



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

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### Authors' contributions

This work was carried out in collaboration between both authors. Author ACCE designed and supervised the study. Author UOM performed the statistical analysis, wrote the protocol, wrote the first draft of the manuscript, managed the analyses of the study and the literature searches. Both authors read and approved the final manuscript.

### Article Information

DOI: 10.9734/IJBCRR/2017/35055

#### Editor(s):

(1) KV Ramanath, Department of Pharmacy Practice, SAC College of Pharmacy, B.G.Nagar, Mandya (Dist), Karnataka, India.

#### Reviewers:

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(3) Ukegbu Chimere Young, University of Nigeria, Nigeria.

Complete Peer review History: <http://www.sciencedomain.org/review-history/20276>

Original Research Article

Received 25<sup>th</sup> June 2017  
Accepted 19<sup>th</sup> July 2017  
Published 29<sup>th</sup> July 2017

### ABSTRACT

**Background/Aim:** Monosodium glutamate (MSG), a flavour enhancing food additive generally regarded as safe by food regulatory bodies at low concentration can induce oxidative stress at high concentration. The heightened search for underutilized plant-sourced food necessitated this study aimed at determining some phytochemicals 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 phytochemicals and vitamins of the sample. In the animal study, twenty four Wistar rats with average weight of  $105.00 \pm 7.00$  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.

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**Results:** The determined vitamins in the sample were vitamin A ( $0.36 \pm 0.02$  IU), vitamin B3 ( $9.03 \pm 0.07$  mg/100 g) and vitamin C ( $369.67 \pm 7.54$  mg/100 g) while the phytochemicals were alkaloids ( $1.13 \pm 0.10$  mg/100 g), saponins ( $5.54 \pm 0.37$  mg/100 g), tannins ( $11.87 \pm 1.87$  mg/100 g), cyanogenic glycoside ( $6.21 \pm 0.22$  mg/100 g) and phytates ( $30.62 \pm 1.54$  mg/100 g). 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 ( $P = .05$ ) compared to that of the control rats, and conversely, significantly ( $P = .05$ ) increased GSH, CAT and SOD activities compared to the group 2 rats.

**Conclusion:** The *Solanum melongena* Linn fruit contains the studied phytochemicals and vitamins while the ethanol extract of the fruit could significantly mitigate MSG-induced oxidative stress in the rats.

**Keywords:** *Solanum melongena* Linn fruit; phytonutrients; 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 points 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 sensitivity [2].

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 upregulation 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 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 via 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 in the production of 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 phytochemicals [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 phytochemical content. This plant possess phytochemicals like alkaloids, saponins, cyanogenic glycoside, tannins etc, which has been shown to possess antioxidant potentials and ascorbic acid which makes it possible for them to acts as reducing agents, metal chelator and radical scavenger [6]. The above interesting nutraceutical potentials of

*Solanum melongena* Linn fruit made it imperative to investigate the influence of its ethanol extract on MSG-induced oxidative stress in animal models.

## 2. MATERIALS AND METHODS

### 2.1 Plant Materials and Preparations

Matured egg plant (55 – 80 days) 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 air-dried for two weeks. The air-dried fruits were milled into powder using a laboratory miller (Author Thomas Lab. mill, crypto Model, USA) and stored in an air tight container.

### 2.2 Extraction and Concentration

The powder (4 kg) 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 in a refrigerator at 4°C 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; 100 mg/kg body weight of the extract; Middle dose; 300 mg/kg body weight of the extract, High dose; 500 mg/kg body weight of the extract) for administration while monosodium glutamate was also dissolved in distilled water to make an aqueous solution. The previous report by Thomas et al. [17] formed the basis for the chosen dose of 8000 mg/kg body weight MSG for the intoxication of the rats for 14 days.

### 2.3 Determination of Vitamins (A, B<sub>1</sub> and C)

Vitamin A, B<sub>1</sub> and C were determined spectrophotometrically [18,19,20] respectively.

### 2.4 Determination of Phytonutrients

Five phytochemicals 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 male adult Wistar rats of mean body weight 105.00 ± 7.00 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.

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 water *ad libitum* during acclimatization and throughout the exposure duration. The MSG (99% min) FCC grade E621 used is a product of meihua group, China.

The animals received the treatment as given below;

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

The treatment was per-oral (using oral gastric tube) and was administered daily for 2 weeks (fourteen days). After 14 days 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 0.15 M KCl, centrifuged and the supernatant used for determination of malondialdehyde (MDA), catalase(CAT), superoxide dismutase (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 tribromoanisole (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 glutathione concentration. This method is based on the principle that 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 was read at 412 nm.

### 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 in alkaline solution rapidly generates 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 450 nm.

### 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 610 nm.

## 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

The result presented in Table 3, revealed that rats exposed to ethanol extract of *Solanum melongena* Linn fruit were significantly different ( $P=0.05$ ) for liver homogenate MDA concentration in group 2 ( $4.10 \pm 0.23\%$ ) compared to the group 3,4,5,6 and the control group. Nevertheless, the data revealed no significant difference ( $P=0.05$ ) for group 3, 4, 5 and 6. Significant ( $P=0.05$ ) decrease was observed in the liver homogenate GSH concentration, CAT concentration and SOD concentration for the group 2 ( $3.22 \pm 0.09$  mg/dl,  $2.48 \pm 0.07$  IU/L and  $3.77 \pm 0.08$  IU/L respectively) compared to the control group and the rest of the treatment groups either ethanol extract and/or with MSG (group 3, 4, 5 and 6).

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

**Table 1. Some vitamin composition of *Solanum melongena* Linn fruit**

Parameters	Concentration (mg/100 g)
Vitamin A	0.36 ± 0.02
Vitamin B <sub>1</sub>	0.50 ± 0.04
Vitamin C	369.67 ± 7.54

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

The vitamin B<sub>1</sub> (0.50 ± 0.04 mg/100 g) is comparable to the value (0.45 ± 0.02 mg/100 g) reported by Eze and Kanu, [31], for *Solanum aethopicum* but higher than the value (0.08 mg/100 g) reported by Fowomola, [32] for mango seed. This suggests that *Solanum melongena* Linn fruit could play vitamin B1 mediated roles; it is required to catabolize carbohydrates, fats and proteins and in ATP production. *S. melongena*, in this study recorded a significant amount of vitamin C (369 ± 4 mg/100 g) compared to the other estimated vitamins. *S. melongena* serves as a rich source of vitamin C: an effective antioxidant to stand against oxidative stress and improves immune function of the cells [33], aids the formation of bile which plays a detoxifying roles in the body, protects from cataracts and scurvy [34] and may prevent the oxidation of low density lipoprotein cholesterol (LDL-c) [35].

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

The tannin content of the present study (11.87 ± 1.87 mg/100 g) is comparable to the value (12.82 ± 0.14 mg/100 g) reported by Agoreyo et al. [8] for *S. melongena* but significantly higher compared to the value (2.12 ± 0.00%) and (0.17 ± 0.07 mg/100 g) reported by Egbuonu and Nzewi, [36] for processed bitter yam and Oyeyemi et al. [39] for *Solanum anguivi* respectively. This concentration suggests that *S. melongena* is relatively a rich source of tannin: an anti-diarrheal and anti-inflammatory agent [40,41]. The saponin content in this study was

(5.54 ± 0.37 mg/100 g). This value is not comparable to the values (1.29 ± 0.11 mg/100 g) reported by Oyeyemi et al. [39] for *S. anguivi* and 14.00 ± 0.23% reported by Eze and Kanu, [31] for *S. aethopicum*. This difference may be due to species differences. Saponins are of pharmacological importance, they inhibit microbial growth and possess anti-haemolytic potentials [42,38].

**Table 2. Some phytochemical composition of *Solanum melongena* Linn fruit**

Phytochemicals	Concentration (mg/100 g)
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

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

The cyanogenic glycoside (6.21 ± 0.22 mg/100 g) is higher than the value (5.71 ± 0.29%) reported by Oyeyemi et al. [39] for *S. anguivi*. This suggests that *S. melongena* Linn fruit could be toxic if consumed in high concentration, considering its significant cyanogenic glycosides concentration. Cyanogenic glycoside is a potent poison and should be treated with caution [43]. However, they could be useful in the management of malignant cells in the stomach [43]. Phytates recorded the highest value (30.62 ± 1.54 mg/100 g) among all the determined phytochemicals. Agoreyo et al. [8], reported a comparable value (26.19 ± 0.15 mg/100 g) for *S. melongena*. This reveals that *S. melongena* is rich in phytate. Phytates act as anticancer, anticarcinogenic agent and also play 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) compared to the control. This indicates lipid peroxidation and suggests oxidative stress conditions in the rats upon MSG-induced toxicity. Group 3 and group 4, 5 and 6 which were co-administered with ethanol extract (in high, medium and low dose) showed a significant (P=0.05) reduction for MDA compared to the control group. This suggests that the plant extract may have enhanced the mechanism of

**Table 3. *In vivo* antioxidant results of rats in different groups daily administered with MSG (8000 mg/kg b.w.) and ethanolic extract of *Solanum melongena* Linn fruit at different concentration (300, 100, 300 and 500 mg/kg b.w) for two weeks**

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 (8000 mg/kg bw MSG)	4.10±0.23	3.22±0.09	2.48±0.07	3.77±0.08
Group 3 (300 mg/kg bw Extract)	1.34±0.17*	5.25±0.09*	4.78±0.16*	8.03±0.05*
Group 4 (8000 mg MSG + 100mg/kg bw Extract)	1.79±0.22*	5.12±0.18*	5.09±0.27*	7.16±0.17*
Group 5 (8000 mg MSG + 300mg/kg bw Extract)	1.55±0.23*	5.21±0.14*	5.20±0.12*	7.56±1.20*
Group 6 (8000 mg MSG + 500mg/kg bw Extract)	1.18±0.09*	5.63±0.11*	6.40±0.85*	7.61±1.18*

Values are expressed as mean ± SEM for four replications. \*values are significantly different at (P=0.05)

defence to inhibit free radical oxidation on lipids [45]. This report is in agreement with the previous study reported by Hanza and Al Harbi, [46] on how MSG may induce oxidative stress and lipid peroxidation and also comparable to the report by Sivakrishan and Kottaimuthu, [47] on paracetamol-induced liver toxicity.

The present investigation further revealed significant (P =.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 Hanza and Al Harbi, [46] and Egbuonu, [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 GSH concentrations was up-regulated 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, [47] and Hanza and Al Harbi, [46] on *In vivo* antioxidant activities. Interestingly, group 3, 4, 5 and 6 showed an increased activity of the enzymes, GSH, SOD and CAT, an indication that the ethanol extract of *Solanum melongena* Linn fruit could improve the *In-vivo* antioxidant defence system.

#### 4. CONCLUSION

*Solanum melongena* Linn fruit contains the studied phytochemicals 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.

#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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