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Ameliorative Potentials of Egg Plant (*Solanum melongena* Linn) Fruit Ethanolic Extract on Monosodium Glutamate- Intoxicated Rats' Lipid Profile, Haematology and Heart Histology

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Authors' contributions

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

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Original Research Article

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ABSTRACT

Aim: This study evaluated the ameliorative potentials of ethanolic extract of *Solanum melongena* Linn fruit on monosodium glutamate (MSG)-intoxicated rats' lipid profile, haematological parameters and heart histology using standard protocols.
Methodology: Twenty four Wistar rats that weighed 105.00 ± 7.00 g. The rats were assigned into six groups and fed thus: Group 1 (control, feed and 1 ml/kg body weight (bw) distilled water only), Group 2 (8000 mg/kg bw MSG), Group 3 (300 mg/kg bw sample extract), Group 4 (8000 mg/kg bw MSG +100 mg/kg bw sample extract), Group 5 (8000 mg/kg bw MSG+ 300 mg/kg bw sample extract) and Group 6 (8000 mg/kg bw MSG+ 500 mg/kg bw sample extract) daily for 14 days.
Results: There were significant (P =.05) increase in total cholesterol, triacylglyceride (TAG), very

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low density lipoprotein (VLDL) and low density lipoprotein cholesterol (LDL-c) and significantly (P =.05) decrease in high density lipoprotein cholesterol (HDL-c) in only MSG fed group compared to the control group. Interestingly, MSG co-administration with ethanolic extract of *Solanum melongena* Linn fruit for group 4, 5 and 6 showed significant (P=.05) reduction in serum total cholesterol, TAG, VLDL and LDL-c and increased HDL-c. There was no significant (P =.05) difference in the haematological parameters (Red blood cell, hemoglobin and hemacrit) except for white blood cell count which was significantly (P =.05) reduced in the MSG fed group. The histological results revealed that MSG ingestion in rats induced toxic injuries in their hearts at 8000 mg/kg body weight and effects were slowly being ameliorated as the concentration of the ethanolic extract of *Solanum melongena* Linn fruit increased.

Conclusion: This study confirmed general adverse influence of MSG at a high concentration (8000 mg/kg body weight) and demonstrated the ameliorative role of ethanolic extract of *Solanum melongena* Linn fruit, notably at 300 mg/kg of body weight, on the studied monosodium glutamate-intoxicated bio-functions in rats.

Keywords: Solanum melongena Linn fruit; MSG–intoxication; lipid profile; haematology and heart histology.

1. INTRODUCTION

Monosodium glutamate (MSG) is a food additive comprising glutamate, an amino acid, and a sodium salt. MSG is produced through fermentation of molasses [1]. MSG is subject to abuse. MSG has been accepted world wide as a flavour enhancer and is approved without a daily recommended range as it is generally regarded as a safe product by regulatory bodies. Geha et al. [2] reported that the estimated average daily intake per person in industrialized countries ranges from 0.3-1.0 g. This report is dependent on individual preference. Another report by Shi et al. [3] read that daily MSG consumption ranges from 0.5 mg/kg to 3 g/kg body weight. Reports indicated that MSG consumption could alter cardiac function [4,5]. This effect is shown experimentally on animals. However, the actually mechanism has not been fully elucidated. MSG catabolic intermediate could play pivotal role in inducing toxicity in rats [6].

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 minerals, vitamins, protein, carbohydrate and moisture [7]. About 25 species have been reported in Nigeria, each based on fruit colour, shape and size [8]. The leaves and fruits serve as vegetables and are used in traditional medicine [9]. *Solanum* species includes *S. tuberosum, S. aethiopicum, S. carolinense, S. lycopersicum, S. macrocarpon* and *S. Nigrum* [8]. *Solanum melongena* fruit is usually cooked to make soup or stew, especially in the southern and western parts of Nigeria [8]. The extracts of *Solanum melongena* were effective against a number of diseases, including high blood pressure, hepatosis and microbes [10,11], and acts as an antioxidant [12], analgesic [13], anti-diabetic [14], anti-pyretic [15], hypolipidaemic agent [16] and as well blood purifier [17], owing to its phytochemical content which includes alkaloids, tannins, saponins [7]. Hence, the need to evaluate the possible ameliorative potentials of ethanolic extract of *Solanum melongena* Linn fruit in MSG- intoxicated animal model in high concentration.

The heart as an organ pumps blood through the blood vessels, provide oxygen to the cells and mediate the removal of metabolic waste [18]. Lipid profile parameters are essential in the assessment of the heart function as they reflect the nature of the activities going on the cardiovascular system in relation to lipid/ lipoprotein related causes of cardiovascular diseases; artherosclerosis, hyperlipidaemia, myocardiac infarction etc [18]. Cardiovascular disease is among the leading cause of death in the world. MSG inadvertent use could cause cardiomyopathy, artheroma, myocardial infarction and ischemia among other CVD [19,20]. Hence the need to investigate the possible ameliorative potentials of ethanolic extract of *Solanum melongena* Linn fruit on MSG intoxicated rats' lipid profile, haematological and heart histological changes. The outcome of the findings will contribute to knowledge that may be viable to the management of heart diseases related to these bio-indicators.

2. MATERIALS 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 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 percentage yield of the extract was 1.43%. The extract was placed into a sample bottle and stored in a refrigerator at about 4°C until it was required for experiment. The ethanolic 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. [21] 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 Animal Study Design

Twenty-four adult male 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 [22]. 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 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.

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 oral gastric tube) and was administered daily for 2 weeks (fourteen days).

2.4 Blood and Organ Harvesting for Histological Studies

The rats were sacrificed on the 15th day after overnight fast by decapitation and the blood samples collected respectively into clean heparin bottles for hematological study and polystyrenes tubes. The blood in the polystyrene tubes were centrifuged at 4000 rpm for 10 minutes respectively and the respectively sera were used for the lipid profile assessment. The heart was excised and the histological examinations of the heart were done using the method of Drury et al. [23].

2.5 Determination of Serum Total Cholesterol Concentration

Serum total cholesterol concentration was determined using the method of Allain et al. [24].

The method is based on the principle that total cholesterol is determined after enzymatic hydrolysis and oxidation of cholesterol to free fatty acids by cholesterol esterase and to cholest-4-en-3-one and hydrogen peroxide by cholesterol oxidase. The hydrogen peroxide combines with 4-aminoantipyrine to form a chromophore (quinoneimine dye). The absorbance was read at 505 nm using labomed spectrophotometer Model UV-2502.

2.6 Determination of Serum Triacylglycerol Concentration

Serum triacylglycerol concentration was determined using the method of Albers et al. [25]. This method is based on the principle that triglycerides are determined after enzymatic hydrolysis with lipases. The indicator is a quinoneimine formed from hydrogen-peroxide, 4-aminophenazone and 4-chlorophenol under the catalytic influence of peroxidase. The absorbance was read at 540 nm using labomed spectrophotometer Model UV-2502.

2.7 Determination of Serum High Density Lipoprotein-cholesterol Concentration

Serum HDL-cholesterol concentration was determined using the method of Albers et al. [25]. This method is based on the principle that low density lipoprotein (LDL-c) and very low density lipoproteins (VLDL) are precipitated from serum by the action of polysaccharide in the presence of divalent cation. Then, high density lipoprotein-cholesterol (HDL-cholesterol) present in the supernatant is determined. The HDL-cholesterol was then measured using labomed spectrophotometer Model UV-2502 by means of the coupled reaction.

2.8 Determination of Serum Low Density Lipoprotein-cholesterol Concentration

LDL-C was determined using the Friedeward formula [26].

$$\text{LDL-Cholesterol} = \frac{\text{Total cholesterol - HDL-c - TAG}}{5}$$

2.9 Determination of Serum Very Low Density Lipoprotein-cholesterol Concentration

VLDL-C was determined using the Friedeward formula [26].

$$\text{VLDL-Cholesterol} = \text{TAG}/5$$

2.10 Determination of Haematological Parameters Using Impedance Method on Diatron Abacus Junior Haematology Analyzer

Diatron Abacus Junior Haematology Analyzer was used for the haematological analysis. This machine operates by Impedance method for the determination of volume and number of cells. In this method, a known dilution volume is drawn through a small aperture. Constant current is passed through the aperture from one side to the other. When a cell passes through the aperture, it causes a change in resistance, which generates a voltage pulse. The amplitude of the voltage pulse is proportional to the ratio of cell volume per aperture volume. This is used to determine the volume of cells. The number of cells can be obtained by counting the pulses. The instrument uses one cell-counter probe: the aperture size is 80 µm and has a reference electrode assembly.

2.11 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=.05. Results were expressed as Mean ± standard error of the mean (SEM).

3. RESULTS AND DISCUSSION

The determination of the serum total cholesterol, serum TAG, serum LDL and serum VLDL-C (Table 1), revealed that rats fed with MSG alone (group 2) are significantly higher (P=.05) compared to the group 3, 4, 5, 6 (which were either fed with ethanol extract only and/or with MSG) and the control group while the serum HDL decreased significantly (P=.05) in the same group 2 (7.41± 0.18 mg/dl) compared to the group 3,4,5,6 and control group. Furthermore, the data for group 3, 4, 5 and 6 showed no significant difference (P=.05) among them.

The haematological assessments of the rats fed groups (Table 2) above revealed no significant difference (P=.05) for the RBC ($\times 10^{12}$ cell/L), HGB (%) and HCT (%) except for WBC ($\times 10^{9}$ cell/L) which showed significant (P=.05)

decrease for the group 2 (8.44 ± 0.30 10^9 cell/L) compared to the group 3, 4, 5, 6 and the control group which recorded ranges of 10.19 ± 0.92 (×10^9 cell/L) to 12.81±1.67 (×10^9 cell/L)

Table 1. Lipid profile (Total cholesterol, TAG, HDL-C, LDL-C and VLDL-C) of rats in different groups daily administered with MSG (8000 mg/kg b.w) and ethanolic extract of *Solanum melongena* Linn fruit (SMLF) at different concentration (300, 100, 300 and 500 mg/kg b.w) for two weeks

Parameters	Total CHOL (mg/dl)	TAG (mg/dl)	HDL-C (mg/dl)	LDL-C (mg/dl)	VLDL-C (mg/dl)
Group 1 (Feed + 1 ml/kg distilled water)	67.59±3.69*	92.84±1.63*	12.45±0.41*	39.69±0.70*	18.57 ± 0.33*
Group 2 (8000 mg/kg bw MSG)	80.14±1.70	113.54±2.83	7.41±0.18	47.55±2.13	22.71 ± 0.57
Group 3 (300 mg/kg bw ethaolic extract of SMLF)	70.76±1.53*	92.94±2.10*	12.23±0.12*	38.03±0.58*	18.56 ±0.42*
Group 4 (8000 mg/kg bw MSG + 100 mg/kg bw ethanolic extract of SMLF)	69.59±2.04*	95.81±2.21*	12.04±0.32*	39.03±1.36*	19.16 ±0.44*
Group 5 (8000 mg/kg bw MSG + 300 mg/kg bw ethanolic extract of SMLF)	73.16±1.17*	92.09±1.71*	12.49±0.44*	37.56±0.45*	18.42 ±0.34*
Group 6 (8000 mg/kg bw MSG + 500 mg/kg bw ethanolic extract of SMLF)	75.11±2.30*	86.32±2.71*	12.75±0.15*	35.31±1.02*	17.26 ±0.54*

*Values are expressed as mean ± SEM for four replications. *values are significantly different at (P=.05)*

Table 2. Haematological parameters of rats in different groups daily treated with MSG (8000 mg/kg b.w) and ethanol extract of *Solanum melongena* Linn fruit at different concentration (300, 100, 300 and 500 mg/kg b.w) for two weeks using Diatron Abacus Junior haematology analyzer

Parameters	WBC (×10^9 cell/L)	RBC (×10^{12} cell/L)	HGB (g/l)	HCT (%)
Group 1 (Feed + 1 ml/kg distilled water)	10.19±0.92*	6.99±0.10	127.00±2.80	41.19±1.07
Group 2 (8000 mg/kg bw MSG)	8.44±0.30	7.00±0.32	123.75±5.09	39.98±2.05
Group 3 (300 mg/kg bw ethaolic extract of SMLF)	12.55±0.78*	7.64±0.25	121.75±4.87	39.26±1.05
Group 4 (8000 mg/kg bw MSG + 100 mg/kg bw ethanolic extract of SMLF)	10.45±1.26*	7.49±0.26	129.75±3.88	41.32±1.08
Group 5 (8000 mg/kg bw MSG + 300 mg/kg bw ethanolic extract of SMLF)	12.81±1.67*	7.22±0.09	123.5±1.66	40.57±0.92
Group 6 (8000 mg/kg bw MSG + 500 mg/kg bw ethanolic extract of SMLF)	11.72±1.44*	6.99±0.16	118.75±1.49	37.90±0.94

*Values are expressed as mean ±SEM for four replications. *values are significantly different at (P=.05)*

3.1 Histological Changes ((H&E × 400)

MSG is a food enhancer that has been reported to alter cardiac functions. This investigation was done to profile the risk of heart diseases on the rats. From the present study (Table 1) MSG treatment daily for 2 weeks significantly (P=.05) increased the total cholesterol, TAG, LDL-C and VLDL-C. These increase may lead to cardiovascular diseases (CVD) on the rats [4]. CVD is among the leading cause of death in the world today and is characterized by narrowed or blockage of blood vessels by cholesterol, which might lead to an attack on the heart [19]. The results showed an altered cholesterol metabolism. However, an increased TAG concentration was also observed compared to total cholesterol concentration in all the groups. TAG is the more concentrated form of energy storage and circulates in the plasma via lipoproteins (very low density lipoprotein (VLDL))/ or in lipid droplets or both. This elevation implies that MSG treatment groups may have shifted their energy source from lipolysis to gluconeogenesis. When VLDL-C is impaired, the transportation of TAG is affected and this in turn leads to hyperlipidemia and concomitant reduction in cholesterol level [27]. MSG may have induced these alteration in the MSG only fed group compared to the control either via apoptotic mechanism [28] or its catabolic products counteraction with other substances in the cell [29]. Again, increase in 5-hydroxy-3-methylglutaryl-Coenzyme A (HMG-CoA) reductase: the rate determining enzyme in biosynthesis of cholesterol and/or a decrease in cholesterol catabolism may result in alteration of bile production [30]. Furthermore, there is a significant (P =.05) increase in LDL- cholesterol. This suggests an increased risk of CVD as associated with artherosclerosis [31]. This implies that MSG fed rats would be at risk of CVD. This may have occurred via induced alterations in metabolic organs (heart) operations. Interestingly, the extract at 300mg/kg bw appears to have shown the best ameliorative results among the experimented doses. This result is comparable to the findings reported by Azab et al. [30] on sodium nitrate induced hyperlipidemia in guinea pig. A significant (P =.05) decrease in HDL-cholesterol was observed for the MSG fed group compared to the control group. However, a marked increase was observed for group 3, 4, 5 and 6 compared to group 2. This suggests that the plant extract possess the potentials to reduce the risk of artherosclerosis. This could be attributed to its phytochemical contents such tannins, alkaloid, saponins (7) to scavenge free radical that may be generated during cholesterol metabolism. The result also revealed a gradual increase of the HDL-c concentration upon administration of the studied ethanolic extraction. HDL-c transports cholesterol away from the heart to the liver, where it is broken down to bile [4]. Hence, its increase may account for the observed reduction in the total cholesterol among the groups compared to group 2. These results are in line with the studied histological results.

Haematological studies are useful in the diagnosis of many diseases and in determining the physiological status of animals [32]. From the results (Table 2), no significant difference (P =.05) was observed for red blood cell count (RBC), haemoglobin (HGB) and haemacrit (HCT). MSG and the extract did not cause any observable changes in the rats as it appears. The function of RBC to transport oxygen via haemoglobin was not interfered. Again, the iron content of *Solanum melongena* Linn fruit as reported by Agoreyo et al. [7], may have played physiological roles in the maintenance of RBC, HCT and HGB in the rats fed groups. Furthermore, the ethanolic extract could have blood purifying potentials [17]. This is in line with the report by Elatrash and El-haleim [33], on protective role of *Ginkgo biloba* on MSG- induced liver and kidney toxicity in rats.

For the serum WBC, the results were significantly (P=.05) different for the MSG group compared to the control group. This suggests that MSG may have induced significant toxic effect that altered the immune function of the Wistar rats. This finding agrees with the reported findings by Egbuonu et al. [34] on sub-chronic esculetin-induced alteration in some haematological parameter. Again, previous studies on MSG induced toxicity in rats have shown reduced WBC count for the MSG feed groups [33,35]. The group 3, 4, 5 and 6 showed an increased concentration for WBC compared to group 2. This reveals an interestingly outcome as the extracts appears to improve immune response to fight infection and defend the body against invasion by foreign organisms and thus, improves wellbeing.

The histological results provided insight into the oxidative stress condition that may have affected the examined organs (heart). In the present study photomicrographs revealed distorted compact myocardial muscle bundles with thick

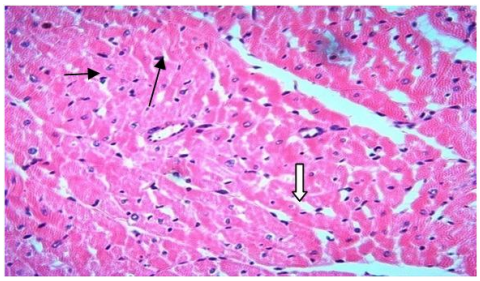

Plate 1. Photomicrograph of the heart section of rats in normal control (Group 1), showing compact myocardial muscle bundles with thin fibrocollagenous stroma (white arrow) and narrow lumina blood vessels (black arrow)

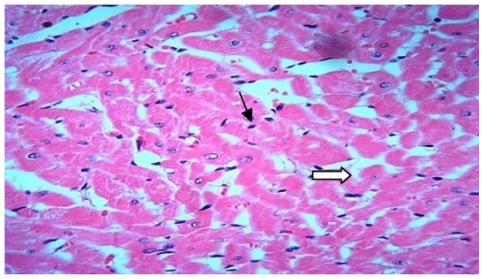

Plate 2. Photomicrograph of the heart section of rats treated with MSG (Group 2) showing compact myocardial muscle bundles with thick fibrocollagenous stroma, mild plumpy myocytes (white arrow) and narrow lumina blood vessels lined by endothelial cells (black arrow)

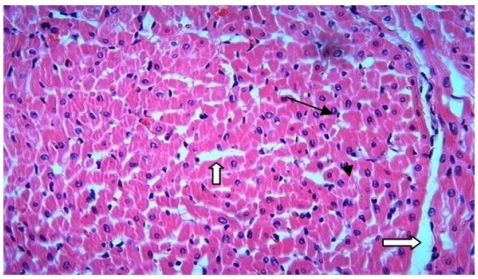

Plate 3. Photomicrograph of the heart section from rats treated with plant extract (Group 3) showing loosely arranged myocardial muscle bundles with thin fibrocollagenous stroma (white arrows) and narrow lumina blood vessels lined by endothelial cells (black arrows)

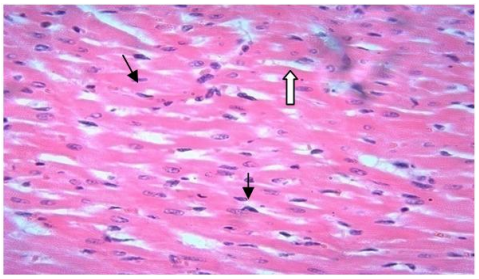

Plate 4. Photomicrograph of the heart section from rats treated with MSG and 100 mg/kg of the plant extract (Group 4) showing compact myocardial muscle bundles with thick fibrocollagenous stroma (white arrow). The myocytes are mildly plump with narrow lumina blood vessel lined by endothelial cells (black arrows)

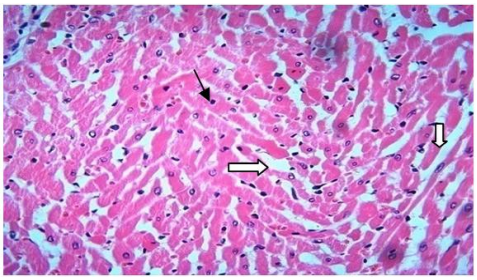

Plate 5. Photomicrograph of the heart section from rats treated with MSG and 300 mg/kg of the plant extract (Group 5) showing compact myocardial muscle bundles with thick fibrocollagenous stroma (white arrows) and narrow lumina blood vessel lined by endothelial cells (black arrows)

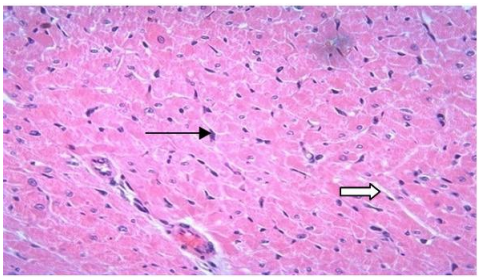

Plate 6. Photomicrograph of the heart section from rats treated with MSG and 500 mg/kg of the plant extract (Group 6) showing loosely arranged myocardial muscle bundles with thin fibrocollagenous stroma (white arrow) and with narrow lumina blood vessel lined by endothelial cells (black arrow)

fibrocollagenous stroma, mild plumpy myocytes and narrow lumina blood vessels lined by endothelial cells in plate 2 compared to plate 1, 3,4,5 and 6. This result is comparable to the

study by Zahkouk et al. [36], on the physiological and histological studies on the heart of male albino rats exposed to electromagnetic field and the protective role of silymarin and/or vitamin e. The mild plump myocytes and the thick fibrocollagenous stroma observed in this study may be due to increased total cholesterol, TAG and possibly LDL-c oxidation and decreased in the synthesis of fibrocollagenases respectively [37]. Nevethe less, these effects were ameliorated gradually upon administration of ethanolic extract of *Solanum melongena* Linn fruit and were comparable to the observed investigated results of the lipid profile of the rats.

4. CONCLUSION

This study confirmed general adverse influence of MSG at a high concentration (8000 mg/kg body weight) and indicated the ameliorative role of ethanolic extract of *Solanum melongena* Linn fruit notably at 300 mg/kg of body weight in rats. Thus, ethanolic extract of *Solanum melongena* Linn fruit may be useful in the management of MSG intoxication related to the studied parameters in the rats, warranting further studies to index the major component of the ethanolic extract of *Solanum melongena* Linn fruit.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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