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GC-MS Profile and protective effect of *Abelmoschus esculentus L,* Moench*,* leaf on monosodium glutamate-induced organ pathologies

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Abstract

Abelmoschus esculentus is a nutritional herb that is consumed in some cultures in Africa and Asia. Its leaf extract has been demonstrated to possess optimal antioxidant activity. This study evaluated the Gas Chromatography-Mass Spectrometry (GC-MS) profile of the ethanol extract of *Abelmoschus esculentus* leaf as well as its protective effect on Monosodium glutamate (MSG)-induced vital organs toxicity. The ethanol extract of *Abelmoschus esculentus* leaf (EAEL) was profiled for its compounds by the GC-MS method using the Agilent 5977B A GC with Agilent 8860 with an Elite-5MS column (30 m x 0.320 mm i. d. x 0.25 µm df). The effects of the extract (76.92 mg/kg; *p.o*) on liver, kidney, heart and uterine histology as well as functions were evaluated using female. The GC-MS revealed the presence of several compounds with 9-Octadecenoic acid, Hexadecanoic acid methyl ester, Methyl stearate, 9,12,15-Octadecatrienoic acid and Pentadecanoic acid being prominent. EAEL reversed some of the MSG-induced histological anomalies in the 4 tissues and ameliorated the biochemical parameters affected by MSG treatment. This study reveals the active compounds in the ethanol extract of *Abelmoschus esculentus* leaf as well as their potentials in reversing MSG-induced organ anomalies.

Keywords: *Abelmoschus esculentus*, Monosodium glutamate, Wistar rats, Gas Chromatography-Mass Spectrometry (GC-MS)

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Introduction

Abelmoschus esculentus L. Moench (Okra) is a culinary plant cultivated in the tropical, subtropical and warm temperate regions (Grubben and Denton, 2004). The pod which is the more consumed part of the plant, is known for its nutritional and medicinal benefits, including its anti-cancer and antidiabetic activities (Liu *et al.,* 2018). The leaves however have been less scientifically investigated and much of its nutritional as well as medicinal potentials remain largely unexplored. The proximate analyses and mineral composition of the leaves have revealed the presence of crude protein and lipids, carbohydrates, potassium, sodium, calcium, iron, zinc and manganese (Idris *et al.,* 2009). *Abelmoschus esculentus* leaves have been used in the preparation of salads, soups and other dishes (Roy *et al.,* 2014) while the Igbo-Ora people of Oyo State, Nigeria, traditionally consume okra leaves, known as "ewe ilasa," which are linked to a remarkably high rate of twin births (Omonkhua *et al.,* 2020).

The toxic effect of MSG on liver, heart (Reddy *et al.,* 2021; Banerjee *et al.,* 2021), kidney (Hussin *et al.,* 2021) and uterus (Kayode *et al.,* 2021) have been reported. Liver enzymes activities (Alkaline phosphatase (ALP) and Alanine aminotransferase) have been reported to increase at 1% and 5% MSG inclusion in diet (Akanya *et al.,* 2015) as well as an alteration in liver architecture (Shrestha *et al.,* 2018). Cardiac tissue cytotoxicity and tissue damage have also been observed in rats exposed to 600 mg/kg of MSG (Banerjee *et al.,* 2021). Reduction in glomerular filtration rate, due to induced enlargement of the intraglomerular mesengial cells, has been attributed with long term consumption of MSG (Hussin *et al.,* 2021). Increased fibroblast count density in the myometrium as well as increased levels of estrogen and progesterone were observed in female rats fed with MSG for 2 weeks, indicating the potential of MSG to cause fibrosis and leiomyomas (Oyebode *et al.,* 2020). *Abelmoschus esculentus* leaf is abundant with inorganic and organic compounds, including phenolic compounds (Caluête *et al.,* 2015), which are well known for their strong antioxidant and anti-inflammatory properties. To the best of our knowledge, there is no previous data on the GC-MS analysis of the ethanol extract of the plant's leaf nor its effect on MSG-induced organ anomalies. This study aimed to address these gaps.

Material and methods

Collection and extraction of plant

Abelmoschus esculentus leaves were obtained from Igbo-Ora town at the Ibarapa Local government area of Oyo state, Nigeria. Authentication and deposition of the leaves were done at the University of Medical Sciences Herbarium with the herbarium number UNIMED P.B.T.H No 035. The pulverized leaves (200 g) were macerated in 2 L of absolute ethanol in an amber bottle and 10 ml of hydrochloric acid (Hcl) included. This was placed on a shaker and filtered after 48 hours. A 1% yield was obtained.

GC-MS analysis

GC-MS analysis of the ethanol extract of *Abelmoschus esculentus* leaf was performed using an Agilent 5977B GC/MSD system coupled with Agilent 8860 auto-sampler, a Gas Chromatograph interfaced to a Mass Spectrometer (GC-MS), equipped with an Elite-5MS (5% diphenyl/95% dimethyl polysiloxane) fused capillary column (30 m × 0.25µm ID × 0.25 µm df). For GC-MS detection, an electron ionization system was operated in electron impact mode with an ionization energy of 70 eV. Helium gas (99.999%) was used as a carrier gas at a constant flow rate of 1 ml/min, and an injection volume of 1µl was employed (a split ratio of 10:1).
The injector temperature was maintained at 300 °C, the ion-source temperature was 250 °C, and the oven temperature was programmed from 100 °C (isothermal for 0.5 min), with an increase of 20 °C/min to 280°C (2.5 min), Mass spectra were taken at 70 eV; a scanning interval of 0.5 s and fragments from 45 to 450 Da. The solvent delay was 0 to 3 min.

Experimental animals

Eighteen female Wistar rats weighing (90-100 g) were procured from and housed at the Animal House of the University of Medial Sciences, Ondo state. The animals were kept at room temperature (25 ± 1 ºC) with access to feed (standard rat diet) and water *ad libitum*. The study was approved by UNIMED Research Ethics Committee (UAREC) with the number
UNIMED-AREC/Apv/2024/003.

Research protocol

The animals were divided into 3 groups (n=6) and treated as follows; Group A (control; Distilled water only), Group B (MSG 100 mg/kg; *i.p* for 14 days and then withdrawal for another 14 days) and Group C (MSG 100 mg/kg; *i.p* for 14 days plus 76.92 mg/kg *p.o.* EAEL for another 14 days). All treatments were administered once daily for 14 days. At the termination of the study (29^{th} day), the rats were bled through the retro orbital vein and their organs harvested for biochemical and histological analysis.

Biochemical analysis

The blood samples were centrifuged at 300 rpm for 10 min and decanted into serum bottles. The samples were analyzed for Total protein (TP), creatinine, urea, Alanine transferase

(ALT) and Follicle Stimulating Hormone (FSH) levels. Serum urea was analysed using the Berthelot's method (Wilcox *et al.,* 1966), Serum creatinine was determined using Jaffe method (Blass *et al.,* 1974), ALT assay was done using RANDOX reagent while FSH serum levels were assessed using a commercial ELISA prepared by Accubind, UK (Hornbeck, 2015).

Statistical analysis

The data collected was carried out using Multiple unpaired T tests with Welch correction on each row. 2 stage linear step up procedure of Benjamini, Krieger and Yekutieli. (Graph-Pad Prism 10; Graph-Pad Software Inc., CA, USA). Results were presented in triplicates and expressed as Mean ± Standard Deviation with $P < 0.05$ considered significant

Results

Gas Chromatography-MassSpectometry

A total of 23 bioactive compounds with different phytochemical activities were identified from the ethanol extract of Abelmoschus esculentus leaf (Figure 1). The major compounds obtained from the GC-MS analysis are 9-Octadecenoic acid (29.763 %), Hexadecanoic acid, methyl ester (13.866 %), 9-Octadecenoic acid (9.577 %), Methyl stearate (9.281 %), 9,12,15-Octadecatrienoic acid (8.797 %), and Pentadecanoic acid (5.906 %) (Table 1, Figure 2).

Total protein content

MSG significantly ($P < 0.05$) altered the TP contents of all the tissues except the liver, however, treatment with EAEL resulted in significant ($P < 0.05$) increase in TP content in the tissues except kidney, compared to the control (Table 2).

Biochemical assay

Exposure to MSG significantly ($P < 0.05$) increased creatinine, urea and FSH levels, while reducing the ALT. Treatment with EAEL however, caused a significant ($P < 0.05$) reduction in creatinine, urea and FSH levels while increasing ALT levels (Table 3).

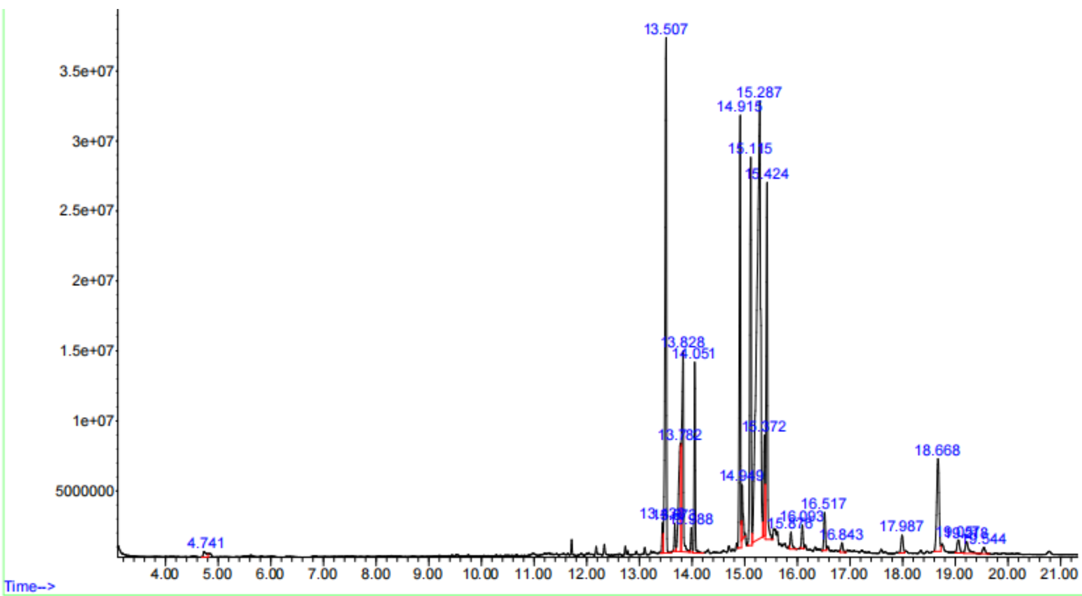


Figure 1: Gas Chromatography-Mass spectometry chromatogram, of ethanol extract of *Abelmoschus esculentus* leaves.

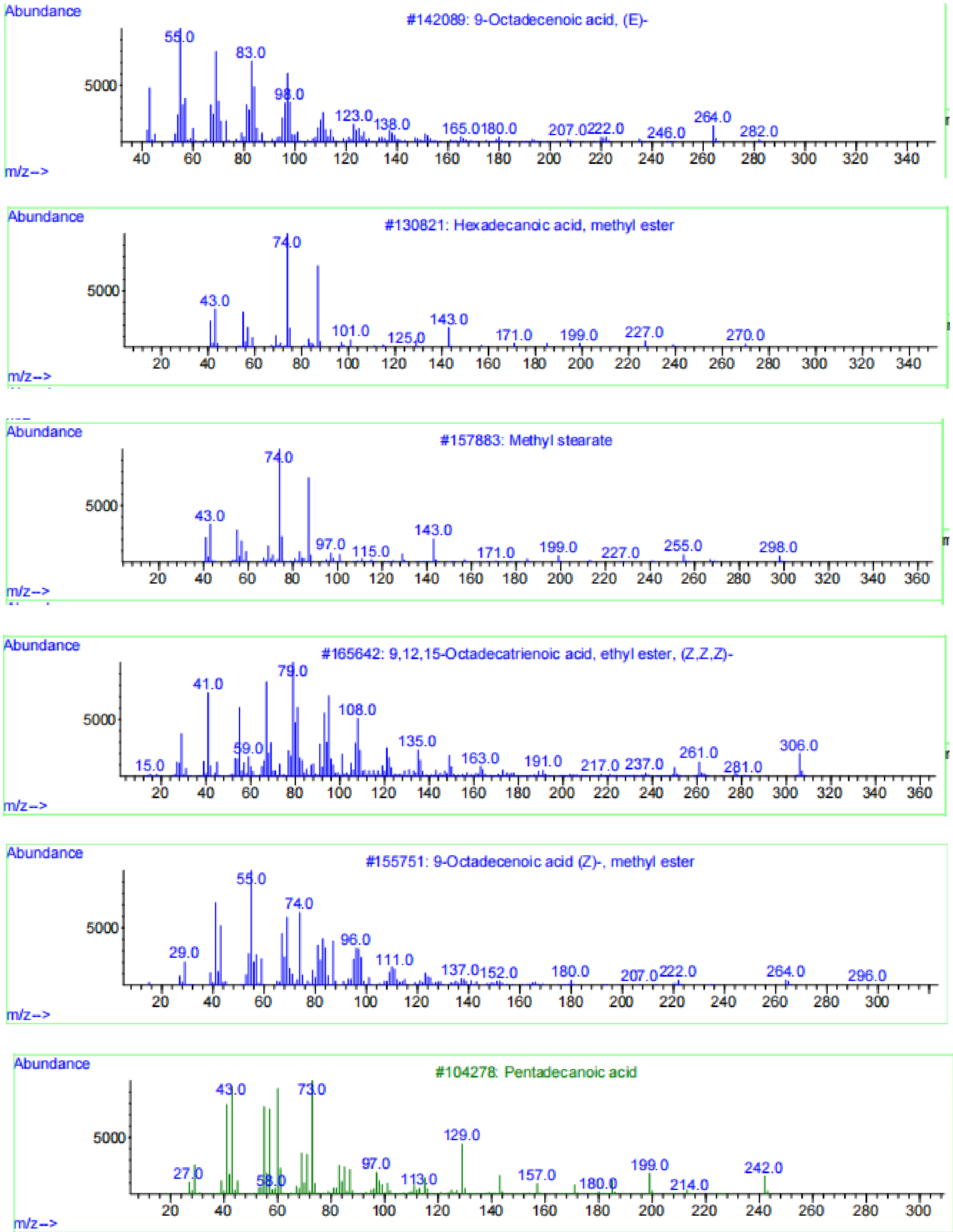


Figure 2. Chromatogram of the 6 major compounds identified in the GC-MS chromatogram of EAEL

Table 1: Bioactive compounds found in ethanol extract of Abelmoschus esculentus leaves

Retention Time	Compound Name	Formula	% Of Total (%)	Mol Wt (G/Mol)
4.741	Benzoic acid, Methyl ester (Methyl benzoate)	C_8H_8O	0.388	136.1479
13.438	Pentadecanoic acid, 14-methyl-, methyl ester (methyl 14-methylpentadecanoate)	$C_{17}H_{34}O$	0.598	270.4507
13.507	Hexadecanoic acid, methyl ester (Methyl palmitate)	$C_{17}H_{34}O_2$	13.866	270.45
13.673	1-Hexandecen-3-ol, 3,5,11,15-tetramethyl	$C_{20}H_{40}O$	0.524	296.5
13.673	Isophytol			
13.782	n-Hexadecanoic acid (Palmitic acid)	$C_{16}H_{32}O_2$	4.476	256.4241
13.828	Pentadecanoic acid (Pentadecylic acid)	$C_{15}H_{30}O_2$	5.906	242.3975
13.988	Hexanoic acid, undec-2-enyl ester	$C_{17}H_{32}O_2$	0.536	268.441
14.051	Hexadecanoic acid, ethyl ester (Ethyl palmitate)	$C_{18}H_{36}O_2$	3.693	284.4772
14.915-14.949	9-Octadecenoic acid (Z)-, methyl ester	$C_{19}H_{36}O$	9.577 1.277	296.4879
15.115	Methyl stearate		9.281	298.5
	(methyl octadecanoate)	$C_{19}H_{38}O_2$		
15.287	9-Octadecenoic acid, (E)- Oleic acid (elaidic acid)	$C_{18}H_{34}O_2$	29.763	282.4614
15.287	6-Octadecenoic acid			
15.372	Linoleic acid ethyl ester (Ethyl linoleate)	$C_{20}H_{36}O_2$	2.314	308.4986
15.424	9,12,15-Octadecatrienoic acid, ethyl ester	$C_{20}H_{34}O_2$	8.797	306.4828

15.876	9,12-Octadecadienoic acid (Z, Z)- (Linoleic acid)	$C_{18}H_{32}O_2$	0.543	280.4455
16.093	9-Octadecenal, (Z)- Cyclohexane (olealdehyde or oleylaldehyde)	$C_{18}H_{34}O$	0.630	266.46
16.517	Glycidyl palmitate	$C_{19}H_{36}O_3$	1.094	312.49
16.843	Methyl 18-methylnonadecanoate	$C_{21}H_{42}O_2$	0.324	326.6
	Hexadecanoic acid			
17.987	9,17-Octadecadienal	$C_{18}H_{32}O$	0.663	264.45
18.688	Glycidyl oleate	$C_{21}H_{38}O_3$	4.154	338.5246
	9-Octadecenal (Oleic acid)	$C_{18}H_{34}O$		266.4620,
19.057	cis-10-Heptadecenoic acid	$C_{17}H_{32}O_2$	0.606	268.43
19.218	Palmitoyl chloride (Hexadecanoyl-chloride)	$C_{16}H_{31}ClO$	0.738	274.870
	Pentadecanoic acid			
19.544	Docosanoic acid, methyl ester (Behenic acid)	$C_{23}H_{46}O$	0.303	354.6101

Table 2: Total protein content in liver, heart, kidney and uterus (g/L)

Groups	Liver	Heart	Kidney	Uterus
DW	1.67±0.11^{c}	1.73±0.02$^{b, c}$	2.45±0.09^{b}	1.66±0.11$^{b, c}$
MSG (100 mg/kg)	1.79±0.02^{c}	1.96±0.07^{a}	1.7±0.13$^{a, c}$	1.23±0.02$^{a, c}$
MSG + EAEL	2.56±0.03$^{a, b}$	1.97±0.01^{a}	2.47±0.08^{b}	2.02±0.03$^{a, b}$

DW=Distilled water, MSG=Monosodium glutamate, EAEL= Ethanol extract of Abelmoschus esculentus leaf. Superscript a,b,c indicates significant difference from DW, MSG and MSG + EAEL groups respectively, within the same column

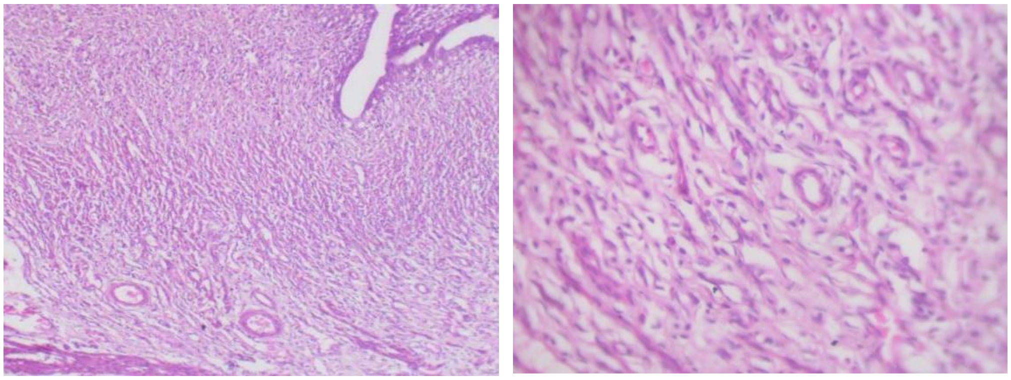
Table 3: Effect of the extract on Creatinine, BUN, FSH and ALT levels of MSG-intoxicated rats

	DW	MSG (100 mg/kg)	MSG + EAEL
Creatinine	95.4±0.82^{b}	137.35±7.76^{a,c}	47.8±15.68^{b}
Urea	5.31±0.49^{b}	68.2±12.33^{a,c}	16.3±6.40^{b}
ALT	35.0±2.45^{b,c}	8.0±3.27^{a,c}	19.0±1.63^{a,b}
FSH	1.16^{b}	1.37^{a,c}	1.05^{b}

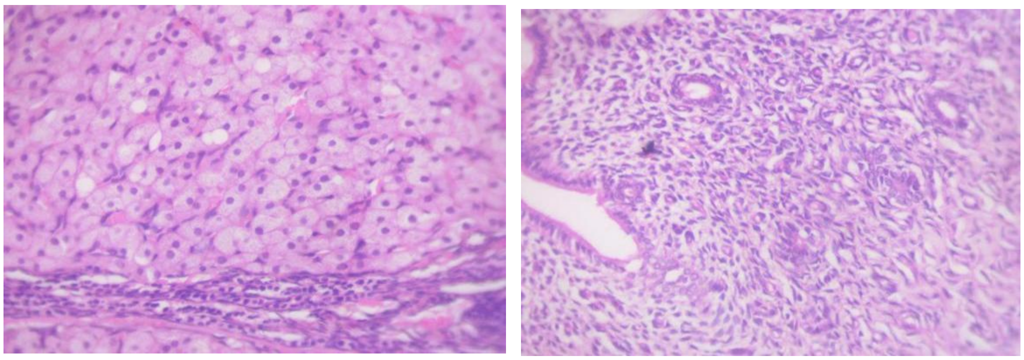
DW= Distilled water, MSG= Monosodium glutamate, EAEL= Ethanol extract of Abelmoschus esculentus leaf. Superscript a,b,c indicates significant difference from DW, MSG and MSG + EAEL groups respectively, within the same row

Effects of the extract on vital organ Histology
Uterus

Uterine samples of the control group appeared apparently normal with normal uterine anatomy with no significant lesions (Figure 3A). In the group that received MSG alone there was mild epithelial hyperplasia, mild stromal cellular infiltration by predominantly mononuclear cells, marked vascular congestion with concurrent localized vasculitis and myometrial degeneration (diffuse and marked), thickened and hyperplastic stroma, single cell necrosis (mild) but marked to moderate diffuse degeneration of myocytes (swollen cytoplasmic appearance) within the myometrium mixed reaction. Some glands appeared collapsed and atrophied while some are hypertrophied (Figure 3B). Treatment with EAEL displayed mild mixed cell infiltration in the endometrium and myometrium, mild capillary congestion, prominent smooth muscle layer with organization of connective tissue and collagen deposition. mild simple cystic hyperplasia, mild fibroplasia within the myometrial layer, mild random single cell necrosis as well as glandular hyperplasia and hypertrophy (Figure 3C).



A



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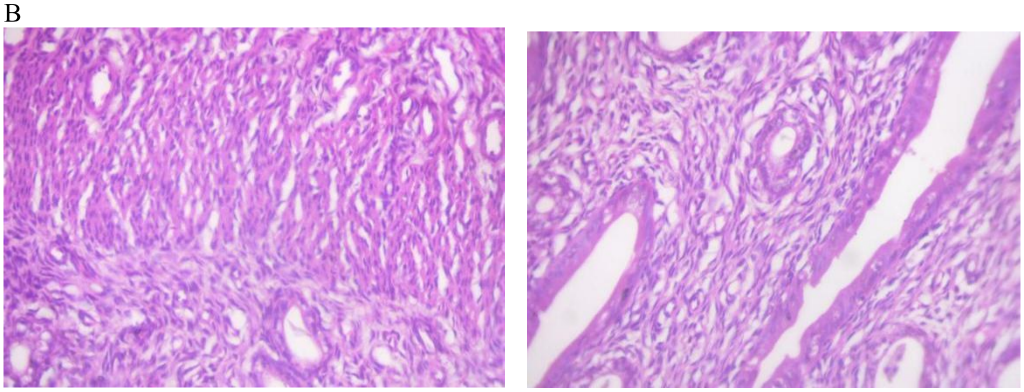
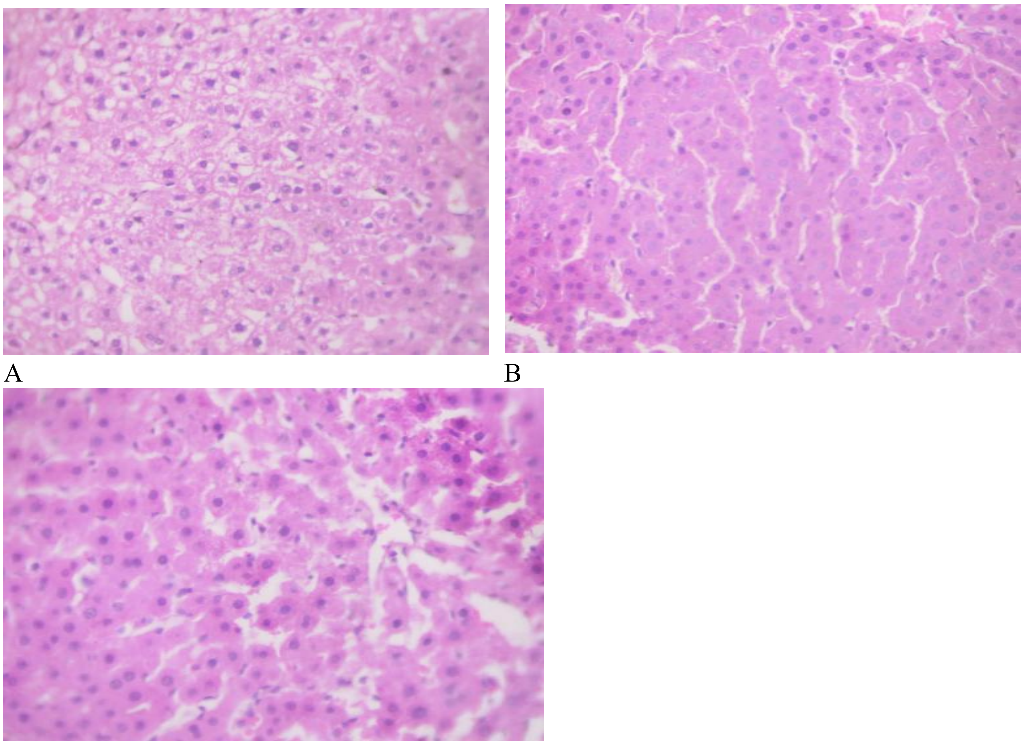


C

Figure 3: Histology of uterine tissues of control (3A), MSG exposed (3B) and EAEL treated (3C) rats

Liver

Liver tissues of the control had no significant lesions (Figure 4A). Those exposed to MSG showed mild single random hepatocellular necrosis, mild to moderate mixed cell inflammation in the midzonal and periportal areas, marked congestion of the central vein and vessels within the portal area, mild ductular proliferations as well as marked multifocal thinning of hepatic cords especially in areas around the central vein (Figure 4B). The Liver tissues of the group that received EAEL had focal areas of necrotic hepatocytes characterized by deep staining eosinophillic cytoplasm and dark nuclei, mild hepatocellular vacuolar degeneration also seen in periportal hepatocytes, multifocal mixed cell inflammation within centrilobular areas and periportal areas, moderate congestion of the central vein and vessels within the portal area, mild in the periportal, multifocal thinning of hepatic cords especially in areas around the central vein and a mild increase in binucleation of hepatocytes also observed in section (Figure 4C).



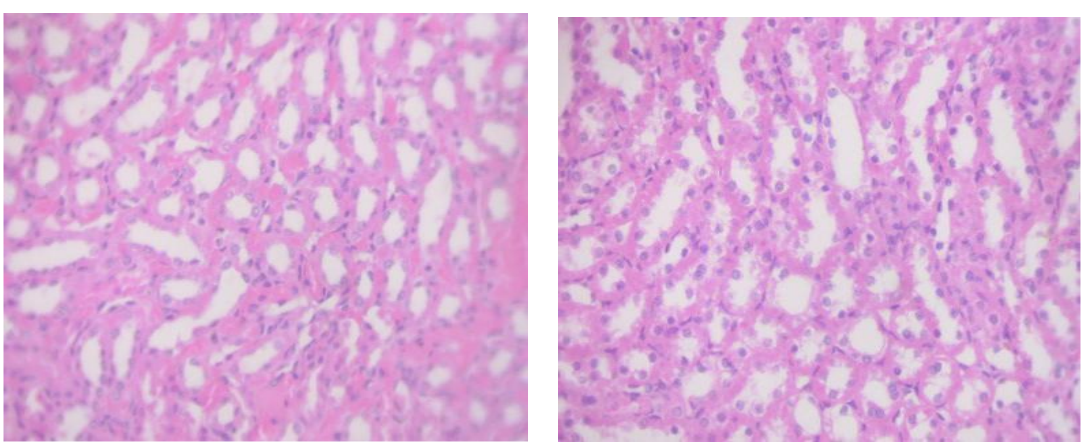
A B

C

Figure 4: Histology of liver tissues of control (4A), MSG exposed (4B) and EAEL treated (4C) rats

Kidney

There were no visible lesions in kidney tissues of the control (Figure 5A). However, there were intact tubules with well vascularized capillaries and congested renal cortical sinusoids. The kidney tissues of those exposed to MSG showed mild glomerular hypercellularity, mild random single cell necrosis, mild mixed cell inflammation in the glomeruli, tubules and intersitium (Figure 5B) while tissues of those treated with EAEL demonstrated mild glomerular tuft atrophy, mild tubular epithelial hyperplasia, mild cast formation in the distal collecting tubule, mild interstitial edema and mild interstitial fibrosis (Figure 5C).



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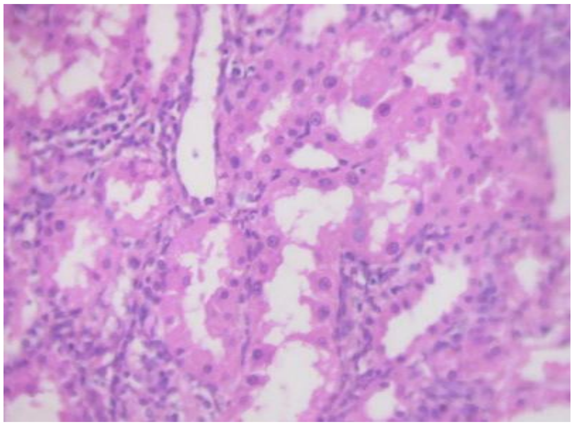

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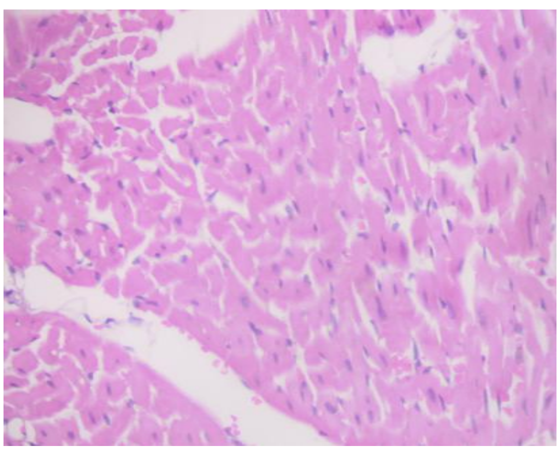
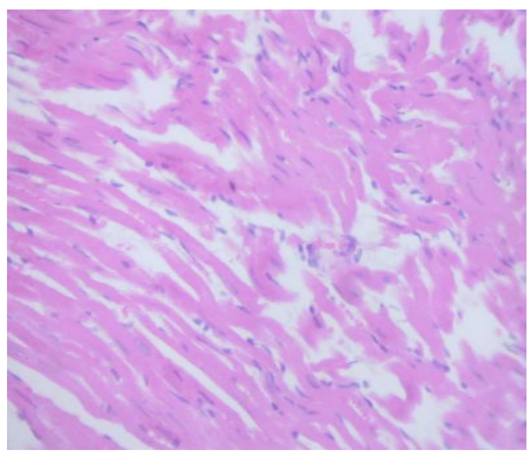
Figure 5: Histology of kidney tissues of control (5A), MSG exposed (5B) and EAEL treated (5C) rats

Heart

There were no visible lesions in the heart tissues of the control group (Figure 6A). Heart tissues of the MSG exposed group had mild random single cell myocardial necrosis characterized by deep staining eosinophillic cytoplasm and karyolysis, mild myocardial hypertrophy, mild inflammation within the myocardium and endocardium characterized by mixed cell inflammation within the myocardial interstitium, mild myocardial cappilary congestion (Figure 6B). Only mild myocardial hypertrophy was observed in heart tissues of those treated with EAEL (Figure 6C).



A



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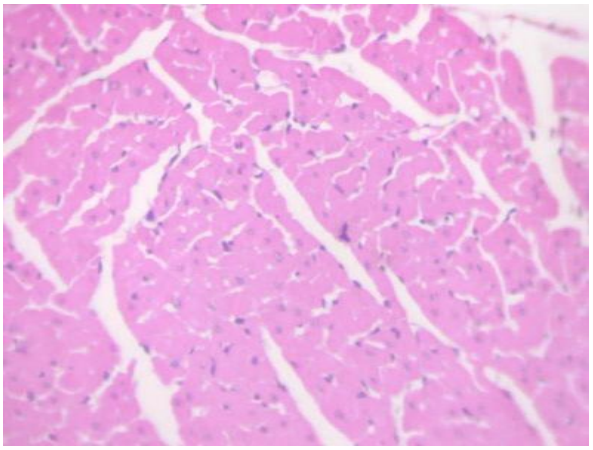

C

Figure 6: Histology of cardiac tissues of control (6A), MSG exposed (6B) and EAEL treated (6C) rats

Discussion

Previous proximate analyses and mineral composition study of *Abelmoschus esculentus* leaf (AEL) revealed good source of essential minerals and nutrients (Idris *et al.,* 2009). The prominent compounds detected in this study have been earlier reported to possess anti-inflammatory, antiandrogenic, and anemiagenic (9-Octadecenoic acid) (Surender *et al.,* 2008); antibacterial activities (Pu *et al.,* 2010), as well as antioxidant, antitumor, anticancer, anticoronary, hypocholesterolemic (Hexadecanoic acid, methyl ester) (Tyagi and Agarwal, 2017; Adenike *et al.,* 2019; Choudhary *et al.,* 2019), nematicidal, anti-nociceptive, intestinal lipid metabolism regulatory, anti-fungal (Methyl stearate) (Pinto *et al.,* 2017, Adan *et al.,* 2019;) and antimicrobial properties (9,12,15-Octadecatrienoic acid) (Alli and Mangamoori, 2014).

The presence of the above bioactive compounds explains the reversal of some of the MSG-induced biochemical and histological anomalies by EAEL. Total protein values which were significantly reduced in kidney and uterine tissues of MSG exposed animals, were increased by EAEL treatment. This effect of the extract has been reported by Borokini *et al.* (2023). Reduction in TP in liver homogenate is indicative of liver dysfunction and immunosuppression (Srivastava and Reddy, 2020, Shareef *et al.,* 2022,), a reduction in protein synthesis in cardiac tissues has been associated with cardiomyopathy in burn patients (Lang *et al.,* 2004). Total protein contents have also been demonstrated to be slightly reduced in the renal tissues of $AlCl_3$ exposed rats (Afolabi *et al.,* 2023), while, reduction in TP was observed in leiomyomas (Ahmed *et al.,* 2019). Increased TP in uterine tissues have been attributed to the luteal phase of oestrus; when the uterus is preparing for implantation (Soleilhavoup *et al.,* 2016) and in early phase of pregnancy (Kayser *et al.,* 2006). In this study, TP was significantly increased by EAEL in all the tissues except the kidney tissues thus suggesting that the extract enhanced liver and cardiovascular function as well as enhance fertility. The restoration of TP levels in the renal tissues as well as a reduction in creatinine and BUN levels is indicative of the nephroprotective activity of EAEL. TPs have been implicated in immune response cells aiding in fighting infections, and AE has been documented to increase immune response in tissues (Ko *et al.,* 2022), the increase in TP levels in the tissues of EAEL treated animals is expected and may suggest a healing process. The anti-inflammatory actions of AE are confirmed by the presence of 9-octadecenoic acid, hexadecanoic acid, methyl ester, methyl stearate and 9,12,15-octadecatrienoic acid, compounds with known anti-inflammatory properties.

Endometrial hyperplasia is usually caused by long term exposure to oestrogen that is not neutralized by progesterone. It is a precursor of uterine cancer and could be responsible for infertility in women (Nees *et al.,* 2022). The infiltration of MN cells, vasculitis and thickening of the stroma are possible pointers to an ongoing inflammatory reaction, fibrosis and other pathological condition in the uterus. Lesions in tissues of EAEL treated group reveal some resolution of the reactions caused by MSG. Liver pathology did not significantly improve except for the slight reduction in congestion of the central vein. Treatment with EAEL, nonetheless, reversed the MSG-induced glomerular hypercellularity, but initiated further hyperplasia, cast formation, oedema and fibrosis in the tubules. The ameliorative effect on myocardial tissues was very evident. An increase was BUN and creatinine levels as seen in the MSG-exposed rats indicates a decline in kidney functions. This has been linked to a constriction of the glomerular capillaries and gates of basement membranes, thus leading to a reduction in filtration capacity of the affected kidney (Hussin *et al.,* 2021). This is evident in the hypercellularity observed in the glomerulus of MSG-exposed animals, Urea and creatinine levels were significantly reduced by EAEL suggesting the potential of the extract in improving renal function. The reduction in ALT values in the MSG-exposed group, contradicts reports from other studies (Shrestha *et al.,* 2018; Mohamed *et al.,* 2013). Reduction in ALT levels has been linked with a loss of weight (Abdalgwad *et al.,* 2020). Moreover, a very low ALT value is a pointer to chronic kidney dysfunction in patients with liver infection (Fabrizi *et al.,* 2001). Borokini *et al.* (2023) reported an increase in ALT levels of female rats given EAEL compared to males. This is similar to our findings. It may be suggested from our study that EAEL precipitated healing in the liver by reversing the possible damage to the hepatocytes by MSG exposure.

Conclusion

Bioactive compounds present in the ethanol extract of *Abelmoschus esculentus* leaves reversed monosodium glutamate-induced organ dysfunction. The leaves contain beneficial therapeutic compounds that could improve health. The anti-inflammatory potential of the leaves is quite pronounced in addition to its nephroprotective, cardioprotective and hepatoprotective potentials.

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Competing interests

The authors have no conflict of interest to declare.

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Data Availability Statement

The authors confirm that the data supporting the findings of this study are available within the manuscript or supplementary information files

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