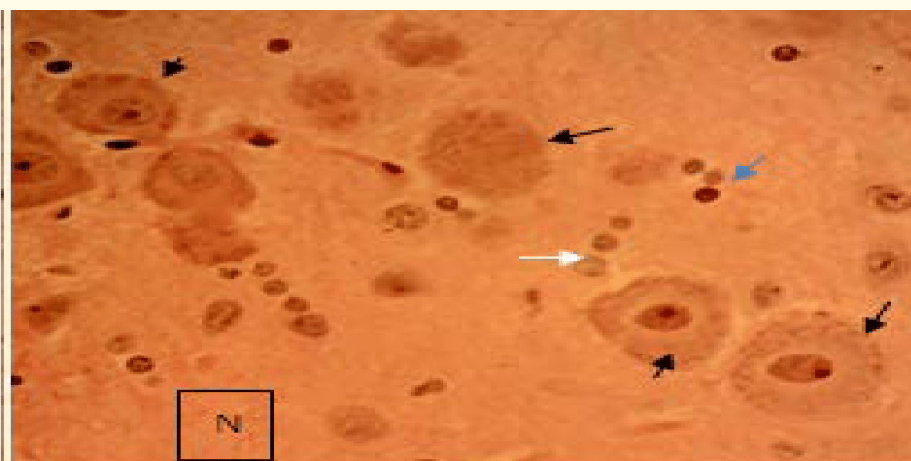
**Plate 1:** Photomicrograph of sections of brain of the rats in the normal group showing normal histo-architecture of the mammalian brain (H & E x 400).



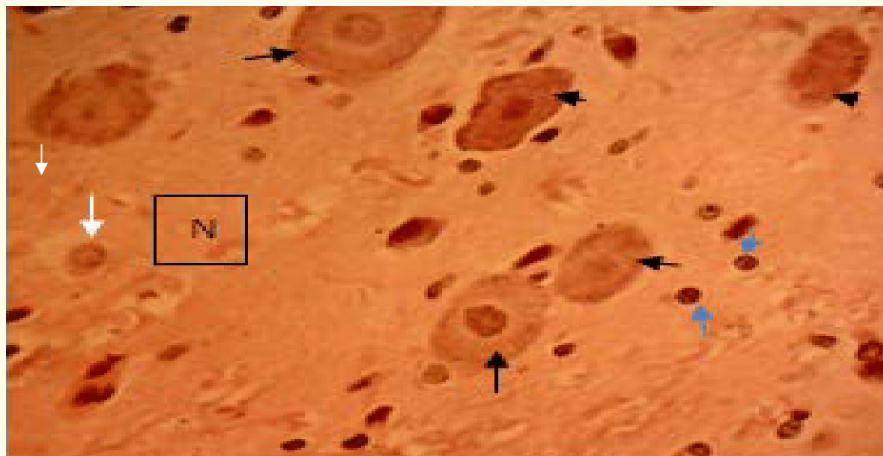
**Plate 2:** Photomicrograph of sections of brain of the rats in the MSG (8 g/kg b.w) group showing hypothalamic neuronal degeneration and necrosis (H & E x 400).



**Plate 3:** Photomicrograph of sections of brain of the rats in the Extract (300 mg/kg b.w) group showing normal histo-architecture of the mammalian brain (H & E x 400).



**Plate 4:** Photomicrograph of sections of Brain of the rats in the MSG (8 g/kg b.w) + extract (200 mg/kg b.w) group showing mild to moderate hypothalamic neuronal degeneration and necrosis (H & E x 400).



**Plate 5:** Photomicrograph of sections of brain of the rats in the MSG (8 g/kg b.w) + extract (400 mg/kg b.w) group showing mild to moderate hypothalamic neuronal degeneration and necrosis (H & E x 400).

Sections of the brain collected from the animals in the extract-only group showed the normal histo-architecture of the mammalian brain. The sections showed brain parenchyma composed of neurons with cell bodies which vary in size and in shape, large amount of pale blue cytoplasm and centrally located vesicular nuclei with nucleolus (black arrow). The neurons are embedded in a framework of glial cells, capillaries, neuropil (N), astrocytes (white arrow) and Oligodendrocytes (blue arrow). Sections of the brains of MSG + low dose extract and MSG + high extract group showed dose-dependent reversal of the neuronal degeneration and necrosis observed in the MSG-only group.

## Discussion

Monosodium glutamate, a common food-flavoring additive, affects the brain and induces oxidative stress. Oxidative stress is a fundamental health-dysfunctions indicator [24,25] while bioactive, notably phenolic, compounds in mango seed kernel suggested that it could be a good source of natural antioxidants [19]. Thus, this study assessed the effect of pulverized underutilized mango (*Mangifera indica* L) seed kernel (MSK) on monosodium glutamate-intoxicated rats' serum antioxidant capacity, brain function and histology. *In vitro*, MSK increased ( $p < 0.05$ ) the DPPH, FRAP and TAC dose-dependently compared to the respective standards, indicating the overall antioxidant potential of MSK. Thus, MSK may elicit antioxidant activity, or sequester free radicals resulting from oxidative stress, by the studied mechanisms (DPPH, FRAP and TAC) involving a combination of scavenging and reducing abilities as suggested in earlier study [29]. In particular, the DPPH ability of MSK was in line with earlier report [38]. For instance,, DPPH scavenge free radicals or exerts antioxidant properties *via* its electron donating capability [39] while total antioxidant capacity (TAC) measures the concentration of electrons donated or radicals quenched by a given antioxidant [31].

The antioxidant capacity of MSK *in vivo* was assessed in normal rats and in MSG-induced oxidative stress rat model according to Mariyamma, *et al* [22]. Results revealed that lower ( $p < 0.05$ ) CAT and SOD activity in MSG-exposed rats as compared to rats in control and MSK-groups dose-dependently increased ( $p < 0.05$ ) in rats concomitantly exposed to MSG and MSK extract. The result confirmed induction of oxidative stress in MSG-treated rats unlike in the MSK-treated rats and the suggested the antioxidant capacity of MSK in mitigating MSG-induced oxidative stress in rats. Generally, SOD converts superoxides to hydrogen peroxides whereas catalase and gluta-

thione peroxidase convert hydrogen peroxide to water and gaseous oxygen. Decreased SOD and CAT activity in MSG only treated rats may have resulted from increased involvement of these enzymes in antioxidant defense response following MSG-induced oxidative stress and deficiency of NADPH resulting from reduction in the redox potential in the cell activity of MSG-treated rats [40]. Thus, the significant ( $p < 0.05$ ) and dose-dependent increase in catalase and superoxide dismutase activity in rats concomitantly exposed to MSG and MSK extract is an indication of the antioxidant potential of mango seed kernel at improving catalase and SOD activity.

The higher ( $p < 0.05$ ) MDA, GPx and GSH in MSG-exposed rats as compared to rats in control and MSK-groups that dose-dependently reduced ( $p < 0.05$ ) in rats concomitantly exposed to MSG and MSK extract further confirmed the induction of oxidative stress in the MSG-treated rats and the potential benefit of MSK extract against, even MSG-induced, oxidative stress in rats. Increased MDA concentration indicated lipid peroxidation hence oxidative stress in animals [41] and was reported following MSG (4 mg/g body weight) administration, in rats' brain [42]. The observed increase in GSH concentration and GPx activity in MSG-only rats could be due to enhanced GSH synthesis in response to increased oxidative stress and perhaps following MSG-up regulated glutamate concentration. Generally, high glutamate concentration up regulates GSH synthesis by competing with and preventing GSH from acting as a feedback inhibitor of Glutamyl-cysteinyl synthetase enzyme resulting in increased synthesis of GSH. However, the observed increase in GSH activity in MSG-only rats contrasted with the reported reduction by Shivasharan, *et al.* [43] but after only 7-day exposure and at a lower MSG-concentration that may not have induced oxidative stress in the rats. Dose and duration differences could account for significant differences in observation of otherwise similar studies.

Glutathione peroxidase utilizes reduced glutathione as its co-substrate to scavenge  $H_2O_2$  formed by the action of SOD [44], thus the observed similar trend in GSH and GPx response was not surprising. This further confirmed MSG-induction of oxidative stress in the rats. Higher GSH concentration, hence imbalance in GSH to oxidized glutathione (GSSG) ratio indicated cellular oxidative stress [44]. Decreased GPx could be resultant to its role as second line of antioxidant defense mechanism following perhaps the overwhelmed antioxidant capacity of SOD and CAT, the first line of antioxidant defense mechanism. CAT may not have effectively converted hydrogen peroxide generated by SOD to water and gaseous oxygen hence involving GPx as a second line of defense leading to its depletion. The decreased activity of GSH and GPx in the MSK extract-only group agreed with earlier report [45], though for mango leaf extract. A decreased GSH and GPx activity in the extract-treated group further suggested the antioxidant capacity of MSK even in normal rats.

To assess the effect of MSK on the brain function of normal and MSG-intoxicated rats, the serum uric acid (UA) concentration was determined in addition to assessing the brain histology. Higher ( $p < 0.05$ ) UA concentration in MSG-exposed rats as compared to rats in control was even increased ( $p < 0.05$ ) in MSK-treated rats and was not lowered ( $p < 0.05$ ) in rats concomitantly exposed to MSG and MSK extract. This suggested preserved brain (and attendant endocrine) function in the rats especially exposed MSK alone followed by those exposed concomitantly to MSG and higher dose of MSK. Generally, uric acid serves as indicator of multiple physiological dysfunctions, including as an antioxidant by for instance inactivating superoxide anion, singlet oxygen and per-oxy nitrate [46] but Manfredini, *et al.* [26] related high UA concentration with lowered neurological/brain damage and cerebral infarction induced by per-oxy nitrite, a toxic derivative of the *in vivo* reaction of nitric oxide with superoxide radicals. Also, high uric acid concentration was associated with reduced brain damage in rat models [47,48].

Thus, the significantly ( $p < 0.05$ ) lower Uric Acid concentration in MSG-only rats compared to the extract-only and MSG + higher MSK extract co-treated groups might have resulted from a first line defense response to increased oxidative stress in the MSG-challenged rats and the ameliorative restoration attempt by MSK at a higher concentration. This position was seemingly supported by the apparent compromised brain integrity due to the hypothalamic neuronal degeneration and necrosis in the brain histology of MSG-only rats. Essentially, the hypothalamic neuronal degeneration and necrosis in the brain histology of MSG-exposed rats contrasted those of MSK- and control- rats, suggestive of a compromised brain histology and functional integrity. Photomicrograph of organ histology collaborated the seric chemistry results [2,49-50] as in the present study.

In a previous similar study, MSK-related modulation of MSG-induced effects was reported in rats [18,51] and attributed to high vitamin C content in mango seed kernel which may enhance the antioxidant potential of MSK [18]. Also, paradoxical response of MSK on MSG-intoxicated rats was reported [18] and attributed to high sodium content of MSK. Thus, the high sodium content of MSK could explain any apparent similar effect as the MSG group whereas the high antioxidant vitamin C content may explain the observed capacity of MSK to mitigate the MSG-intoxication in the rats seric antioxidant parameters, brain function and histology.

## **Conclusion**

The study confirmed a definite MSG-induction of oxidative stress in, probably via enhanced oxidant generation that distorted brain histology of, rats. It further indicated *in vitro* antioxidant potential of MSK and demonstrated the antioxidant capacity of MSK in improving and modulating the brain histology and serum antioxidant capacity of normal and MSG-intoxicated rats, respectively probably by way of enhanced oxidant scavenging activity. Further studies are warranted to explore and harness MSK antioxidant potential in managing, especially MSG-induced, health-dysfunctions presented with oxidative stress in animals.

## **Conflict of Interest**

None exists.

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