

Hepatoprotective Potentials of Ethanolic Extract of *Solanum melongena* Linn Fruit on Monosodium Glutamate- Intoxicated Rats' and Liver Section Histology

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Authors' contributions

This work was carried out in collaboration among all authors. Author ACCE designed the study. Author UOM performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors UOM and OJ managed the analyses of the study and the literature searches were done by authors NKA and IDN. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/IBRR/2019/45935

Editor(s):

- (1) Dr. Anamika Dhyani, Laboratory of Biochemistry & Molecular and Cellular Biology, Hemocentro-UNICAMP, Brazil.
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Reviewers:

- (1) Gunjegaonkar Shivshankar, M JSPM's Charak College of Pharmacy and Research, India.
(2) S. Murugesan, University of Madras, India.

Complete Peer review History: <http://www.sciencedomain.org/review-history/27897>

Original Research Article

Received 04 October 2018
Accepted 06 December 2018
Published 21 December 2018

ABSTRACT

This study evaluated the hepatoprotective potentials of ethanolic extract of *Solanum melongena* Linn fruit on monosodium glutamate-intoxicated rats and liver section histology using standard protocols. Twenty four Wistar rats that weighed 105.00 ± 7.00 g were divided into 6 groups of 4 each. The six groups were thus fed: 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

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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) daily for 14 days. The MSG only fed group showed a significant ($P=0.05$) increase in the activities of serum alanine aminotransferases (ALT), aspartate aminotransferases (AST), alkaline phosphatases (ALP) and total bilirubin compared to the control group. MSG and ethanolic extract of *Solanum melongena* Linn fruit co-treated groups at different concentration were significantly ($P=0.05$) reduced to become comparable to the control group. The concentration of serum albumin and total protein were significantly ($P=0.05$) reduced for group 2 compared to group 1. The co-administration of MSG and ethanolic extract of *Solanum melongena* Linn fruit resulted to a significant ($P=0.05$) increase notably at 500 mg/kg body weight sample extract and the same was observed for the histology. This suggests the improved synthetic function of the liver. MSG at a high concentration (8000 mg/kg body weight) adversely affected the rats liver biofunction and the hepatoprotective potentials of ethanol extract of *Solanum melongena* Linn fruit was notably at 500 mg/kg of body weight in rats. However, further studies in this direction are warranted.

Keywords: *Solanum melongena* Linn fruit; MSG –intoxication; hepatoprotective; liver; histology.

1. INTRODUCTION

Solanum species (eggplants) belongs to the family of Solanaceae and the plant genus *Solanum*. *Solanum melongena* is known by Igbo(s): Afufa; Yoruba(s): Igba and Hausa(s): Yalo. *Solanum melongena* is an economically important vegetable crop that is widely cultivated in the tropical region. *Solanum melongena* fruit is usually cooked to make soup or stew, especially in the Southern and Western parts of Nigeria [1]. This fruit has been reported to be rich in carbohydrate, fibres, vitamins and minerals. The extracts of *Solanum melongena* were effective against a number of diseases, including high blood pressure, hepatitis and microbial attacks [2,3] and acts as an antioxidant [4,5], analgesic [6], antidiabetic [7], antipyretic [8] and hypolipidaemic agent [9,10] owing to its phytochemical component. Hence, the need to evaluate the possible hepatoprotective potentials of ethanolic extract of *Solanum melongena* Linn fruit in MSG- intoxicated animal model in high concentration.

Monosodium glutamate (MSG) is a food additive comprising glutamate, an amino acid, and a sodium salt. MSG is produced through fermentation of molasses [11]. MSG is subject to abuse. Reports indicated that consumption of MSG could alter liver function [12,13]. Egbuonu et al. [14], reported that MSG could intoxicate the kidney. Other reports by Onyema [15], Sharma and Deshmukh [13] and Mbah and Egbuonu [5,10], indicate that MSG could adversely affect male reproductive organs, haematological parameters, cardiac health and serum antioxidant potentials. All these effects are

shown experimentally on animals. MSG has been accepted worldwide as a flavour enhancer and is approved without a daily recommended range as it is generally regarded as a safe product by regulatory bodies. However, there is no stated daily recommended range by the food and drug administration considering its sodium content though it has an umami taste which is one major reason for its inadvertent use. MSG influences the appetite by improving the palatability of food [16]. Daily MSG consumption ranges from 0.5 mg/kg to 3 g/kg [17]. Glutamate produced in the body plays a pivotal role in human metabolism, but could be toxic in excess [18,19]. Glutamate could impair memory and learning ability [19], steatohepatitis and results to body weight increase which affects the body physiology [16]. The actual mechanism has not been fully elucidated. However, glutamate catabolic intermediate could play pivotal role in inducing toxicity in rats' liver.

The liver is a large, meaty organ that seat on the right side of the belly. It is one of the most vital organs of the body. The main work of the liver is to filter the blood coming from the digestive tract, before passing it to the rest of the body [20]. It also functions as a centre for metabolism of nutrients, haematological functions, storage of minerals and vitamins, secrete bile, make blood clotting proteins and excretion of waste metabolites. Hence, if the liver is damaged or loss, these functions are impaired, thus demonstrating the liver's great importance. Thus, the hepatoprotective potentials of ethanolic extract of *Solanum melongena* fruit using normal and monosodium glutamate intoxicated Wistar rats, based on changes in the rats liver

function parameters is warranted. In a bid to confirm of its adverse effects, the histological elevation is critical to observed if is any pathological changes observed under a microscope after staining.

2. MATERIALS AND METHODS

2.1 Plant Materials and Preparations

Matured eggplant fruits were bought from 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 and Biotechnology 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 and stored in an airtight container.

2.2 Extraction and Concentration

The powder (4 kg) was immersed in 3000 ml absolute ethanol for 72 hrs with interval shaking. The extract was filtered with No 1 Whatman filter paper. The filtrate was concentrated using a water bath at 60°C and was further dried in an oven set at 50°C. The extract yield was 1.54% w/w. The extract was placed into a sample bottle and stored at 4°C in the refrigerator until it was required for the 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. [18] formed the basis for the chosen dose of 8000mg/kg body weight MSG for the intoxication of the rats for 14 days.

2.3 Reagents and Chemicals

Reagents used for all the experiments were commercial kits and products of Randox, (USA) and Teco (TC), (USA).

2.4 Animal Study Design

Twenty-four periadolescent 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 h day/night cycle throughout the period of the experiment. All the animals received humane care in accordance with the guidelines of the National Institute of Health, USA for the ethical treatment of laboratory animals [21]. 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. The animals were randomly grouped into six groups of four animals, as shown in the table below after one week acclimatization. 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 used is a product of Meihua group, China.

The animals received the treatment as given below:

Group 1: Feed + 1 ml/kg body weight of distilled water only.

Group 2: 8000 mg/kg body weight (bw) of MSG only.

Group 3: 300 mg/kg bw of ethanolic extract of *Solanum melongena* Linn fruit.

Group 4: 8000 mg/kg bw of MSG + 100 mg/kg bw ethanolic extract of *Solanum melongena* Linn fruit.

Group 5: 8000 mg/kg bw of MSG + 300 mg/kg bw ethanolic extract of *Solanum melongena* Linn fruit.

Group 6: 8000 mg/kg bw of MSG + 500 mg/kg bw ethanolic extract of *Solanum melongena* Linn fruit.

The treatment was per-oral (using orogastric tube) and was administered daily for 14 days. At the end of the experiment, after an overnight fast, the rats were sacrificed to obtain blood samples by cardiac puncture using sterile capillary tubes (containing no anticoagulant) as much blood is needed for the examination. Blood samples were separately centrifuged for 10 minutes at 3,000 rpm at room temperature, and the serum was separated and stored in a deep freezer for the determination of the concentration of alanine aminotransferase, alkaline phosphatase, aspartate aminotransferase, total bilirubin, total protein and albumin in the rats serum.

2.5 Liver Function Parameters

Serum Alanine Aminotransferase (ALT)

activity: The assay of ALT activity was by the method of Reitman and Frankel [22]. This method is based on the principle that the pyruvate formed from the alanine aminotransferase catalyzed reaction between α -ketoglutarate and L-alanine is coupled with 2,4-dinitrophenylhydrazine in alkaline medium to form coloured hydrazone. The colour intensity was measured against the blank using UV-VIS Spectrophotometer Labomed UV-2502 at 540 nm.

Serum Alkaline Phosphatase (ALP) activity:

The assay of ALP activity was by the method of Reitman and Frankel [22]. The principle of this method is based on the reaction involving serum alkaline phosphatase and a colorless substrate of phenolphthalein monophosphate, giving rise to phosphoric acid and phenolphthalein which at alkaline pH values turn pink and was determined using UV-VIS Spectrophotometer Labomed UV-2502 at 540 nm.

Serum Aspartate Aminotransferase (AST)

Activity: The assay of AST activity was by the method of Reitman and Frankel [22]. This method is based on the principle that the oxaloacetate formed from the aspartate aminotransferase catalyzed reaction between α -ketoglutarate and L-aspartate is coupled with 2,4-dinitrophenylhydrazine in alkaline medium to form coloured hydrazone. The colour intensity is measured against the blank using UV-VIS Spectrophotometer Labomed UV-2502 at 546 nm.

Serum Total Bilirubin: This was done according to the method of Jendrassik and Grof [23] as contained in Randox assay kits. This method is based on the principle of direct (conjugated) bilirubin reacts with diazotized sulphanilic acid in alkaline medium to form a blue coloured complex. Total bilirubin is determined in the presence of caffeine, which releases albumin bound bilirubin, by the reaction with diazotized sulphanilic acid. The absorbance was read using UV-VIS Spectrophotometer Labomed UV-2502 at 545 nm.

Serum Total Protein: Total protein content in blood plasma was determined using Peterson's modifications of the Micro-Lowry method [24] using a protein assay kit. This method is based on the principle that Cu^{2+} complexes into

functional groups in the protein's peptide bonds. The formation of a Cu^{2+} -protein complex requires two peptide bonds and produces a violet-coloured chelate product which is measured by absorption spectroscopy at 540 nm.

Serum Total albumin: This was determined by the method described by Doumas et al. [25]. This method is based on the principle that serum albumin is measured by its quantitative binding to the indicator 3,3',5,5'-tetrabromo-m cresol sulphonephthalein (bromocresol green, BCG). The albumin-BCG-complex absorbs maximally at 578 nm, the absorbance being directly proportional to the concentration of albumin in the sample.

Histopathological Examination: The histopathological examination of the liver tissues of Wistar rats were done using the method of Drury et al. [26]. This procedure requires the following; fixation and washing of the tissues, dehydration, clearing with xylene, infiltration with paraffin, embedding with paraffin, paraffin section, mounting, staining with haematoxylin and microscopic observation of the slides at x400 magnification.

2.6 Statistical Analysis

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

3. RESULTS AND DISCUSSION

The results in Table 1 showed significantly ($P=0.05$) higher serum ALT, AST, ALP and Total bilirubin activities in groups 2, 3, 4, 5 and 6 compared to the control group. The high serum concentration for these significantly increased group resulted from the ingestion of MSG and the extract as seen in group 3. The data also showed that ethanolic extract of *Solanum melongena* Linn fruit reduced the observed increases ($P=0.05$) revealed in the MSG only fed group. Significantly ($P=0.05$) lower serum ALB and total protein were observed for MSG only fed group (26.85 ± 1.30 g/dl and 3.48 ± 0.08 g/dl) respectively compared to groups 3, 4, 5, 6 and the control group.

Table 1. Effects of daily treatment of rats with mono-sodium glutamate (8000 mg/kg bw) and ethanolic extract of *Solanum melongena* Linn fruit at different concentration (100, 300 and 500 mg/kg bw) on some liver function parameters

Treatment	ALT (IU/L)	AST (IU/L)	ALP (IU/L)	ALB (g/dl)	Total protein (g/dl)	Total Bilirubin (mg/l)
Group 1	43.83 ± 0.91	77.00 ± 1.56	55.67±2.93	58.41±1.09	7.64±0.29*	0.92±0.05
Group 2	98.63 ± 1.45*	201.88 ± 1.51*	132.75±2.50*	26.85±1.30*	3.48±0.08	1.58±0.05*
Group 3	60.38 ± 2.14*	95.75 ± 2.14*	63.13±2.54	59.75±2.08	7.76±1.48*	0.97±0.03
Group 4	84.25 ± 1.87*	139.75 ± 3.45*	92.25±2.71*	37.11±0.83	6.41±0.13*	1.29±0.05
Group 5	71.00 ± 1.46*	127.50 ± 3.89*	77.50±2.59	46.54±2.20	6.83±0.12*	1.19±0.03
Group 6	66.00 ± 2.88*	116.88 ± 3.23*	70.88±0.83	53.44±2.46	7.01±0.05*	1.05±0.03

Values are expressed as mean ± S.E.M for four replicates. *values are significantly different at (P =.05)

Histology results

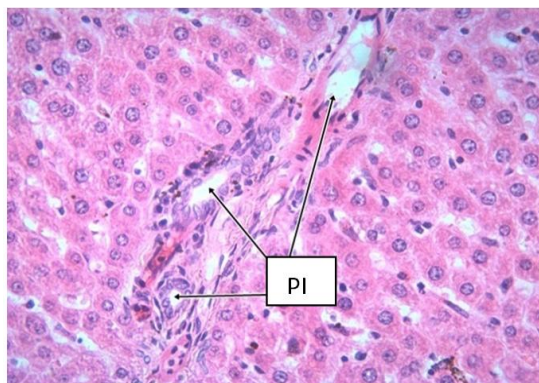


Plate 1. Photomicrograph of the liver (H & E x400) section architecture from rats in normal control (Group 1), showing mild portal inflammation (P) but without interface hepatitis or steatosis

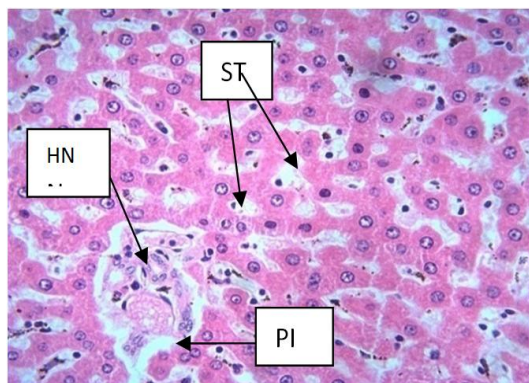


Plate 2. Photomicrograph of the liver (H & E x400) section from rats exposed to MSG (Group 2) showing mild portal inflammation (PI), mild lobular hepatitis and confluent necrosis (HN) and steatosis (ST)

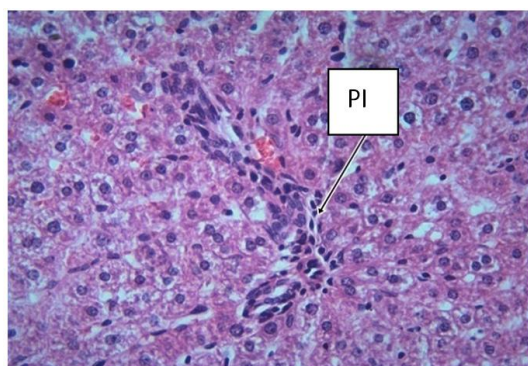


Plate 3. Photomicrograph of the liver (H & E x400) section from rats treated with the extract (Group 3) showing a mild portal inflammation (PI) without interface hepatitis nor interstitial fibrosis

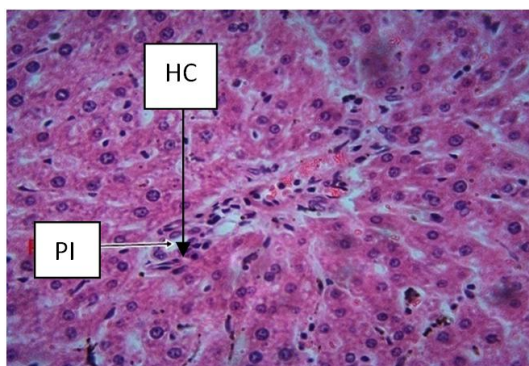


Plate 4. Photomicrograph of the liver (H & E x400) section from rats treated with MSG and 100mg/kg body weight of plant extract (Group 4) showing portal inflammation (PI) and mild lobular hepatitis with occasional confluent necrosis (HC) but no portal inflammation

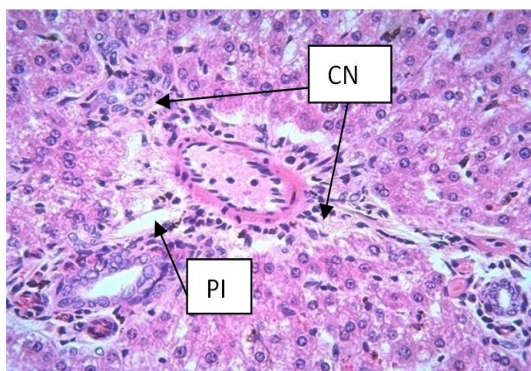


Plate 5. Photomicrograph of the liver (H & E x400) section from rats treated with MSG and 300mg/kg of the plant extract (Group 5) showing mild portal inflammation (PI) and focal confluent necrosis (CN)

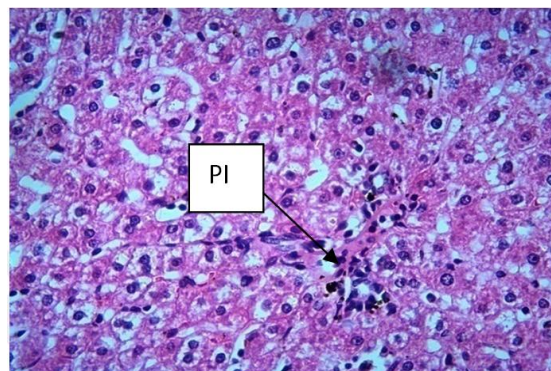


Plate 6. Photomicrograph of the liver (H & E x400) section from rats treated with MSG and 500mg/kg of the plant extract (Group 6) showing mild portal inflammation (PI). There is neither lobular hepatitis nor interstitial fibrosis

4. DISCUSSION

The liver is one of the most vital organs in the body. It acts as a central hub of the cell and detoxifies toxic substances amongst other functions. Impairment of the normal liver function is reflected by alteration in some biochemical indices [14,20]. In the present study, serum ALT, AST and ALP activities in the MSG only rat fed group were significantly ($P=0.05$) higher compared to the control group. These agree with previous reports by Thomas et al. [18]; Egbunu et al. [14] and Madila et al. [27], on the induction of MSG-intoxication in rats. A pointer to the induced toxic effect suffered by the liver, which resulted to the leakage of the liver enzyme biomarkers into the systemic circulation. However, the increases may not be attributed to the liver only as these tested parameters are non-hepatic specific. AST, ALT and ALP are associated with skeletal and cardiac muscles, kidneys and the bone [14]. The observed results do not represent a hepatocellular nor cholestatic pattern of liver toxicity considering that ALT is less to ALP levels in all the groups. These imply that there is more to MSG-intoxications as the observed result for ALT and ALP is not usual. It may have resulted from an infiltrative liver disease and could also be related to possible effects of glutamate catabolic products [6]. Recall that ALP is always present in bile secreting cells in the liver and as such its high concentration as revealed in the study could suggest that there might be blockage of bile flow out of the liver. This holds true considering the observed high concentration of bilirubin in the same group 2 of this study. AST and ALT levels

are almost triple in group 2 compared to the control group. Nonetheless, AST is less liver specific than is ALT level. Comparison of group 2 to groups 3, 4, 5 and 6, showed an interestingly outcome: that *Solanum melongena* fruit ethanolic extracts possess hepatoprotective potentials over MSG induced effects and it was found to be dose dependent. Hence, a reduction in the concentration of the leaked ALT, AST and ALP were observed. This outcome is in line with the reports by Hanzah et al. [28], on hepatoprotective effects of *Solanum melongena* extract on CCL4 induced toxic rats. This further suggests that *Solanum melongena* Linn fruit ethanolic extract would improve hepatic integrity in rats' exposure to MSG in high concentration.

ALB is a part of the total protein levels, which determines the synthetic functions of the liver. The results for ALB and Total protein showed a significant ($P=0.05$) reduction for the MSG only fed group compared to the control group. This suggests that MSG exposure led to the decrease in the total protein and ALB produced by the liver. The study further revealed that the synthesis functions of the liver was improved notably at 500 mg/kg body weight of ethanolic extract of *Solanum melongena* Linn fruit upon comparison to groups 3, 4, 5 and 6. This is a pointer that the extract was able to ameliorate the effects of MSG intoxication in rats' liver. Upon comparison of group 2 results to those of groups 3, 4, 5 and 6, the dose dependent potentials of the ethanolic extract to ameliorate MSG intoxication on rat liver were seen. This report agreed with the reports of Madiha et al. [27] and

Tawfik and Al-Badr, [29]. Furthermore, a significant ($P=0.05$) changes in the serum total bilirubin level for the MSG fed group was recorded compared to the control group. This agrees with reported findings by Tawfik and Al-Badr, [29]. However, the concomitant administration of MSG plus the extracts revealed that the extract could protect the rats from MSG-induced toxic effect on the reticuloendothelial system. Bilirubin is produced by the breakdown of red blood cells and myoglobin by the reticuloendothelial system. This broken bilirubin is then transported to the liver by bounding tightly to ALB, where it is biotransformed through a glucuronidation and sulfation and then excreted as bile. This study showed an increased bilirubin concentration in group 2, which suggests a problem with the liver function compared to the control group and to the groups 3, 4, 5 and 6. These results agree with the results observed for ALB and Total protein as they were decreased upon binding of ALB to the produced bilirubin for transportation and the total protein for making available pigment containing proteins, which were broken by the liver to produced bilirubin.

The histological results provided insight into the nature of the examined organ (Liver). The histological photomicrographs of the section of the liver in the different treatment groups: Plates, 4, 5 and 6 revealed gradual regeneration from portal inflammation, lobular hepatitis and confluent necrosis compared to the group 2 (plate 2). Plate 2 confirmed that MSG at high concentration induced intoxication in the rats which may be through oxidative stress and thus, the observed histomorphological changes [5]. These correlated with serum chemistry results. Plate 3 is of group 3 that were fed with 300mg/kg body weight ethanol extract of *Solanum melongena* Linn fruit were found to be comparable to that of the control (plate 1), it points that the observed higher ALT, AST, ALP and total bilirubin, did not exact any definite adverse effects on the rat liver at that concentration. The gradual regeneration is an indication of the hepatoprotective potential of the ethanolic extract *Solanum melongena* Linn fruit. The hepatoprotective roles were revealed. notably at 500 mg/kg body weight of ethanolic extract of *Solanum melongena* Linn fruit and 8000 mg/kg body weight of MSG (Plate 6) and it appears to be concentration dependent and may be attributed to the bioactive compound present in the plant [28,30]. A similar finding was reported by Hanaa and Saleh [31], on the effect of MSG in rat liver and the ameliorating effect of

Guanidino ethane sulfonic acid (GES). Hence, component of this extract should be an index in further warranted studies.

5. CONCLUSION

MSG at a high concentration (8000 mg/kg body weight) adversely affected the rats liver biofunction and the hepatoprotective potentials of ethanol extract of *Solanum melongena* Linn fruit was notably at 500 mg/kg of body weight in rats. Thus, the ethanol extract of *Solanum melongena* fruit may be useful in the management of MSG intoxication in rats. However, further studies in this direction are needed.

ETHICAL APPROVAL

All the animals received humane care in accordance with the guidelines of the National Institute of Health, USA for the ethical treatment of laboratory animals. 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

CONSENT

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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