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Protective efficacy of *Murraya koenigii* aqueous extract against monosodium glutamate-induced hepatotoxicity in Wistar rats

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The present work was designed to study the potential effect of antioxidant rich aqueous extract of *Murraya koenigii* (AE-MK) on monosodium glutamate-induced hepatotoxicity in Wistar rats. The study was conducted on thirty adult Wistar rats, classified into six groups. MSG (1000 mg/kg, p.o) was administered to induce hepatotoxicity. The MSG treated group showed significant (P <0.05) increase in % change in body weight, relative organ weight, SOD, MDA, cholesterol, bilirubin, triglycerides, LDL levels; and AST, ALT activity while significant (P <0.05) decrease in glutathione peroxidase, albumin, HDL level and ALP activity in liver tissue as compared to control group. AE-MK (200, 400 mg/kg) significantly (P <0.05) reversed all the above parameters as compared to MSG treated rats. Histopathological changes observed in MSG treated rat liver tissue were cytoplasmic vacuolation, sinusoidal congestion, and cellular aggregates around the portal area. These changes were reversed with AE-MK (200, 400 mg/kg). The total phenol content was found to be 62 µg of gallic acid equivalent /mg of extract and free radical scavenging activity by DPPH method was found to be 74.16%. The study suggests that antioxidant rich aqueous extract of *M. koenigii* has protective effect against MSG-induced hepatotoxicity.

Keywords: Hepatotoxicity, Monosodium glutamate, *Murraya koenigii*, Oxidative stress.

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Introduction

Monosodium glutamate (MSG), a salt of glutamic acid present in the food as flavour enhancer is used as food additive in the form of hydrolysed protein or purified monosodium salt[1]. Glutamate is non-essential amino acid that usually exists in two forms, namely bound and free. In a bonded form, it is tasteless while free glutamate has umami taste[2]. MSG act on the glutamate receptors and release neurotransmitters which play a vital role in normal physiology as well as pathological processes[3]. In a metabolic reaction several types of reactive species are generated which are either oxygen or nitrogen derived, and they are known as pro-oxidant which can attack macromolecules causing tissue damage[4].

Antioxidant are produced endogenously or received exogenously to counteract their effects. In a normal cell there is balance between pro-oxidant and oxidant, when production of oxygen species is increased or level of antioxidants is diminished, this balance can be shifted towards pro-oxidants. This state is called oxidative stress and leads to cell damage if the stress is prolonged[5].

Chronic administration of MSG induces oxidative stress in experimental animals. Subsequently, it was documented that MSG produces oxygen derived free radicals[6,7]. Several studies were carried out to study the toxicity of MSG on various organs in laboratory animals which includes neurotoxicity[8], hepatotoxicity[9], cardiotoxicity[10], reproductive organ toxicities[11], obesity and metabolic disorders[12,13], and nephrotoxicity[14].

Murraya koenigii, known as curry tree or curry-leaf tree, a tropical to subtropical tree of the family Rutaceae, is native to India, Sri Lanka, Bangladesh, and Andaman Island. It is well known as a food flavouring herb[15].

Different parts of *M. koenigii* such as leaves, root, bark, and fruits are known to promote various biological activities. This includes antibacterial activity, antifungal activity, antiprotozoal activity, antioxidant activity, anti-lipid peroxidase activity, anti- hypertensive activity[16].

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Various phytochemical studies on the leaves, stem bark, roots, and fruits have shown the presence of large concentration of bioactive compounds such as mahanine, koenine, koenigine, murrayanol, murrayagetin, marmesin-1"-O-rutinoside, girinimbine, murrayazolidine[16-18]. In addition to that, three monomeric and five binary carbazole alkaloids namely mukoenine-A, B, and C and murrastifoline-F.bis-2- hydroxy-3-methyl carbazole, bismahanine, bi koeniquinone-A, and bismurrayaquinone-A were also extracted from the bark[17-18] (Table 1).

In view of the above literature and the presence of rich antioxidant phenolic content in the leaves of *M. Koenigii*, the present study was designed to evaluate the possible hepatoprotective activity.

Materials and Methods

Plant collection and identification

Leaves of *M. koenigii* were collected in the month of August 2020, and authenticated in Department of Pharmacognosy, MGV's Pharmacy College, Nashik (Herbarium Voucher number MGV/23/20).

Extract preparation

Leaves were washed, sun dried, and crushed into fine powder. For the aqueous extraction, 50 g of weighed curry leaves powder was added to 500 mL of distilled water. The mixture was boiled for thirty minutes, cooled, and kept aside for 24 h. The extract was filtered through muslin cloth and evaporated. The aqueous extract of *M. koenigii* was air-dried to obtain the product (5.6 % w/w)[21].

In vitro antioxidant activity

Determination of total phenol content

The total phenolic content of the plant extract was determined using spectrophotometric method (UV- 2600, Shimadzu). The reaction mixture was prepared by mixing 0.5 mL of aqueous solution of extract, 2.5 mL of 10 % Folin-Ciocalteu's reagent. Blank was concomitantly prepared, containing 0.5 mL methanol, 2.5 mL of 10% Folin-Ciocalteu's reagent. The samples were then incubated at room temperature for 45 min. The absorbance was determined using spectrophotometer at λ_{max} 765 nm. The samples were prepared in triplicate and the mean value of absorbance was recorded. The same procedure was repeated for the standard solution of gallic acid for the calibration curve. Based on the measured absorbance, the concentration of phenolics was observed as (mg/mL) from the calibration line. The equivalent content of phenolics in *M. koenigii* extract was expressed in terms of gallic acid equivalent (mg of GA/g of extract)[22].

Estimation of free radical scavenging activity

The antioxidant activity of *M. koenigii* extract were measured in comparison to standard antioxidant ascorbic acid depending on the scavenging effect of 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radicals. Briefly, different concentrations (10, 20, 40 µg/mL in methanol) of ascorbic acid solution as well as *M. koenigii* extract (10, 20, 40 µg/mL) solution were mixed with 3 mL of 0.1 mM DPPH solution. The mixture was kept in dark for 30 min to measure the absorbance at 517 nm using spectrophotometer (UV 2600, Shimadzu). The scavenging activity against DPPH was calculated using the equation[23].

$$\% \text{ Scavenging activity} = \frac{(\text{Absorbance of blank} - \text{Absorbance of sample})}{\text{Absorbance of blank}} \times 100$$

Table 1 — The major bioactive compounds of *Murraya koenigii* and its pharmacological activity[19,20].

Phytoconstituents	Parts of plant	Pharmacological activity
Mahanine	Leaves, stem bark, seed	Anti-microbial, anti-cancer, Effect on bronchial disorders
Mahanimbine	Leaves, stem bark, seed and fruits	Antioxidant and anti-diabetic
Koenimbine	Leaves, seed and fruits	Cytotoxicity and anti-diarrhea
Koenigine	Leaves and stem bark	Antioxidant
Girinimbine	Roots, stem bark, and seeds	Anti-tumor and anti-trichomona
Isomahanine	Leaves, seed and fruits	Antioxidant, hyperlipidemic and anti-microbial
Isolongifolene	Leaves	Antioxidant and neuroprotective
Murrayafoline A	Roots	Cytotoxicity and anti-inflammatory
Murrayazoline	Stem and bark	Cytotoxicity and anti-tumor
Murrayacinine	Stem and bark	Anti-diabetic and hyperlipidemic
Mahanimbilol	Stem, leaves and roots	Vasodilation and Anti-Trichomona

Chemicals

Monosodium glutamate (MSG) (Sigma), vitamin C (Research lab, Mumbai), DPPH, TCA, TBA (Sigma). Biochemical kits for alanine transferase (ALT or SGPT), aspartate amino transferase (AST or SGOT), alkaline phosphatase (ALP), cholesterol, bilirubin, triglycerides, albumin were obtained from Pawar Agencies, Nashik.

Animals

Laboratory Wistar rats of either sex weighing between 180-220 g procured from Lacsmi Biofarms Pvt. Ltd., Pune (India), maintained under standard laboratory conditions of 25±1 °C, relative humidity of 45-55% and photoperiod (12 h dark/ 12 h light) were used for experiment. Commercial pellet diet (Jay Trading Co. Panchavati, Nashik, India) and water were provided *ad libitum*. The experiments were carried out according to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi, India. The protocol was approved by the Institutional Animal Ethical Committee (MGV/PC/CPCSEA/XXXII/01/2020/03).

Experimental design

All animals were divided into six groups; each group consisted of 5 animals and were treated for 14 days. MSG (1000 mg/kg) was given after 1 hour of AE-MK and vitamin C administration. Group I received distilled water (10 mL/kg, p.o), Group II received MSG (1000 mg/kg, p.o), Group III received AE-MK (100 mg/kg, p.o) + MSG (1000 mg/kg), Group IV received AE-MK (200 mg/kg, p.o) + MSG (1000 mg/kg), Group V received AE-MK (400 mg/kg, p.o) + MSG (1000 mg/kg), Group VI received vitamin C (300 mg/kg, p.o) + MSG (1000 mg/kg). All the animals were sacrificed on the 15th day for biochemical and histopathological investigation.

Percent change in body weight, relative organ weight (Liver)

Body weight of each animal was determined before treatment and before sacrifice. Liver of each animal were dissected out and weighed. Relative organ weight (ROW) was determined by using formula:

$$\text{ROW} = \frac{\text{Absolute organ weight(gm)}}{\text{Body weight of the rat on the day of sacrifice}}$$

Blood collection and separation

At the end of treatment period, the animals were sacrificed 24 h following the last given dose. Blood samples were withdrawn by cardiac puncture. Serum was separated by centrifugation at 3000 rpm for 10 min. The serum sample was used for measurement of biochemical assay.

Biochemical assay

Aspartate aminotransferase (AST or SGOT)

The method is based on the principle that oxaloacetate (oxaloacetic acid) catalyzed reaction between alpha ketoglutarate and aspartate is coupled with chromogen (2,4 dinitrophenyl hydrazine) in alkaline medium to form coloured hydrazone. The concentration of the coloured hydrazone is proportional to the aspartate aminotransferase activity. The oxidation rate is measured kinetically by monitoring the change in absorbance at 340 nm[24,25].

Alanine aminotransferase (ALT or SGPT)

Alanine transaminase (ALT) catalyses the transamination of L-alanine and α- ketoglutarate to form pyruvate and L-glutamate. In subsequent reaction, lactate dehydrogenase reduced pyruvate to lactate with simultaneous oxidation of NADH to NAD. The oxidation rate is measured kinetically by monitoring the change in absorbance at 340 nm[25,26].

Alkaline phosphatase (ALP)

Alkaline phosphatase catalyses the hydrolysis of colourless p-Nitrophenyl phosphate (pNPP) to yellow coloured p-Nitrophenol and phosphate. The change in absorbance due to yellow colour formation is measured kinetically at 405 nm and is proportional to ALP activity in the sample[27].

Albumin

At pH 3.6, albumin acts as a cation and binds to the anionic dye bromocresol green, forming a green coloured complex. The absorbance of final colour is measured at 630 nm. The colour intensity of the complex is proportional to albumin concentration in the sample[28,29].

Bilirubin

Bilirubin reacts with diazotized sulphanilic acid to form a coloured azobilirubin compound. The unconjugated bilirubin couples with the sulphanilic acid in the presence of a caffein benzoate accelerator. The absorbance is measured at 546 nm. The intensity of the colour formed is directly proportional to the amount of bilirubin present in the sample[30].

Cholesterol

Cholesterol esterase hydrolyses esterified cholesterol to free cholesterol. The free cholesterol is

oxidized to form hydrogen peroxide which further reacts with phenol and 4-aminoantipyrin by the catalytic action of peroxidase to form a red coloured quinoneimine dye complex. Intensity of the colour formed is directly proportional to the amount of cholesterol present in the sample[31,32].

Triglycerides

Triglycerides are hydrolysed by lipoprotein lipase to produce glycerol and free fatty acids. In the presence of glycerol kinase, adenosine triphosphate, phosphorylates glycerol to produce glycerol 3-phosphate and adenosine diphosphate. glycerol 3-phosphate further oxidized by glycerol 3-phosphate oxidase to produced dihydroxyacetone phosphate and H_2O_2. Absorbance of coloured dye is measured at 505 nm and is proportional to triglycerides concentration in the sample[33,34].

Antioxidant parameters

Preparation of tissue homogenate

A known amount of tissue was weighed and homogenized in 0.1 M Tris-HCl buffer for estimation of SOD, lipid peroxidation, and glutathione peroxidase activity.

Superoxide dismutase activity (SOD)

The assay of SOD is based on the ability of SOD to inhibit spontaneous oxidation of adrenaline to adrenochrome. To 0.5 mL of supernatant, 2.0 mL of carbonate buffer and 0.5 mL of EDTA solution were added. The reaction was initiated by addition of 0.5 mL of epinephrin and auto-oxidation of adrenaline to adrenochrome was measured at 480 nm. The change in absorbance for every minute was measured against blank. The results were expressed as unit of SOD activity (mg/wet tissue)[35].

Lipid peroxidation (MDA)

Serum malondialdehyde (MDA) concentration, one of the end product of lipid peroxidation was determined based on the principle that thiobarbituric acid reacting substances (TBARS), in this case malondialdehyde, react with thiobarbituric acid (TBA) to give a red or pink colour which absorbs maximally at 535 nm. 0.1 mL of supernatant was treated with 2.0 mL of (1:1:1 ratio) TBA-TCA-HCl reagent (Thiobarbituric acid 0.37%, 0.25 N HCl and 15% TCA) and placed in water bath for 15 min, cooled and centrifuged at room temperature for 10 min at 1000 rpm. The absorbance was measured at 535 nm[36].

Glutathione peroxidase (GPx)

Exactly 0.1 mL of supernatant was mixed with 50 mM phosphate buffer containing 2 mM EDTA. The assay system in a final volume of 1 mL contained 0.05 M phosphate buffer with 2 mM EDTA. 0.025 mM sodium azide, 0.15 mM glutathione, and 0.25 mM NADPH. The reaction was started by the addition of 0.36 mM H_2O_2. The linear decrease of absorbance at 340 nm was recorded using a UV/VIS spectrophotometer. The specific activity of the enzyme was expressed as U/mL[35].

Histopathological examination

After sacrificing the animal the liver tissue was removed immediately and fixed in 10% formalin solution and sent for histopathological examination. The tissues were embedded in paraffin wax, cut into fine thin sections of 3-5 µm thickness and were stained with hematoxylene-eosin and observed for histological changes by taking photograph under 40X magnification[36].

Statistical analysis

The results were expressed as mean±SEM. Statistical analysis was done using one-way analysis of variance, followed by Dunnett's multiple comparison tests. $P < 0.05$ was considered significant.

Results and Discussion

***In vitro* antioxidant activity**

Total phenol content

The total phenol content of AE-MK was found to be 62 µg of gallic acid equivalent /mg AE-MK (Fig. 1).

Free radical scavenging activity

The % scavenging activity of AE-MK was found to be 74.16%. In the DPPH free radical scavenging assay, AE-MK at various concentration produced

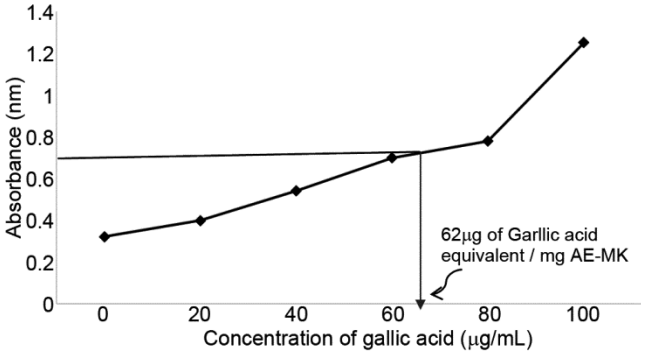

Fig. 1 — Total phenolic content determination of AE-MK by Folin-Ciocalteu's method.

inhibition of DPPH free radicals. The scavenging activity was found to be 74.16% in aqueous extract of *M. koenigii.* It was reported that the aqueous extract may well act as electron donors and convert free radicals into more stable product, leads to termination of free radical chain reaction[37].

Percent change in body weight, relative organ weight

Percent change in body weight

There was a significant (P <0.05) increase in body weight in rats given MSG treatment as compared to vehicle treated group. Treatment with AE-MK (200, 400 mg/kg) and vitamin C (300 mg/kg) caused a significant (P <0.05) decrease in percent change in body weight as compared to MSG-treated group (Table 2). The increase in weight caused by MSG has been ameliorated with AE-MK.

Relative organ weight

There was a significant (P <0.05) increase in relative organ weight of liver tissue in rats given MSG treatment as compared to vehicle treated group. Treatment with AE-MK (200, 400 mg/kg) and vitamin C (300 mg/kg) caused a significant (P <0.05) decrease relative organ weight as compared to MSG-treated group (Table 2). The % Change in body weight, relative organ weight in MSG treated rat was significantly increased which showed the toxic effect of MSG. MSG was able to cause increase in energy intake which could lead to obesity[38] or it may alter metabolism levels of carbohydrate, lipids and proteins in rats[39]. Increase in body weight may be attributed to inflammation and oedema of liver tissue[40].

Table 2 — Effect of *Murraya koenigii* on Monosodium glutamate induced change in body weight, relative organ weight in Wistar rats

Treatment group (mg/kg)	% Change in body weight (Mean±SEM)	Relative organ weight of liver (g) (Mean±SEM)
Control (10 mL/kg)	2.93±0.14	1.51±0.06
MSG (1000)	5.68±0.81#	2.13±0.04#
AE-MK (100) +MSG (1000)	3.85±0.37	1.94±0.07
AE-MK (200) +MSG (1000)	2.93±0.42*	1.75±0.08*
AE-MK (400) +MSG (1000)	2.63±0.07*	1.55±0.07*
Vit. C (300) +MSG (1000)	2.83±0.07*	1.39±0.09*

All values are expressed as Mean±SEM. N= 5, All data were subjected to ANOVA followed by Dunnett's test. *P <0.05 was considered significant as compared to MSG treated group, # P <0.05 was considered significant as compared to control group. MSG = Monosodium glutamate, AE-MK = Aqueous extract of *Murraya koenigii*

Biochemical assay

ALT and AST levels

There was significant **(**P <0.05) increase in AST and ALT levels in rats given MSG (1000 mg/kg) treatment as compared to vehicle treated group. Treatment with AE-MK (200,400 mg/kg, p.o) caused a significant (P <0.05) decrease in AST and ALT level. Vit. C (300 mg/kg, p.o) significantly (P <0.05) decreased AST and ALT levels as compared to MSG (1000 mg/kg) treated group. The current results revealed high statistically significant impairment of liver function as indicated by significant increase in ALT and AST enzymes in MSG group. The increase could be explained by free radical production that reacts with polyunsaturated fatty acids of the cell membrane leading to impairment of mitochondrial and plasma membrane resulting in leakage of enzymes[41] (Table 3).

ALP level

There was significant **(**P <0.05) decrease in ALP level in rats given MSG (1000 mg/kg) treatment as compared to vehicle treated group. Treatment with AE-MK (200, 400 mg/kg, p.o) caused a significant (P <0.05) increase in ALP level. Vitamin C (300 mg/kg, p.o) significantly (P <0.05) increased ALP level as compared to MSG (1000 mg/kg) treated group (Table 3).

Bilirubin, cholesterol, triglycerides

There was significant **(**P <0.05) increase in total bilirubin, cholesterol, and triglycerides levels in rats given MSG (1000 mg/kg) treatment as compared to vehicle treated group. Treatment with AE-MK (200, 400 mg/kg, p.o) caused a significant (P <0.05) decrease in total bilirubin, cholesterol, and triglycerides levels. Vitamin C (300 mg/kg, p.o) significantly (P <0.05) decreased total bilirubin, cholesterol and triglycerides levels as compared to MSG (1000 mg/kg) treated group. Increase in cholesterol level with increase in TGs in MSG treated rats was observed. MSG probably was able to increase the action of 3-hydroxy-3-methylglutaryl CoA (HMGCoA) reductase; the regulating enzyme in the cholesterol synthesis. Increase in enzyme action leads to increase in cholesterol synthesis in MSG treated group[40] (Table 3).

Albumin

There was significant **(**P <0.05) decrease in albumin activity in rats given MSG (1000 mg/kg) treatment as compared to vehicle treated group.

Table 3 — Effect of *Murraya koenigii* on monosodium glutamate induced changes in liver function test

Parameter	Treatment group (mg/kg)					
	Control (10 mL/kg)	MSG (1000)	AEMK (100) + MSG (1000)	AEMK (200) +MSG (1000)	AEMK (400) +MSG (1000)	Vit. C (300) +MSG (1000)
Bilirubin (mg/dL)	0.86±0.03	1.14±0.03$^{\#}$	0.99±0.02*	0.90±0.08*	0.87±0.01*	0.88±0.00*
Cholesterol (mg/dL)	40.09±1.87	59.09±2.06$^{\#}$	55.33±2.27	49.66±1.41*	38.75±2.67*	46.56±2.26*
Triglycerides (mg/dL)	117.70±5.82	175.00±7.37$^{\#}$	162.1±9.29	141.2±3.27*	128.3±5.96*	136.60±4.21*
Albumin (g/dL)	3.70±0.10	2.85±0.20$^{\#}$	2.75±0.25	3.22±0.06	3.88±0.22*	3.5±0.07*
HDL (mg/dL)	126.78±0.004	54.42±12.08$^{\#}$	84.55±11.27	141.63±25.54*	129.75±37.56*	201.73±18.06*
LDL (mg/dL)	17.37±5.99	34.93±2.53$^{\#}$	26.87±3.21	10.53±4.34*	14.01±3.88*	6.55±2.41*
ALP (IU/L)	109.35±9.85	47.44±8.02$^{\#}$	108.22±8.79*	105.73±5.72*	103.45±9.0*	103.70±8.41*
AST (IU/L)	27.99±3.62	60.98±3.71$^{\#}$	50.24±3.90	58.92±7.55	35.34±1.22*	32.85±1.51*
ALT (IU/L)	34.62±1.36	75.48±5.18$^{\#}$	62.02±3.0	57.31±1.74*	36.85±1.79*	28.53±2.18*

All values are expressed as Mean±SEM. N= 5, All data were subjected to One way ANOVA followed by Dunnett's test. **P* <0.05 was considered significant as compared to MSG treated group, #*P* <0.05 was considered significant as compared to control group.
MSG = Monosodium glutamate, AE-MK = Aqueous extract of *Murraya koenigii*

Treatment with AE-MK (200,400 mg/kg, p.o) caused a significant (*P* <0.05) increase in albumin activity. Vit. C (300 mg/kg, p.o) significantly (*P* <0.05) increased albumin activity as compared to MSG (1000 mg/kg) treated group (Table 3).

LDL and HDL level

There was significant **(***P* <0.05) increase in LDL level in rats given MSG (1000 mg/kg) treatment as compared to vehicle treated group. Treatment with AE-MK (200,400 mg/kg, p.o) caused a significant (*P* <0.05) decrease in LDL level. Vit. C (300 mg/kg, p.o) significantly (*P* <0.05) decreased LDL level as compared to MSG (1000 mg/kg) treated group.

A significant **(***P* <0.05) decrease was noted in HDL level in rats given MSG (1000 mg/kg) treatment as compared to vehicle treated group. Treatment with AE-MK (200,400 mg/kg, p.o) caused a significant (*P* <0.05) increase in HDL level. Vit. C (300 mg/kg, p.o) significantly (*P* <0.05) increased HDL level as compared to MSG (1000 mg/kg) treated group (Table 3).

In vivo antioxidant parameters

There was a significant (*P* <0.05) increase in SOD activity and lipid peroxidation (MDA) in MSG treated rats as compared to vehicle treated group. Treatment with AE-MK (400 mg/kg, p.o) and vitamin C (300 mg/kg, p.o) significantly decreased SOD activity and lipid peroxidation (MDA) as compared to MSG treated group. (Table 4). SOD is considered to be the primary step of defence in the antioxidant system. It catalyses dismutation of superoxide radicals to molecular oxygen and hydrogen peroxide[39] while glutathione peroxidase is the enzyme responsible for

Table 4 — Effect of *Murraya koenigii* on Monosodium glutamate induced changes on antioxidant status in the liver

Treatment group (mg/kg)	SOD (U/mg)	GPx (U/mL)	MDA (nMoles/mg)
Control (10 mL/kg)	0.0020±0.00	0.40±0.00	3.10±0.13
MSG (1000)	0.0072±0.00$^{\#}$	0.27±0.00$^{\#}$	8.9±0.02$^{\#}$
Extract (100) +MSG (1000)	0.0070±0.00	0.27±0.00	7.75±0.08*
Extract (200)+ MSG (1000)	0.0071±0.00	0.36±0.00*	2.67±0.13*
Extract (400) +MSG (1000)	0.0012±0.00*	0.34±0.00*	2.27±0.42*
Vit.C (300) + MSG (1000)	0.0018±0.00*	0.35±0.00*	3.67±0.24*

All values are expressed as Mean±SEM. N= 5, All data were subjected to ANOVA followed by Dunnett's test. **P* <0.05 was considered significant as compared to MSG treated group, $^{\#}$*P* <0.05 was considered significant as compared to control group.
MSG = Monosodium glutamate, AE-MK = Aqueous extract of *Murraya koenigii*

the conversion of lipid peroxidase to their congruent alcohol and it reduces free H_2O_2 reaction. The activity of both enzymes is sufficient for removal of ROS in normal homeostasis. The SOD activity was found to be significantly increased after MSG treatment, which may be the response of the organ toward the increased production of reactive oxygen species. The increase in lipid peroxidation may be attributed to direct effect of increase generation of ROS and an attempt by the tissue to restore their normal oxidative state[42]. Treatment with AE-MK (200, 400 mg/kg, p.o) significantly decreased the SOD and lipid peroxidation whereas showed significant increase in glutathione peroxidase as compared to MSG treated rats.

In case of glutathione peroxidase, MSG treated group showed significant **(***P* <0.05) decrease in GPx

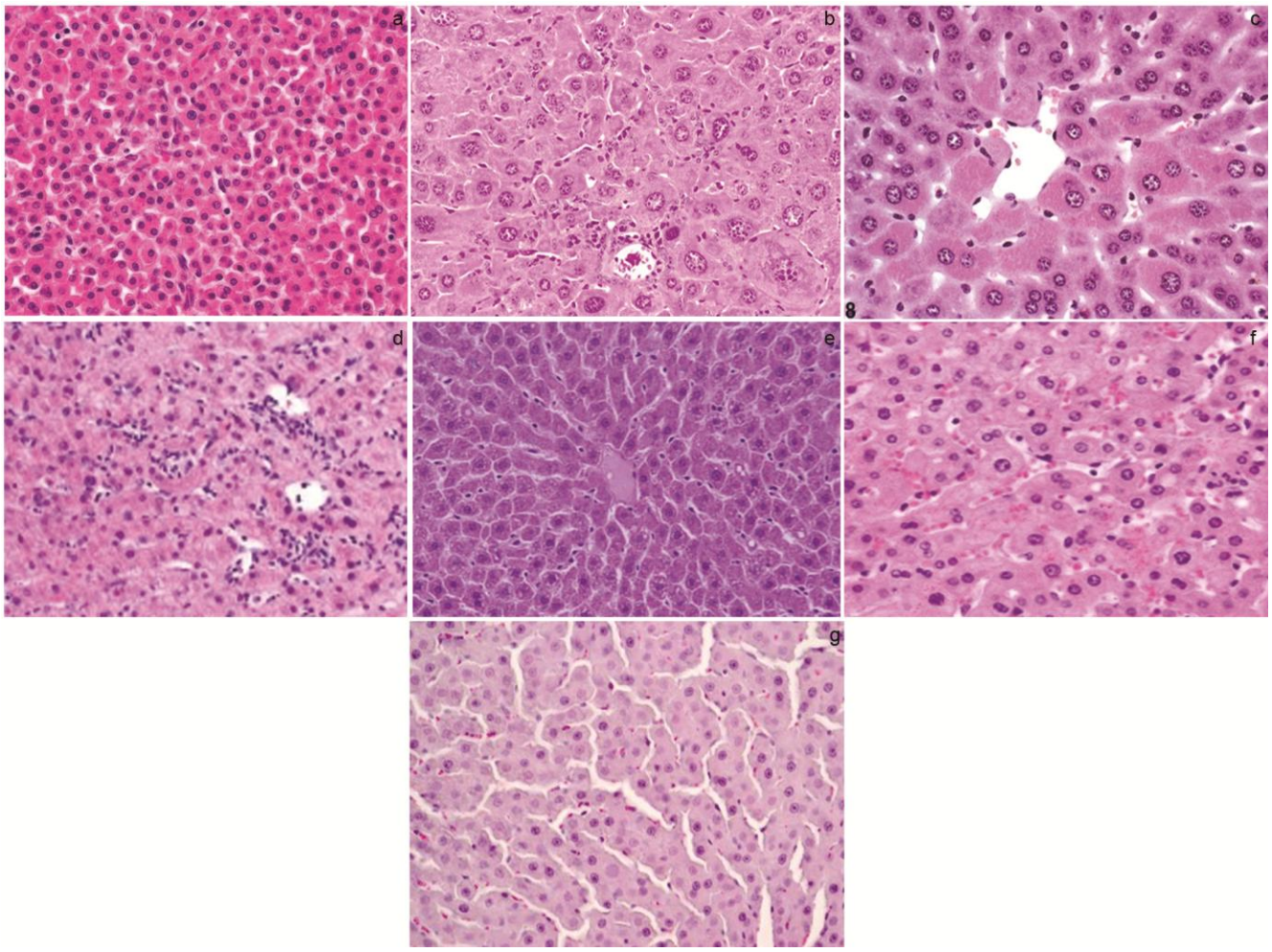

Fig. 2 — a) Section of H and E stained control group showing normal layer of hepatocytes (40X), b) Section of MSG (1000 mg/kg) treated rat liver tissue (40X) showing cellular aggregates around the portal area, c) Section of MSG (1000 mg/kg) treated rat liver tissue (10X) showing cytoplasmic vacuolation and sinusoidal congestion, d) Section of AE-MK (100 mg/kg) and MSG (1000mg/kg) treated rat liver tissue (40X) showing cytoplasmic vacuolation and cellular aggregation around the portal area, e) Section of AE-MK (200 mg/kg) and MSG (1000 mg/kg) treated rat liver tissue (40X) showing mild cytoplasmic vacuolation and cellular aggregation around the portal area, f) Section of AE-MK (400 mg/kg) and MSG (1000 mg/kg) treated rat liver tissue (40X) showing mild cytoplasmic vacuolation, sinusoidal congestion and cellular aggregates around the portal area, g) Section of Vit. C (300 mg/kg) and MSG (1000 mg/kg) treated rat liver tissue (40X) showing mild cytoplasmic vacuolation, sinusoidal congestion and cellular aggregates around the portal area.

activity in rats as compared to vehicle treated group. Treatment with AE-MK (100, 200, 400 mg/kg, p.o) in MSG (1000 mg/kg) treated rat caused a significant (P <0.05) increase in GPx activity. Vitamin C (300 mg/kg, p.o) significantly (P <0.05) increased GPx activity as compared to MSG (1000 mg/kg) treated group.

Histopathology

Histopathological studies of H and E stained liver section of the control group showed the normal architecture. On the contrary, MSG treated section of liver represented sign of cellular aggregates around the portal area, cytoplasmic vacuolation, sinusoidal congestion as compared to control group. AE-MK and vitamin C treatment in rats have shown to ameliorate the above pathological effects (Fig. 2).

Conclusion

The present study concluded that hepatotoxicity caused by MSG is mainly due to oxidative stress and increased production of ROS. Aqueous extract of *M. koenigii* has ameliorated hepatotoxicity induced by MSG thus showing hepatoprotective activity in laboratory rats. This hepatoprotective effect may be attributed to its ability to scavenge free radicals and inhibit ROS accumulation.

Conflict of interest

There is no conflict of interest.

References

1 Kazmi Z, Fatima I, Perveen S and Malik S, Monosodium glutamate: Review on clinical reports, *Int J Food Prop*, 2017, **20**, 1807-1815.

2 Thuy L N, Salanța L C, Tofana M, Socaci S A, Farcas A C, *et al.*, Mini review about monosodium glutamate, *Bulletin UASVM Food Sci Technol*, 2020, **77**(1), 1-12.

3 Niaz K, Zaplatic E and Spoor J, Extensive use of monosodium glutamate: A threat to public health?, *EXCLI J*, 2018, **17**, 273-278.

4 El-Shabrawi M H, Naglaa M K, Fawzi A H, El-Guindi M A and Sobhy G A, Serum superoxide dismutase activity in acute and chronic paediatric liver diseases, *Arab J Gastroenterol*, 2014, **15**, 72–75.

5 Irshad M and Chaudhari P S, Oxidant-antioxidant system: Role and significance in human body, *Indian J Exp Biol*, 2002, **40**, 1233-1239.

6 Mai A AL-Mosaibih, Effects of monosodium glutamate and acrylamide on the liver tissue of adult wistar rats, *Life Sci J*, 2013, **10**(2), 35-42.

7 Singh K and Aeluwalia P, Studies on the effect of monosodium glutamate [MSG] administration on some antioxidant enzymes in the arterial tissue of adult male mice, *J Nutr Sci Vitaminol*, 2003, **49**, 145-148.

8 Mohan M, Gangurde S and Kadam V, Protective effect of *Solanum torvum* on monosodium glutamate induced neurotoxicity in mice, *Indian J Nat Prod Resour*, 2017, **8**(4), 351-359.

9 Kadam V, Gangurde S and Mohan M, Protective effect of *Solanum torvum* on monosodium glutamate induced hepatotoxicity and nephrotoxicity in rats, *Indian J Nat Prod Resour*, 2019, **10**(1), 31-42.

10 Konrad S P, Farah V, Rodrigues B, Wichi R B, Machado U F, *et al.*, Monosodium glutamate neonatal treatment induces cardiovascular autonomic function changes in rodents, *Clinics*, 2012, **67**(10), 1209-1214.

11 Oladipo I C, Adebayo E A and Kuye O M, Effects of monosodium glutamate in ovaries of female sprague-dawley Rats, *Int J Curr Microbiol App Sci*, 2015, **4**(5), 737-745.

12 Furuya D T, Polleto A C, Favaro R R, Martins J O, Zorm T M, *et al.*, Anti-inflammatory effect of atorvastatin ameliorates insulin resistance in monosodium glutamate–treated obese mice, *Metab Clin Exp*, 2010, **59**, 395–399.

13 Tawfik M S and Nawal A B, Adverse effects of monosodium glutamate on Liver and Kidney functions in adult Rats and potential protective effect of Vitamins C and E, *Food Nutr Sci*, 2012, **3**, 651-659.

14 Sathaye S, Bagul Y, Gupta S, Kaur H and Redkar R, Hepatoprotective effects of aqueous leaf extract and crude isolates of *Murraya koenigii* against *in vitro* ethanol-induced hepatotoxicity model, *Exp Toxicol Pathol*, 2011, **63**, 587-591.

15 Singh S, More P K and Mohan S M, Curry leaves (*Murraya koenigii Linn. Sprengal*)- A mircale plant, *Indian J Sci Res*, 2014, **4**(1), 46-52.

16 Ajay S, Rahul S, Sumit G, Paras M, Mishra A, *et al.*, Comprehensive review: *Murraya koenigii Linn*, *Asian J Pharm Life Sci*, 2011, **1**(4), 417-425.

17 Balakrishnan R, Vijayraja D, Song-Hee J, Palanivel G and Dong-K C, Medicinal profile, phytochemistry, and pharmacological activities of *Murraya koenigii* and its primary bioactive compounds, antioxidants, 2020, **9**, 101.

18 Muthulinggam N and Subramanian P, *Murraya koenigii* (curry leave)- A review on its potential, *Int J Pharm Tech Res*, 2015, **7**, 566-572.

19 Jain M, Gilhotra R, Pal R and Mittal J, Curry leaf (*Murraya Koenigii*): A spice with medicinal property, *MOJ Biol Med*, 2017, **2**, 236-256.

20 Al-Manhel A J and Niamah A K, Effect of aqueous and alcoholic plant extracts on inhibition of some types of microbes and causing spoilage of food, *Pak J Food Sci*, 2015, **25**(3), 104-109.

21 Singleton V L, Orthofer R and Lamuela-Raventos R M, Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu Reagent, *Meth Enzymol*, 1999, **299**, 152-178.

22 Dahlia I A, Islam M B, Zaman S, Islam M A, Jalil M A, *et al.*, *In-vitro* DPPH free radical scavenging activity of the plant *Murraya koenigii* Linn (Curry leaf) in Rajshahi, *Bangladesh J Complement Altern Med Res*, 2017, **3**(4), 1-9.

23 Roberts W L, *et al.*, Reference information for the clinical laboratory in *Teitz fundamentals of clinical chemistry*, edited by C A Burtis, E R Ashwood, D E Bruns, 6th Edn., (St. Louis, Mo.: Saunders Elsevier) 2008, 856.

24 Gustafsson J E, Automated serum determination by use of the immediate reaction with bromocresol green reagent, *Clin Chem*, 1978, **24**(2), 369-373.

25 Jendrassik L and Grof P, Simplified photometric methods for the determination of Bilirubin, *Biochem J*, 1938, **297**, 81-89.

26 Herbert K, Lipids, in *Clinical chemistry: Theory, analysis and corelation*, (Toranto), 1984, 1182-1230.

27 McGowan M W, Artiss J D, Strandbergh D R and Zak B, A peroxidase-coupled method for the colorimetric determination of serum triglycerides, *Clin Chem*, 1983, **29**, 538-542.

28 Saggu H, Cooksey J, Dexter D, Wells F R, Lees A, *et al.*, A selective increase in particulate superoxide dismutase activity in parkinsonian substantia Nigra, *J Neurochem*, 1989, **53**, 692-697.

29 Niehaus W G and Samuelss B, Formation of malonaldehyde from phospholipid arachidonate during microsomal lipid peroxidation, *European J Uiochem*, 1968, **6**, 126-130.

30 Firdaus S B, Ghosha D, Chattyopadhyay A, Duttaa M, Paul S, *et al.*, Protective effect of antioxidant rich aqueous curry leaf (*Murraya koenigii*) extract against gastro-toxic effects of piroxicam in male Wistar rats, *Toxicol Rep*, 2014, **1**, 987-1003.

31 Ishak K, Histological grading and staging of chronic hepatitis, *J Hepatol* 1995, **22**, 69-99.

32 Lee J C, Kim H R, Kim J U and Jang Y S, Antioxidant property of an ethanol extract of the stem of *Opuntia ficus-indica* var. Saboten, *J Agric Food Chem*, 2002, **50**, 6490−6496.

33 Mozes S, Sefcikova Z, Lenhardt L and Racek L, Obesity and changes of alkaline phosphatase activity in the small intestine of 40- and 80-day-old Rats subjected to early postnatal overfeeding or monosodium glutamate, *Physiol Res*, 2004, **53**, 177-186.

34 Yeda S D, Fernandes A H, Kleber E C, Fernanda M, Bartolome O R, *et al.*, Toxicity of hypercaloric diet and monosodium glutamate: Oxidative stress and metabolic shifting in hepatic tissue, *Food Chem Toxicol*, 2004, **42**, 313–319.

35 Park J S, Choi M A, Kim B S, Han I S, Kurata T, *et al.*, Capsaicin protects against ethanol-induced oxidative injury in the gastric mucosa of rats, *Life Sci*, 2000, **67**, 3087–3093.

36 Onyema O O, Farombi E O, Emerole G O, Ukoha A I and Onyeza G O, Effect of vitamin E on Monosodium glutamate induced hepatotoxicity and oxidative stress in rats, *Indian J Biochem Biophys*, 2006, **43**, 20-24.

37 Poli G, Cottalasso D, Pronzato M A, Chiarpotto E, Biasi F, *et al.*, Lipid peroxidation and covalent binding in the early functional impairment of Liver golgi apparatus by carbon tetrachloride, *Cell Biochem Funct*, 1990, **8**, 1-10.

38 Atef H, El-Morsi D A, El-Shafey M, El-Sherbiny M, El-Kattawy H A, *et al*., Monosodium glutamate induced hepatotoxicity and oxidative stress: Pathophysiology, biochemical and electron microscopic study, *Med J Cairo Univ*, 2019, **87**, 397-406.

39 Bhasin P, Singla N and Dhawan D K, Protective role of zinc during aluminum-induced hepatotoxicity, *Environ Toxicol*, 2014, **29**(3), 320-327.

40 Qitao R, Hanyu L, Yuji I, Wenbo Q, Tomas A P, Reduction in glutathione peroxidase 4 increases life span through increased sensitivity to apoptosis, *J Gerontol, Biol Sci*, 2007, **62**(9), 932–942.

41 Gupta R C, *Toxicology of Organophosphate and Carbamate Compound*, (Elsevier Academic Press), 2016, 768.

42 El-Aziz A D and Hamza R Z, Monosodium glutamate induced hepatotoxicity and the possible mitigating effect of vitamin C and propolis, *J Adv Med Pharm Sci*, 2016, **7**(4), 1-10.