Effect of L-Glutamic acid on histology and functions of Liver and kidney of Rats and Protective Role of Zingibar Officionale

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Abstract

Background: Monosodium glutamate (MSG) or L-Glutamic acid is the sodium salt of glutamic acid is toxic to human and experimental animals. Liver and kidney may be susceptible to injury resulting from toxic substances. Ginger has immuno-modulatory, antitumorigenic, anti-inflammatory, anti-apoptotic, antihyperglycemic and anti-lipidemic actions.

Objective: This study has been carried out to evaluate the protective effects of aqueous extract of Zingiber Officinale in decreasing and ameliorating effects of Monosodium glutamate induced alteration in hepatic and renal tissues and their functions of rats.

Patients and Methods: Twenty four adult rats were divided into three equal groups and maintained under standard laboratory conditions. The ginger extract and the MSG were given orally once daily for 21 days. Group I Control: distilled water Group II: received MSG (4.0g/kg B.W) Group III: received 4.0g/kg B.W of MSG +100mg/Kg of ginger. After the last dose blood was collected.

Results: Monosodium Glutamate /L-Glutamate caused loss of normal histological architecture of liver and kidneys of rats of Group II with significant changes in biochemical parameters compared with control groups ($P < 0.0001$). Zingibar Officinale /Ginger is an excellent antioxidant substance that ameliorated and prevented the toxic effect of MSG in a group III treated with combination of MSG and Ginger histologically and functionally.

Conclusion: Zingibar Officinale (ginger) caused decrease the hepatotoxic and nephrotoxic effects resulted from oxidative damage induced by MSG because of their antioxidant effects.

Key words: Monosodium glutamate, ginger, antioxidant, liver, kidney

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Introduction

Monosodium Glutamate (MSG) or L-Glutamic acid known as AJI-NOMOTO is the sodium salt of glutamic acid [1].

Glutamate is one of the most common amino acids found in nature and is the main component of many proteins and peptides

of most tissues, it is also produced in the body and plays an essential role in human metabolism [2,3]. Now MSG can be produced commercially by bacterial fermentation [4]. Despite its taste stimulation and improved appetite enhancement, reports indicate that MSG is toxic to human and experimental animals [5]. The hepatocytes have metabolic functions that deal with very essential processes such as detoxification, deamination, transamination, removal of ammonia in the form of urea, biosynthesis and release of the non-essential amino acids and plasma proteins, gluconeogenesis, storage of glycogen, conversion of carbohydrates and proteins into lipids, synthesis of lipoproteins, phospholipids and cholesterol, oxidation of fatty acids, storage of iron in the form of ferritin as well as storage of vitamins A, D and B12. Several functional tests have been formulated to explore hepatic status [6,7,8,9,10]. Several enzymes have been determined to explore hepatic status such as alanine amino transferase (ALT) and aspartate amino transferase (AST) and because the liver is involved in the performance of these varied functions, it may be susceptible to injury resulting from toxic substances [11].

The Kidneysarenormally involved with the removal of toxic metabolites and waste products from the blood and regulation of the amount of fluid and electrolytes balance in the body. To test functions of the kidneys routine urinalysis is used to measure serum urea, creatinine, sodium, potassium and bicarbonate [7,12]. However several studies in animals have shown that MSG is toxic to various organs such as liver, brain, thymus, and kidneys [1,2].

ZingebarOffecionale (Ginger) is a strong anti-oxidant substance prevents generation of free radicals. It is considered a safe herbal medicine with only few and non-significant side effects (13). The main pharmacological actions of ginger include immuno-modulatory, antitumorigenic, anti-inflammatory, anti-apoptotic,
antihyperglycemic, anti-lipidemic and anti-emetic actions.

This study has been carried out to evaluate the protective effects of aqueous extract of ZingiberOfficionale on Monosodium glutamate induced histomorphometricalteration in hepatic and renal tissues and their functions in rats.

Patients and Methods

Twenty four adults male rats weighing between 180-210g were maintained in a well-ventilated animal house under standard condition of humidity, temperature and a constant light: dark lighting schedule, they were allowed to acclimatize for one week prior to the start of the experiment. The animals were fed with pelletized food and water. They were obtained from the animal house of veterinary college, University of Sulaimani. The animals were housed in clear polypropylene cages lined with wood chip beddings. The health and reproductive status of the animals were assessed and only healthy animals were selected for the experiment.

Statistical analysis

Synthetic monosodium glutamate (MSG) of 98% purity was obtained from Aldrich chemistry, France, for use in the study. A stock solution was prepared by dissolving (1.0g) MSG granule in (1.0) ml distilled water. From this and based on the animals weight, 4.0g/kg B.W dosage were administered to the animals in group (II and III) using gavage tube [14].

The aqueous extracts of Zingiber Officinale (Ginger) was prepared by dissolving 1 g of dried powderof MSG in 50ml of distilled water and the final extract concentration obtained was 20mg/ml. Extract

was stored in air- tight container and refrigerated throughout the experiment(15).

Experimental design:The twenty four rats were divided into three groups of eight animals per each.The ginger extract and the MSG were given orally once a day for 21 days. They were administered as follows: Control Group I: received distilled water Group II: received MSG (4.0g/kg B.W) Group III: received MSG 4.0 g/Kg BW and ginger 100g/kg .

Blood was collected; serum was prepared by centrifugation and used for enzyme analysis. Statistical analysis of the data was performed by using SPSS (Version 18), using independent paired t-test.

Results

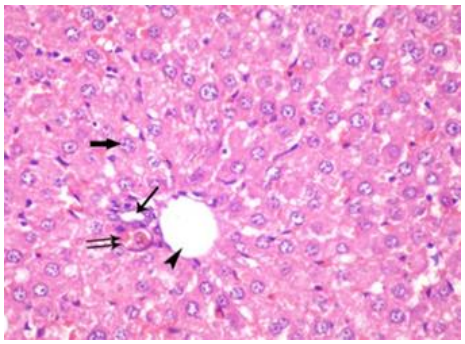

Figure (1): A photomicrograph of a section in the liver of control group showing the portal area with branches of portal vein (arrow head) and hepatic artery (double arrows).H&E X200

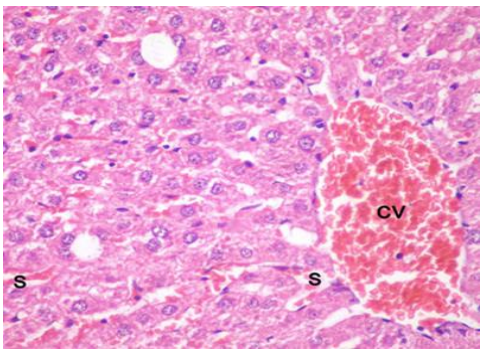

Figure (2):A photomicrograph of a section in the liver of MSG treated group II showing dilated congested central vein (CV) and blood sinusoids (s) [H&E ×400].

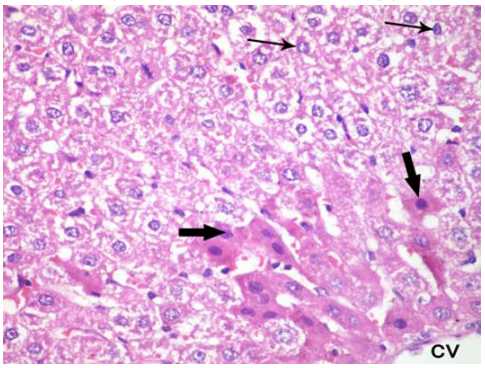

Figure (3): A photomicrograph of a section in the liver of MSG treated group II showing that most of the peripheral hepatocytes (thin arrows) appear with cytoplasmic vacuolization.[H&E ×400].

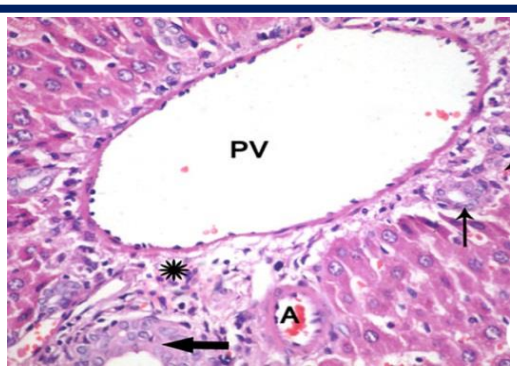

Figure (4):A photomicrograph of a section in the liver of MSG treated group II showing enlarged portal area with branches of dilated portal vein (pv), congested hepatic artery (A), cellular infiltration (*) and numerous bile ductules (thin arrows).[H&E ×400].

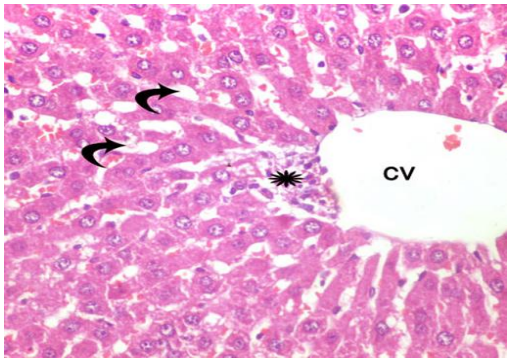

Figure (5):A photomicrograph of a section in the liver of MSG and ginger treated group III showing preservation of nearly normal hepatic lobular architecture with the presence of slightly dilated congested central vein (cv) and blood sinusoids (curved arrows) with few cellular infiltration (*) [H&E ×400].

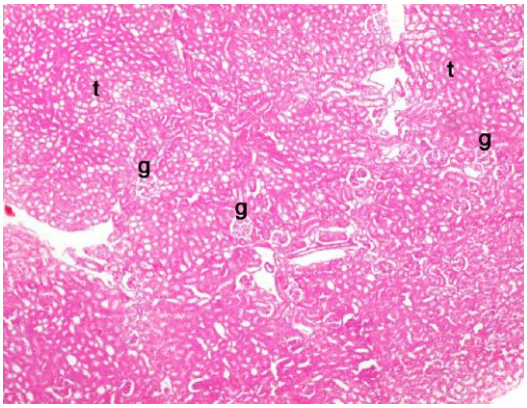

Figure (6): A photomicrograph of a section in a control rat's kidneyshowing normal glomerular tuft (g) in the cortex and tubules (t). (H & E X40)

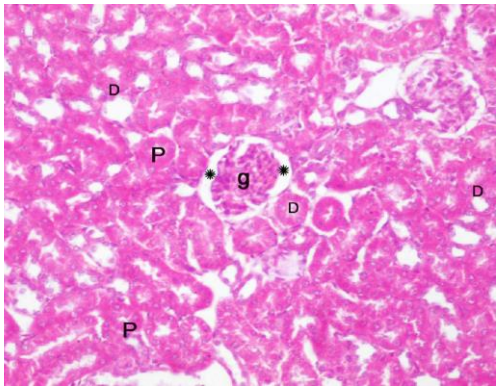

Figure (7): A photomicrograph of a section in a control rat's kidney showing renal corpuscle formed of glomerulus (g) surrounded with Bowman's capsule and preserved renal space(*).Note the proximal (P)and distal (D)convoluted tubules(H&E X100).

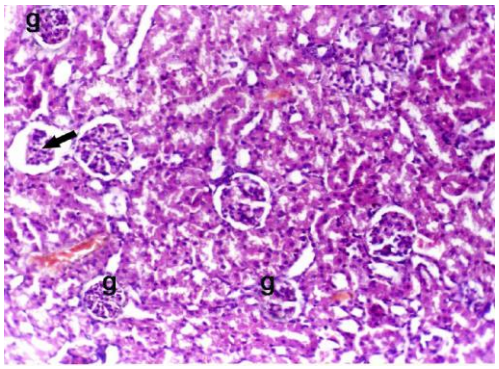

Figure (8): A photomicrograph of a section from the kidney of a rat from groupII.Someglomeuli showing shrinkage (arrow) and the others showing swelling with partial loss of the Bowman's spaces (g).(H & E X100).

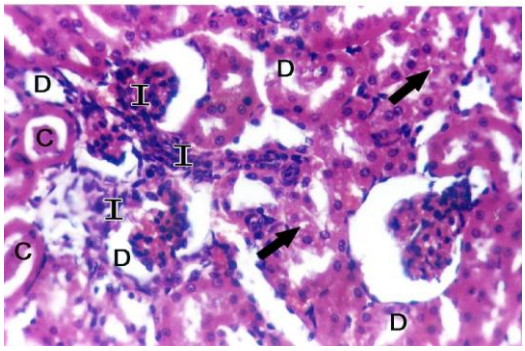

Figure (9): A photomicrograph of a section from the kidney of a rat of MSG group showing monocellular inflammatory cell infiltrates (I). Tubular dilatation (D), hyaline casts (C) andvacuolization of tubular cells are also observed (arrows) (H & EX200).

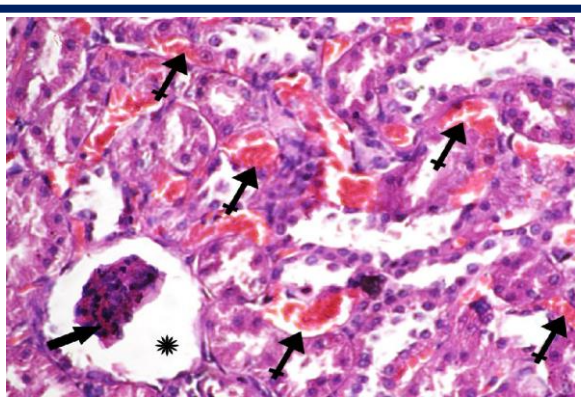

Figure (10):A photomicrograph of kidney section form a rat of MSG group Ishowing severe shrinkage of the glomerulus (arrow) with increased in Bowman's spaces (*). There is also dilated congested interstitial blood vessels and interstitial hemorrhage in the inter tubular spaces (crossed arrows). (H &E X400).

The biochemical parameters were significantly higher in the MSG group II than control groups (P < 0.0001) In group treated with combination of MSG and Ginger the results were.

Table (1): Comparison of means of liver function test parameters between Groups I, II, and III.

	Group I Mean±SD	Group II Mean ±SD	Group III Mean ±D
Bilirubin	0.1667±0.02108	0.1667±0.02108	0.2000±0.00000
Cholesterol	63.3333±1.28236	57.6667±1.97765 *↓	45.8000±1.39284 *↓
AST	234.00±1.57056	153.17±1.35195*↓	225.00±1.46059* ↓
ALT	57.6667±0.91894	33.5000±0.99163*↓	53.8333±0.98036 *↓
Albumin	1.3667±0.02108	1.2667±0.02108*↓	1.3333±0.02108

Table (2): Comparison of means of renal function test parameters betweenGroups I, II, and III.

	Group I Mean±SD	Group II Mean ±SD	Group III Mean ±SD
Uric acid	1.3000±0.03651	2.6667±0.10220*↑	1.6833±0.04773*↑
Urea	15.6667±0.21082	17.5000±0.42817*↑	15.1667±0.30732
creatinine	0.7667±0.02108	0.9000±0.03651*↑	0.7333±0.03333
Na	133.83±0.60093	147.33±0.76012*↑	138.50±0.42817*↑
K	5.3000±0.09661	6.4167±0.10138*↑	5.6333±0.09545*↑
Cl	114.67±0.42164	102.67±0.88192*↓	110.00±0.57735*↓

Table (3): Comparison of means of liver function test betweenGroup II and III.

	Group II Mean ±SD	GroupIII Mean ±SD
Bilirubin	0.1667±0.02108	0.2000±0.00000
Cholesterol	57.6667±1.97765 *↑	45.8000±1.39284*↓
AST	153.17±1.35195*↓	225.00±1.46059*↑
ALT	33.5000±0.99163*↓	53.8333±0.98036*↑
Albumin	1.2667±0.02108	1.3333±0.02108

Table (4): Comparison of means of renal function test between Group II and III.

	Group II Mean ±SD	Group III Mean ±SD
Urea	17.5000±0.42817*↑	15.1667±0.30732*↓
creatinine	0.9000±0.03651*↑	0.7333±0.03333↓
Na	147.33±0.76012*↑	138.50±0.42817*↓
K	6.4167±0.10138*↑	5.6333±0.09545*↓
Cl	102.67±0.88192*↓	110.00±0.57735*↑

Discussion

Monosodium Glutamate is one of the most extensively researched food additives in the world [9,12].Results of studies continue to support the finding that at levels normally consumed as flavor enhancer, MSG is safe for the general population [6].Oxidative stress is caused by excessive production or a decreased elimination of free radicals in cells, the majority of which are oxygen radicals and other reactive oxygen species (ROS)[6] .The abundance of long-chain polyunsaturated fatty acids in the composition of renal lipids makes kidney susceptible to damage by ROS[13].This makes kidney tissues prone to damage by different mechanisms such as the promotion of lipid peroxidation, protein modification, and DNA damage, leading to cell death [7,12]. Accordingly, the involvement of ROS has been reported in glomerular, tubular and tubulo-interstitial alterations [8,9].

Inthis study the liver of experimental animals showed changes in histological pattern evidentby disruption of hepatic cords, presence of inflammatory cells within and around the centralvein with uneven sizes of nucleus in hepatocytes. Few reports on alteration in liverhistology and/or biochemistry have been documented although these studies used doses thatwere way above the dose we chose for this study [10,15,16]. Monosodium glutamate may have acted as toxins to the hepatocytes, thereby affecting their cellular integrity and causing defect in membrane permeability and cell volume homeostasis. The atrophic and degenerative changes observed in this experiment may have been caused by the cytotoxic effect of MSG on the liver. This obviously will affect the normal detoxification processes and other functions of the liver.

Kidney microanatomy in group II that received MSG compared to control showed dilatation of the Bowman's space, contraction of the renal glomerulus andhypercellularity which are in keeping with renal injury, this corroborates results of studiescarried out in 2007 by Eweka [1] .He investigated the effects of MSG on the kidney of adultWistar rats given 3g and 6g of MSG thoroughly mixed with growers mash for the period offourteen days, results of kidney microanatomy showed varying degrees of cytoarchitecturaldistortion and reduction in the number of renal corpuscles in the treated groups which was atvariance with that of the control group.

Monosodium glutamate can induce changes in the renal cytoarchitecture, increase glomerular hyper-cellularity, infiltration of inflammatory cells in the renal cortex, edema of tubular cells, and eventually degeneration of renal tubules [17-18]. These results exactly confirm the results that we found.The formation of ROS in the kidney exposed to MSG was seen as a major contributor to their nephrotoxic effects leading to cellular and functional damage [13]. MSG supplementation either by injection or oral intake has been shown to alter renal antioxidant system markers, including lipid peroxidation byproducts and

kidney function in rats (15,17)]. In our study the rats in group III which treated with MSG and Ginger showed better results in their liver and renal function tests and their cytoarchitecture had been less affected, this has occurred because of antioxidant role of Ginger.Moreover, some studies have found the ameliorating effect of vitamin C, E, and Quercetin a plant pigment on MSG-treated kidneys(17, 19, 20). The mechanism whereby these antioxidants exert such effects is yet to be fully elucidated. However, these antioxidants seem to play a key role against renal inflammatory responses through a diminution of the activity of inflammatory enzymes [1]. These important findings add further prospective to the therapy of MSG-induced renal oxidative stress using antioxidants.However, altered kidney function and pathology but not the renal stones were reported by Paul et al. (2012) after 6 months of oral MSG treatment with higher dose (20). Cellular infiltration was noticed in the portal areas in liver sections of group II in this study. These observations were in accordance with the results obtained by other studies that referred cellular infiltration to ROS production which indirectly regulate chemokine receptor expression and promote cytokine IL-6 and IL-8 which are key modulators of inflammatory response(21,22). Other investigators considered that cellular infiltration as a prominent immune response of the body tissues by movement of fluids and leukocytes from the blood into the extravascular tissues(23,24).

The effects observed in both the liver and kidneys could have occurred because theseorgans are involved in the metabolism of glutamate or as in another study it may be dueparticularly in the liver exacerbation of trans-fat induced fatty liver disease in rats by amechanism that includes increased central adiposity and alterations in both hepatic andwhite adipose tissue gene expression [25, 26].

In conclusion, ginger can decrease the damage tolivercells from oxidative damage induces by MSG, and itis dependent on their antioxidant effects.

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