

Original Research Article

Ethanol Extract of *Solanum melongena* Linn Fruit Mitigated Monosodium Glutamate-Induced Oxidative Stress

ABSTRACT

Background/Aim: Monosodium glutamate (MSG), a flavour enhancing food additive generally regarded as safe by food regulatory bodies, could induce oxidative stress. The heightened search for underutilized plant-sourced food necessitated this study aimed at determining some anti-nutrients and vitamins in *Solanum melongena* Linn fruit and the influence of the ethanol extract of the sample on MSG-induced oxidative stress. **Study Design/Methodology:** Standard protocols were employed in the determination of the studied phyto-contents of the sample. In the animal study, twenty four Wistar rats with average weight of 105 ± 7.00 g were assigned into six groups and fed thus: Group 1 (control, feed and distilled water only), Group 2 (8000 mg/kg body weight MSG), Group 3 (300 mg/kg body weight the sample extract), Group 4 (8000 mg/kg body weight MSG+ 100 mg/kg body weight the sample extract), Group 5 (8000 mg/kg body weight MSG+ 300 mg/kg body weight the sample extract) and Group 6 (8000 mg/kg body weight MSG+ 500 mg/kg body weight the sample extract). Exposure was oral and daily for 14 days. **Place and Duration of study:** Department of Veterinary Medicine, Michael Okpara University of Agriculture, Umudike, Nigeria and National Root Crop Research Institute, Umudike, Abia State Nigeria, between May and June, 2016. **Results:** The determined vitamins in the sample were vitamin A (0.36 ± 0.02 IU), vitamin B3 (9.03 ± 0.07 mg/100g) and vitamin C (369.67 ± 7.54 mg/100g) while the anti-nutrients were alkaloids (1.13 ± 0.10 mg/100g), saponins (5.54 ± 0.37 mg/100g), tannins (11.87 ± 1.87 mg/100g), cyanogenic glycosidic (6.21 ± 0.22 mg/100g) and phytates (30.62 ± 1.54 mg/100g). The MSG only fed group significantly ($P < .05$) increased malondialdehyde (MDA) concentration but decreased ($P < .05$) glutathione (GSH), catalase (CAT) and superoxide dismutase (SOD) activity compared to the control, suggesting induction and enhanced generation of reactive oxygen species (ROS) which compromised the antioxidant defense of the MSG-fed rats. Co-administration of MSG with ethanol extract of *Solanum melongena* Linn fruit significantly ($P < .05$) reduced the MDA concentration to a value non-significant ($p > 0.5$) when compared to that of the control rats, and conversely similarly significantly ($P < .05$) increased GSH, CAT and SOD activities when compared to the group 2 rats. **Conclusion:** The *Solanum melongena* Linn fruit contains the studied anti-nutrients and vitamins while the ethanol extract of the fruit could significantly mitigate MSG-induced oxidative stress in the rats.

Keywords: *Solanum melongena* Linn fruit, Phyto-nutrients, Vitamins, Reactive Oxygen Species, Antioxidant, MSG-Induced Oxidative Stress.

1. INTRODUCTION

Monosodium glutamate (MSG), a sodium salt of glutamic acid is a food additive with a great flavour enhancing capacity. MSG is produced through fermentation of molasses (1). MSG has been reported to alter mitochondrial lipid peroxidation and antioxidant status in the cells (2). However, the mechanism of glutamate toxicity remains unknown but reports point towards apoptosis, which is a programmed cell death (3). Apoptosis could result to the generation of reactive oxygen species (ROS) which may lead to oxidative stress. Hence, inadvertent or abuse use of MSG could have adverse effects like alteration of the cell defense mechanism via induction of physiological stress, affects the central nervous system, liver function etc (4). This has been shown experimentally on animals and is also dependent on MSG concentration and individual sensitive (2).

Comment [A1]: Safe by food regulatory bodies at low concentration and can induce oxidative stress at higher concentrations

Comment [A2]: I think this should be called phytochemicals not anti-nutrients

Antioxidants are molecules that donate electrons to free radicals to neutralize them. Antioxidants lowers oxidative stress conditions in cells either via preventing oxidation of substrate by ROS or by ameliorating free radicals oxidation via up-regulation of enzymatic antioxidant in the cell like catalase (CAT) and superoxide dismutase (SOD) (5). Oxidative stress conditions describes the redox state of a biological system that is maintained towards more negative redox potentials values. As ROS are generated in the cell increases, there is a concomitant reduction of antioxidant in the cell, which leads to further decrease of the negative potentials that creates an oxidizing environment that encourages oxidative stress. Free radicals (ROS) are reactive species with one or more unpaired electron. They are constantly generated in the cells during ATP production during oxidative phosphorylation. It is vital to understand that free radicals are not harmful at all times, rather their toxicity depends on factors like the type of ROS, their concentration and localization, how they are produced and how they are eliminated (6). Nevertheless, in excess, it is detrimental to the cells as they attack proteins, lipids causing lipid peroxidation and adduct which has been implicated in the aetiology of many adverse health conditions (7).

Solanum species (eggplants) belong to the family of *Solanaceae* and the plant genus *Solanum*. *Solanum melongena* is an economically important vegetable crop that is widely cultivated in the tropical region and a good source of vitamins and phytonutrients (8). The leaves and fruits serve as vegetables and are used in traditional medicine (9). *Solanum melongena* fruit is usually cooked to make soup or stew, especially in the southern and western parts of Nigeria (10). The extracts of *Solanum melongena* were effective against a number of diseases, including high blood pressure, hepatitis and microbes (11; 12), and acts as an antioxidant (13), antidiabetic (14), hypolipidaemic agent (15) and as well as blood purifier (16) owing to its phytonutrients content. This plant possess secondary metabolites like alkaloids, saponins, cyanogenic glycoside, tannins etc, which has been ~~shown~~ demonstrated to possess antioxidant potentials and ascorbic acid which makes it possible for them to acts as reducing agents, metal chelator and radical scavenging (6). In particular, there were no reports that investigated the influence of *Solanum melongena* Linn fruits ethanol extract on MSG-induced oxidative stress in animal models, warranting the present study.

Comment [A3]: I would prefer you use the word phytochemicals than using secondary metabolites. Thanks

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2. MATERIAL AND METHODS

2.1 Plant materials and preparations

Matured egg plant fruits were bought in a local market: Ehere market in Aba, Abia State in the fruiting season of May, 2016. The fruit was identified as *Solanum melongena* Linn in the Plant Science Department, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria. The fruits were washed with clean tap water, crushed into smaller pieces using a knife and were sun-dried for two weeks. The sun-dried fruits were milled into powder using a laboratory miller (Author Thomas Lab. mill, crypto Model, USA) and stored in an air tight container.

Comment [A5]: How do you measure matured eggplant??? What is the duration of growth ?? please specify

Comment [A6]: Why did you sun dry instead of air dry... It seems some vital phytochemicals must have been lost in the process

2.2 Extraction and concentration

The powder (4kg) was immersed in absolute ethanol (98%) for 72 hours with interval shaking. The extract was filtered with No 1 Whatmann filter paper. The filtrate was concentrated using water bath at 60°C and was further dried in an oven set at 50°C. The extract was placed into a sample bottle and stored at a room temperature until it was required for experiment. The ethanol extract of *Solanum melongena* Linn fruit was then dissolved in water and prepared into three different doses (Low dose; 100mg/kg body weight of the extract; Middle dose; 300mg/kg body weight of the extract, High dose; 500mg/kg body weight of the extract) for administration while monosodium glutamate was also dissolved in distilled water to make an

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aqueous solution. The previous report by Thomas et al. (17) formed the basis for the chosen dose of 8000mg/kg body weight MSG for the intoxication of the rats for 14 days.

2.3 Determination of Vitamins (A, B₁ and C)

Vitamin A, B₁ and C were determined spectrophotometrically (18; 19 and 20) respectively.

2.4 Determination of Phytonutrients

Five phytochemicals/nutrients were determined; Alkaloids and saponins by the method described by Okwu and mora (21), cyanogenic glycosides and phytates by the methods described by Onwuka (18) and Young and greaves (22) respectively. Tannins were determined by Folin-Dennis colorimetric method as described by Makkar et al. (23).

2.5 Animal study design

Twenty-four adult Wistar rats of mean body weight 105.00 ± 8.00 g, was obtained from the animal breeding unit of the College of Veterinary Medicine, Michael Okpara University of Agriculture, Umudike. The animals were kept in appropriate cages and in a well ventilated room with free access to standard feed and clean tap water under room temperature with a 12 hour day/night cycle throughout the period of experiment. All the animals received humane care in accordance with the guidelines of the National Institute of Health, USA for ethical treatment of laboratory animals (24). This guideline was approved by the ethical committees of the department of Biochemistry and college of Natural science Michael Okpara university of Agriculture, Umudike, Nigeria.

Comment [A8]: What is the sex of the rats used in this study

After one week of acclimatization, the animals were randomly grouped into six groups of four animals, as shown in the table below. The rats were fed with Vital feed grower mash and were given water *ad libitum* during acclimatization and through the exposure duration. The MSG (99% min) FCC grade E621 a product of meihua group, China.

Comment [A9]: Reference

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The animals received the treatment as given below;

Group	Exposure
Group 1	Received feed + 1ml/kg body weight of distilled water only.
Group 2	8000mg/kg body weight of MSG only.
Group 3	300mg/kg body weight of ethanol extract of <i>Solanum melongena</i> Linn fruit.
Group 4	8000mg/kg body weight of MSG + 100mg/kg body weight extracts of <i>Solanum melongena</i> Linn fruit.
Group 5	8000mg/kg body weight of MSG + 300mg/kg body weight extracts of <i>Solanum melongena</i> Linn fruit.
Group 6	8000mg/kg body weight of MSG + 500mg/kg body weight extracts of <i>Solanum melongena</i> Linn fruit.

The treatment was per-oral (using oral gastic tube) and was administered daily for 2 weeks (fourteen days).

after 14days of the treatment, the rats were sacrificed by decapitation and the liver excised, rinsed in iced-cold sucrose, and a 10% w/v homogenate was prepared using 0.15m KCl, centrifuged and the supernatant used for determination of malondialdehyde (MDA), catalase(CAT), superoxide dimutase (SOD) and glutathione (GSH) activities.

2.6 Quantification of oxidative stress biomarker in liver homogenates

2.6.1 Determination of malondialdehyde levels (MDA)

MDA levels, an index of lipid peroxidation were measured by the double heating method (25). The method is based on the spectrophotometric measurement of the purple colour generated by the reaction of TBA with MDA. The absorbance was measured at 532 nm. More MDA is generated in organs under oxidative stress, thus, more chromogen is produced.

2.6.2 Determination of Reduced Glutathione (GSH)

The method of Ellman (26) was used to determine the serum glutathione concentration. This method is based on the principle there is a development of a yellow colour using the Ellmans reagent 5,5 dithio-bis-2-nitrobenzoic acid (DTNB) to compounds containing sulphhydryl groups. The absorbance is read at 412nm.

2.6.3 Determination of Superoxide Dismutase (SOD) Activity

The superoxide dismutase was determined by the method Marklund and Marklund, (27). This method is based on the principle that pyrogallol autooxides rapidly in alkaline solution generating superoxide ions. SOD inhibits its auto oxidation dismutating the superoxide ions to hydrogen peroxide and molecular oxygen. The activity of inhibition by SOD was measured at 450nm.

2.6.4 Determination of Catalase (CAT) Activity

The catalase activity was determined by the method described by Shina, (28). This method is based on the principle that catalase split hydrogen peroxide. At specific time interval, the reaction was stopped by the addition of dichromate/ acetic acid reagent. When heated in the presence of hydrogen peroxide, dichromate in acetic acid was reduced to chromic acetate and an unstable perchromic intermediate. The remaining hydrogen peroxide was determined by measuring the chromic acetate formed by a wavelength of 610nm.

2.7 Statistical Analysis

Collected data were subjected to statistical Analysis of Variance (ANOVA) with the statistical package for social sciences (SPSS) for Windows version 22.0. The Duncan post hoc test was used to identify the means that differ significantly at $p < 0.05$. Results were expressed as Mean $\pm$ standard error of the mean (SEM).

3. RESULTS AND DISCUSSION

Table 1 shows the results of some vitamins (mg/100g) in the *Solanum melongena* Linn fruit and it revealed that a water soluble antioxidant vitamin (Vitamin C, 369.67 mg/100g) was significantly higher than vitamin B (0.50 mg/100g) followed by vitamin A (0.36 mg/100g), which is the least.

Table 1: Some vitamin composition of *Solanum melonegena* Linn fruit

Parameters	Concentration (mg/100g)
Vitamin A	0.36 $\pm$ 0.02
Vitamin B ₁	0.50 $\pm$ 0.04
Vitamin C	369.67 $\pm$ 7.54

Values are expressed as means $\pm$ S.E.M for triplicate determinations.

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151 The results as shown in table 2 in Table 2 on some phytochemical composition of *Solanum melongena*
 152 Linn fruit revealed that phytates was higher (30.62 mg/100g) was higher than the other determined
 153 phytochemical followed by tannin(11.87mg/100g), cyanogenic glycosides (6.21 mg/100g), saponins
 154 (5.54mg/100g) and alkaloids(1.13 mg/100g) being the least.

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155 **Table 2: Some phytochemical composition of *Solanum melonegena* Linn fruit**

Phytochemicals	Concentration (mg/100g)
Alkaloids	1.13 ±0.10
Saponins	5.54 ±0.37
Tannins	11.87±1.87
Cyanogenic glycosides	6.21 ± 0.22
Phytates	30.62 ± 1.54

156 Values are expressed as means ± S.E.M for triplicate determinations.

157 **Table 3: Effects of daily treatment of rats with mono-sodium glutamate and ethanolic extract of**
 158 ***Solanum melongena* Linn fruit on some antioxidant parameters.**

PARAMETERS	MDA (%TBAR)	GSH (mg/dl)	CAT (IU/L)	SOD (IU/L)
Group 1 (Feed + water)	0.95±0.04	4.93±0.18*	4.87±0.12*	8.02±0.05*
Group 2 (8000mg/kg bw MSG)	4.10±0.23*	3.22±0.09	2.48±0.07	3.77±0.08
Group 3 (300mg/kg bw Extract)	1.34±0.17	5.25±0.09*	4.78±0.16*	8.03±0.05*
Group 4 (8000mg MSG + 100mg/kg bw Extract)	1.79±0.22	5.12±0.18*	5.09±0.27*	7.16±0.17*
Group 5 (8000mg MSG + 300mg/kg bw Extract)	1.55±0.23	5.21±0.14*	5.20±0.12*	7.56±1.20*
Group 6 (8000mg MSG + 500mg/kg bw Extract)	1.18±0.09	5.63±0.11*	6.40±0.85*	7.61±1.18*

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160 Values are expressed as mean ±SEM for four replications. *values are significantly different at (P<0.05).
 161 The result presented in table 3, above revealed that rats exposed to ethanol extract of *Solanum*
 162 *melongena* Linn fruit were significantly difference (P<0.05) for serum MDA concentration in group 2
 163 (4.10±0.23%) compared to the group 3,4,5,6 and the control group. Nevertheless, the data revealed no
 164 significant differences (P>0.05) for group 3, 4, 5 and 6. Significant (P<0.05) decrease was observed in the

165 serum GSH concentration, serum CAT concentration and serum SOD concentration for the group 2
166 (3.22±0.09 mg/dl, 2.48±0.07 IU/L and 3.77±0.08 IU/L respectively) compared to the control group and the
167 rest of the treatment groups either ethanol extract and/or with MSG (group 3, 4, 5 and 6).
168

169 Reports have shown that antioxidants from plants are of nutraceutical values. In this study, oxidative
170 stress induced by MSG and its mitigation using ethanol extract of *Solanum melongena* Linn fruit was
171 investigated. Table 1 showed the result for the vitamin composition. Vitamins are required for normal
172 processes of metabolism including growth and maintenance of health. They act as co-enzymes to modify
173 the enzyme activities. From the result, the Vitamin A content (0.36 ± 0.02 IU) of *S. melongena* is higher
174 than the value (0.078 mg/100g) reported by Akoto et al. (29) for *S. torvum*. This result reveals a potential
175 roles for this plant extract in mediate neural crest development, through ensuring proper positioning of the
176 cells during development of the embryo (30) and in maintaining the integrity of white blood cells. These
177 functions are attributed to their antioxidant potentials.

178 The vitamin B₁ (0.50 ± 0.04 mg/100g) is comparable to the value (0.45 ± 0.02 mg/100g) reported by Eze
179 and Kanu, (31), for *S. aethopicum* but higher than the value (0.08mg/100g) reported by Fowomola, (32)
180 for mango seed. This suggests that *Solanum melongena* Linn fruit could play vitamin B1 mediated roles;
181 it is required to catabolize carbohydrates, fats and proteins and in ATP production. *S. melongena* in this
182 study recorded a significant amount of vitamin C (369 ± 7.54 mg/100g) compared to the other estimated
183 vitamins in this study. *S. melongena* serves as a rich source of vitamin C: an effective antioxidant to stand
184 against oxidative stress and improves immune function of the cells (33), aids the formation of bile which
185 plays a detoxifying roles in the body, protects from cataracts and scurvy (34) and may prevent the
186 oxidation of low density lipoprotein cholesterol (LDL-c) (35).

187 The phytochemical results presented in Table 2 showed five phytonutrients of *Solanum melongena* Linn
188 fruit. Alkaloids recorded (1.31 ± 0.10 mg/100g). This value is comparable to the value (1.16 ±
189 0.09mg/100g) reported by Agoreyo et al.(8) for *S. melongena* and (1.75 ± 0.01%) reported by Egbuonu
190 and Nzewi, (36) for processed bitter yam but lower than the value (5.0 ± 0.77mg/100g) reported by Eze
191 and Kanu, (31) for *S. aethopicum*. Alkaloids are used in local anesthesia, as an active antibacterial
192 (quinine), antifungal and antihypertensive (indole alkaloids) agent (37; 38).

193 The tannin content of the present study (11.87 ± 1.87 mg/100g) is comparable to the value (12.82 ± 0.14
194 mg/100g) reported by (8) for *S. melongena* but significantly higher compared to the value (2.12 ± 0.00%)
195 and (0.17 ± 0.07 mg/100g) reported by Egbuonu and Nzewi, (36) for processed bitter yam and (39) for *S.*
196 *anguivi* respectively. This concentration suggests that *S.melongena* is relatively a rich source of tannin:
197 an anti-diarrheal and anti-inflammatory agent (40; 41). The saponin content in this study was (5.54 ±
198 0.37mg/100g). This value is not comparable to the values (1.29 ± 0.11 mg/100g) reported by oyeyemi et
199 al.(39) for *S. anguivi* and 14.00 ± 0.23% reported by Eze and Kanu, (31) for *S. aethopicum*. This
200 difference may be due to species differences. Saponins are of pharmacological importance, they inhibits
201 microbial growth and possess anti-haemolytic potentials (42; 38).

202 The cyanogenic glycoside (6.21 ± 0.22 mg/100g) is higher than the value (5.71 ± 0.29%) reported by
203 Oyeyemi et al. (39) for *S. anguivi*. This suggests that *S. melongena* Linn fruit could be toxic as a potent
204 poison and should be treated with caution. Glycosides could be useful in the management of malignant

Comment [A13]: Under MDA I strongly believe the asteris sign should come to group 1,3, 4, 5, and 6 because there is a significant difference between group 2 (positive control) and other groups.

cells in the stomach (43). Phytates recorded the highest value (30.62 ± 1.54 mg/100g) among all the determined phytonutrients. Agoreyo et al. (8), reported a comparable value (26.19 ± 0.15 mg/100g) for *S. melongena*. This reveals that *S. melongena* is rich in phytate. Phytates acts as anticancer, anti-calcification agent and also plays a role in the regulation of insulin secretion (44). These said roles are all a function of the antioxidant potentials.

The in-vivo antioxidant potentials of *Solanum melongena* Linn fruit (Table 3) showed that the lipid peroxidation activity in this present study: expressed in TBARS% that measured MDA concentration increased in the MSG fed rats (group 2). This indicates lipid peroxidation and suggests oxidative stress conditions in the rats upon induced toxicity. Group 3, 4, 5 and 6 co-administered with the ethanol extract (in high, medium and low dose) showed a significant ($P \leq .05$) reduction for MDA compared to the control group. This suggests that the plant extract may have enhanced the mechanism of defence to inhibit free radical oxidation on lipids. This report is in agreement with the previous study reported by Hanza and Al Harbi, (45) on how MSG may induce oxidative stress and lipid peroxidation and also comparable to the report by Sivakrishan and Kottaimuthu, (46) on paracetamol-induced liver toxicity.

Comment [A14]: reference

The present investigation further revealed significant ($P \leq .05$) decreases in GSH, SOD and CAT for the MSG fed group (group 2) compared to the control group. This report agrees with the reported findings by (45) and (33). GSH, an important antioxidant synthesized from glycine, glutamate and cysteine is the most abundant intracellular thiol based antioxidant to scavenge free radicals in the body. The reduction in the concentration of GSH for the MSG fed group (group 2) suggests that MSG at high concentration modulate the generation of free radicals in the body. Furthermore, group 3, 4, 5 and 6 were upregulated and thus scavenge the free radicals generated by the system. The scavenging action may be through conjugation of the reactive end of the free radical via glutathione – S – transferase catalyzed reaction to maintain cellular antioxidant defences. The SOD and CAT protected cells from superoxides and hydrogen peroxides respectively. These reduced activities suggest that the enzymes may have done defensive works for the cells against the invasion of free radicals generated from the increased lipid peroxidation. This report is comparable to the findings by Sivakrishan and Kottaimuthu, (46) and Hanza and Al Harbi, (45) on *In vivo* antioxidant activities. Interestingly, group 3, 4, 5 and 6 showed an increased activity of the enzymes, GSH, SOD and CAT. This suggests that the ethanol extract of *Solanum melongena* Linn fruit could improve the *In-vivo* antioxidant defence system.

234

235 5. CONCLUSION

Solanum melongena Linn fruit contains the studied anti-nutrients and vitamins and its ethanol extract at different dosages ameliorated MSG-induced oxidative stress in the rats. Further studies are warranted to determine the specific contribution of the determined phyto-constituents of the *Solanum melongena* Linn fruit in mitigating MSG-induced oxidative stress in rats.

240 ETHICAL APPROVAL

The procedure for this study was in accordance with the guidelines on the care and wellbeing of research animals (NIH, 1985) and was approved by the Ethics Committee of Department of Biochemistry, Michael Okpara University of Agriculture, Umudike, Nigeria.

Comment [A15]: please fix this in the materials and method section

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