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Protective Role of *Ginkgo biloba* on Monosodium Glutamate: Induced Liver and Kidney Toxicity in Rats.

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ABSTRACT

Monosodium glutamate (MSG) was administrated to rats at doses of 1.5 mg/kg body weight for four weeks. The liver functions, the activities of ALT and AST significantly increased in the serum on MSG administration while serum total protein and albumin significantly decreased, MSG had adverse effect on kidney functions as serum urea , serum creatinine and uric acid were significantly increased.Ginkgo biloba (80 mg/kg body weight) co-treatment with MSG, significantly reported the activities of ALT and AST in the serum were reduced to become comparable with control. Consequently, serum total protein, and albumin were significantly increased in the serum, while serum urea , serum creatinine and uric acid were significantly decrease after administration of each ginkgo biloba. Lipid peroxidation level of liver and kidney was increased on MSG administration against the control while lipid peroxidation level decreased significantly in co-treatment with ginkgo biloba. The results showed that MSG at dose 1.5 mg/kg of body weight may cause an adverse effect on the heptic and renal functions which might be due to oxidative stress induced by MSG on the liver and renal tissue. Supplement of ginkgo biloba was capable of ameliorating MSG induced oxidative stress on hepatic and renal functions.

Keywords: *Ginkgo biloba*, monosodium glutamate, liver, kidney

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INTRODUCTION

Monosodium glutamate (MSG) is one of the world's most extensively used food additives which is ingested as part of commercially processed foods. As a flavor enhancer, MSG increases the sapidity of food. MSG produces a flavor that cannot be provided by other foods. It elicits a taste described in Japanese as umami, which is translated to sovery [1,2]. In 1991, the average intake of MSC in united kingdom was 580 mg/day for general population individual and 4.689/day for extreme users [3]. The estimated average daily MSG intake/person in industrialized countries in 0.3-1.0 g, but it depended on MSG content in food an individuals taste preferences . According to a joint inquiry by the governments of Australia and New Zealand in 2003, a typical Chinese restaurant meal contains between 10 and 1500 mg of MSG/100 g [4]. The oral dose that in lethal 5% of subjects LD50 in rats and mice is 15.000-18.000 mg/kg body weight [5].

Studies providing the evidence of MSG toxic effects have raised the increasing interest in MSG intake as flavor enhancer. Neurotoxic effects in brain, obesity and metabolic defects Chinese restaurant syndrome and detrimental effects on sex organs are the most discussed in connection with MSG intake [2].

As liver transaminases were severely depressed, authors hypothesized that MSG could induce liver injury likely as a consequence of incipient non alcoholic steato hepatitis, contributing to inflammation . MSG increased the expression on several genes implicated in adipocytes differentiation, elevated serum free fatty acids, triglycerides, insulin and bile synthesis [6]. Oxidative stress after MSG administration by a gavage at a dose of 0.6 mg/g for 10 days has been shown in the liver of rats, in which MSG induced lipid peroxidation, decreased reduced glutathione-transferas, catalase and superoxide dismutase [7,8].

Ginkgo biloba (GLE) is spelled gingko and also known as the maidenhair tree, is a unique species of tree with no close living relatives [9]. The ginkgo is a living fossil, recognizably similar to fossils dating back 270 million years. Native to china the tree is widely cultivated and was introduced early to human history. It has various uses in traditional medicine and as a source of food. Extracts of ginko leaves contain flavonoid glycosides and terpenoids (*Ginkgolides bilobalides*) and have been used pharmaceutically [10-12].

Ginkgo extract has in addition been found to act as a selective 5-HTIA receptor against in vivo [13]. Ginkgo supplements are usually taken in the range of 4-200 mg/day. In 2010, a meta-analysis of clinical trials has shown Ginkgo it be moderately effective in improving cognition in dementia patients not preventing the onset of Alzheimer's disease in people without dementia [14-16].

MATERIALS AND METHODS

Materials

Animals

Albino rats aged 3-4 months and weighing 100-150 g will be used. Animals were maintained under standard conditions of temperature (20-25 oC) and (50-65%) relative humidity with regular diet 12h dark cycle and allowed free access to standard laboratory food and water. Rats were acclimatized perior to experimental by one week.

Animal grouping

According to the treatment with the liver and kidney by monosodium glutamate and ginkgo biloba respectively, animals, were classified into the following 4 groups:

Group I: it has included 10 rats did not received any treatment.

Group 2: Ginkgo biloba group (GLE) in which, rats were received GLE orally by stomach tube a dose of (80 mg/kg

body weight/twice a week) for four weeks:

Group 3: monosodium glutamate group in which, rats were received MSG orally by stomach tube a dose of (1.5 mg/kg body weight/twice a week) for four weeks.

Group 4: co-treated group in which, rats were received orally MSG (1.5 mg/kg body weight/twice a week) and GLE (80 mg/kg body weight /twice a week) for four weeks.

Methods

Blood samples

At the end of the experimental period, and part of blood was collected from the inferior rena cava of each rat in heparinized and non-heparinized glass tubes, than all samples centrifuged at 1000 rpm for 20 min at 4oC. The samples were stored at -70oC until analysis.

Complete blood picture (CBC) were assayed by automatic methods (sysmex hx-21n automated haematology analyzer; JAPANCARE Co., LTDJ (1998). Including haemoglobin (Hb), white blood cells (WBCs), red blood cells (RBCs), platelets and haematocrite or packed cells volume (PCV).

Serum was separated by centrifugation at 3000 rpm for 15 min. for estimation of total protein, albumin, urea, uric acid,creatinin ,alanine aminotransferase (ALT) and aspartate aminotransferase (AST).

Tissue samples

After decapitation of rats, livers and kidneys were quickly removed and washed by cold saline.

Ten livers from each group were been stored at -20oC and were divided into two parts, each piece was homogenized separately with chilled – glass – teflon proter – Elvheim. One part was homogenized in phosphate buffer (PH 7.2) for estimation of total protein content and AST, ALT activities. The second part 10% w/v liver homogenate in ice-cold 0.9% saline for estimation of lipid peroxidation.

Biochemical investigation in liver tissue

Total protein: The protein concentration was detected by the methods of [17] as modified by [18].

Lipid peroxidation is one of terminal product, for at the time of the decomposition of polyum saturated fatty acids mediated by free radicals. LP was estimated by [19].

Biochemical investigation of kidney function in serum:

Urea concentration -Urea level in serum was assayed by using commercial kit that was supplied by diamond from Egypt [20].

Creatinine concentration: creatinine concentration in serum was assayed by using commercial kit that was supplied by diamond [21].

Uric acid concentration: Uric acid level in serum was assayed by using a commercial kit that was supplied by SPINREACT from SANTA Coloma, Spin [22].

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Biochemical investigation of liver function

Serum alanine aminotransferase (ALT)

All activity assayed by using commercial kit that was supplied by Randox, from Egypt. ALT was estimated by [23].

Serum aspartate aminotransferase (AST)

AST activity assayed by using commercial kit that was supplied by Rondox from Egypt. AST was estimated by [23].

Albumin concentration

Albumin concentration assayed by using commercial kit that was supplied by Diamond, form Egypt, Albumin concentration was estimated according to [21].

RESULTS

The changes blood parameters in different groups.

Red blood cells (RBCs)

RBCs levels, platelets and HB in MSG group were significantly decreased when compared with control group and GLE groups(Table 1).

White blood cells (WBCs)

WBCs levels in MSG group were significantly decreased when compared with control and GLE groups in table 3. The results shows that treatment with GLE along with MSG may show improvement in all WBCs indices.

Liver function in serum*:*

ALT level in MSG group showed significantly increase when compared with control , GLE and co-treatment groups.AST levels of all groups were significantly increase in MSG compared to control and GLE groups. Total proteins level showed significant decrease in MSG and contrasted groups .Albumin levels showed significant decreases in MSG and co-treated groups compared to control and GLE groups (Table 3) .

Kidney functions:

Serum urea showed significant increase in MSG and co-treated groups than control and GLE groups. Serum creatinine in control and ginkgo biloba showed significant decrease as compared to MSG and co-treated groups. Serum uric acid showed significant increase in MSG and MGS with ginkgo biloba groups compared to control and GLE groups (Table 3).

Lipid peroxidation(LP): LP level in MSG only group showed increase as compared to control and GLE groups. Also co-treated treatment showed decrease as compared to MSG only groups (Table 4).

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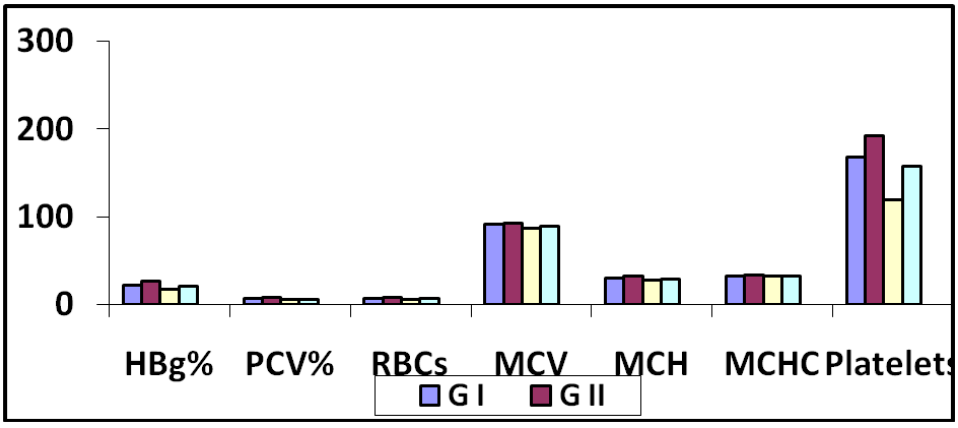
Table I: Changes in blood indices (HB%,PCV%, RBCs, MCV, MCH, MCHC and platelets) levels in different groups .

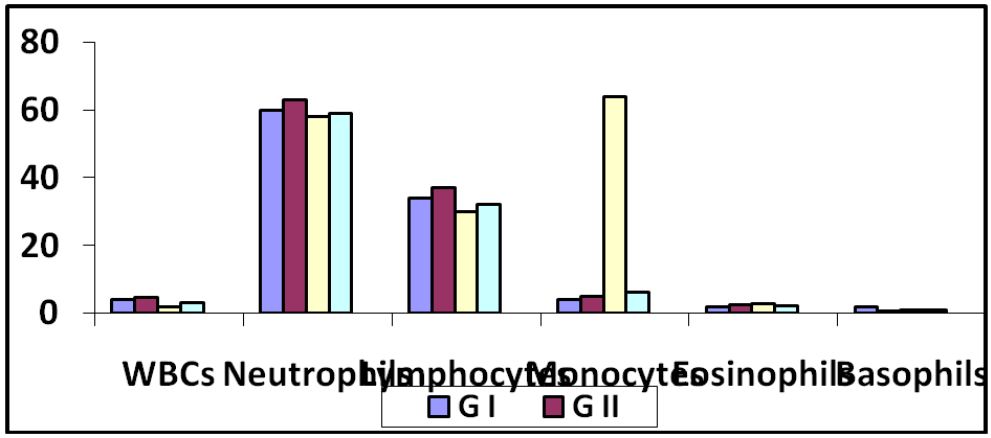
Group	Hb g%	PCV %	RBCs (million/Cm^3)	MCV (P)	MCH (pg)	MCHC (g/dl)	Platelets x 10^3
G I	22.1±0.23	6.7±1.1	7.4±0.39	91±1.1	30±1.1	33±1.2	168±5.7
G II	25.4±0.31	8.1±0.9	7.9±0.5	93±1.7	32±1.9	34±1.6	192±6.1
G III	16.90±0.78	5.66±0.25	5.66±0.25	87.8±0.24	28.4±0.24	32.5±0.2	119±7
G V	20.3±0.5	6.2±0.6	6.9±0.8	89.6±0.3	29.7±0.6	32.9±0.1	158±3
P	P<0.01	P<0.01	P<0.05	P<0.05	P<0.05	P>0.05	P<0.01



Data are expressed as mean ± SE of 10 observation. Significant difference from the control group (G1) at P<0.01. significant differences from monosodium glutamate (G3) at P< 0.01. where G1, control group, G2 GLE; G3, monosodium glutamate and G4, co-treated monosodium glutamate groups with GLE.

Table 2: Changes in WBCs and its components differential cells levels in different groups.

Group	WBCs x 103	Neutrophils %	Lymphocytes %	Monocytes %	Eosinophils %	Basophils %
G I	4±0.49	60±1.7	34±1.45	4±0.33	2±0.33	0
G II	4.5±0.32	63.2±1.9	37.3±1.7	5±0.81	2.5±0.18	0.6±0.3
G III	2±0.92	58.6±1	30.8±0.48	6.8±0.96	2.8±0.37	1±0.7
G V	3.1±0.7	59.1±1.1	32.7±0.41	6.2±0.89	2.1±0.29	0.9±0.8
P	P<0.05	P>0.05	P<0.05	P<0.05	P<0.05	P>0.05



Data are expressed as mean ±SE of 10 rats. Significant difference from the control group (G1) at P<0.05. significant different from monosodium glutamate group (G3) at P<0.05. where G1, control group; G2 GLE; G3, monosodium glutamate; G4, co-treated monosodium glutamate group with GLE.

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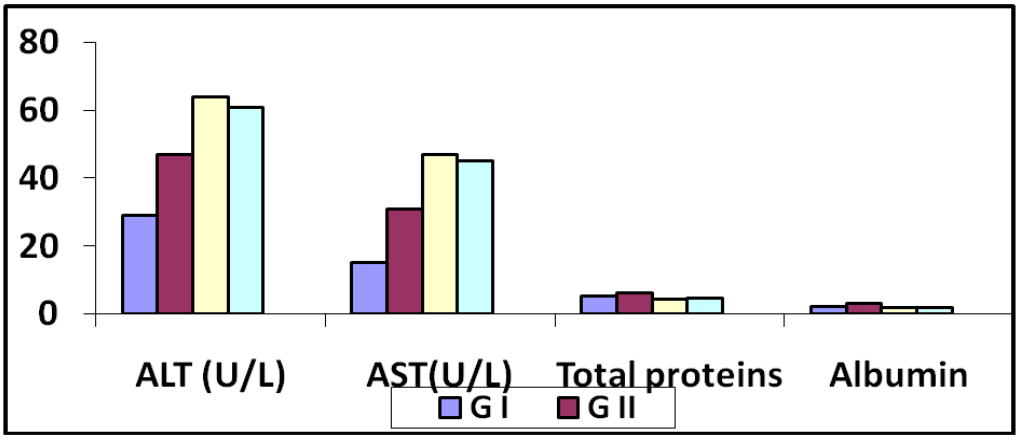
Table 3: Changes in liver total protein, albumin, ALT and AST in different groups.

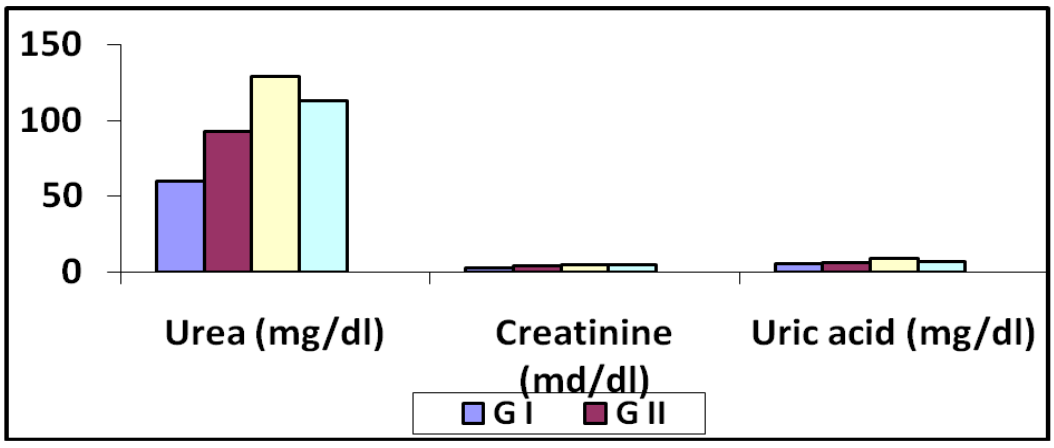
Group	ALT(U/L)	AST(U/L)	Total proteins(mg/g)	Albumin(mg/g)
G I	29.±2.6	15±1.8	5.1±0.26	2.2±0.2
G II	47±3.1	31.±1.7	6.3±0.31	3.1±0.1
G III	64±4.3	47±4.9	4.3±0.17	1.7±0.03
G V	61±4.1	45±3.9	4.6±0.13	1.8±0.01
P	P<0.01	P<0.01	P<0.05	P>0.05



Data are expressed as mean ± SE of 10 rats. Significant difference from the control group (G1) at *p< 0.05. significant difference from monosodium glutamate (G3) at #P<0.05. where G1, control group, G2 GLE group; G3 monosodium glutamate; G4, co-treated monosodium glutamate group with GLE.

Table 4: Changes in kidney urea, creatinine and uric acid in difference groups.

Group	Urea(mg/dl)	Creatinine(mg/dl)	Uric acid(mg/dl)
G I	60±1.7	2.9±3.9	5.90±0.37
G II	93±2.7	4.1±34	6.23±0.37
G III	129±10.7	5.2±0.23	8.96±0.39
G V	113±8.9	4.9±0.39	6.94±0.40
P	P<0.01	P<0.01	P<0.05



Data are expressed as mean± SE of 10 rats. Significant difference from the control group (G1) at *P<0.05 significant difference from monosodium glutamate (G3) at P<0.05. where G1, control group; G2, GLE group; G3, monosodium glutamate; G4, co-treated monosodium glutamate group with GLE.

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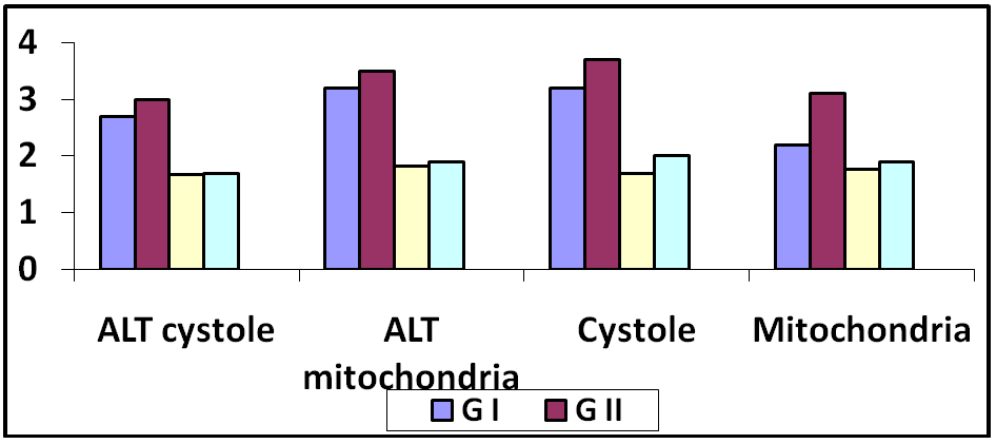
Table 5: Changes in tissue liver ALT and AST in citole and mitochondria homogenate indifferent groups.

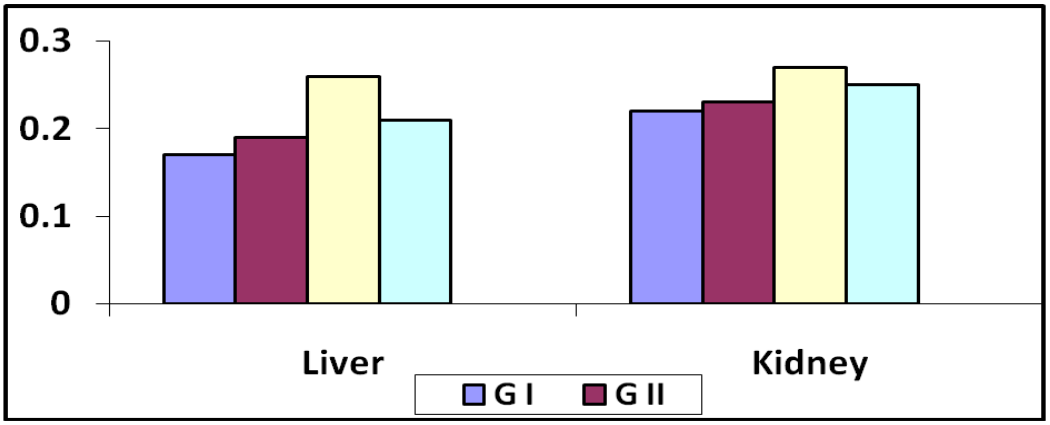
Group	ALT Cystole	ALT Mitochondria	AST Cystole	AST Mitochondria
GI	2.7±0.4	3.2±0.23	3.2±0.43	2.6±0.48
GII	3±0.1	3.5±0.21	3.7±0.49	3.1±0.41
GIII	1.68±0.37	1.82±0.15	1.7±0.21	1.76±0.2
GV	1.7±0.41	1.9±0.12	2±0.31	1.9±0.19



Data are expressed as mean ± SD of 10 observations. Significant difference from the control group (G1) at P<0.05. Significant difference from monosodium glutamate (G3) at P<0.05. Where G1, control group, G2 GLE, G3, monosodium glutamate group, G4, co-treated monosodium glutamate group with GLE

Table 6: Lipid peroxidation (liver lipid peroxidation level m/min/g/wet weight tissue) in liver of albino rat

Group	Liver	Kidney
G I	0.17±0.001	0.22
G II	0.19±0.02	0.23
G III	0.26±0.02	0.27
G V	0.21±0.01	0.25
P	P<0.01	P<0.01



The four groups, G1, control; G2, GLE; G3, monosodium glutamate; G4, monosodium glutamate group with GLE. Each reading represent mean ± SD of 10 rats. The significant of difference was chacked by one way ANOVA and multiple comparison dunnett test (compare all vs. control). The difference checked by one-way ANOVA was significant at P<0.001l; and Dunnet test was significant at P<0.05

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DISCUSSION

The study conducts a hematological and biochemical investigation into whither GLE has a protective and ameliorated effect on monosodium glutamate. The animals studies have shown that MSG per oral administration in doses similar to average human intake [24] and the intake in extreme users [25] led to disturbances in metabolism with increase in more parameters including insulin, fatty acids and triglycerides in serum and it also affected the liver functions resulting in elevation of transaminase levels and bile synthesis [2].

The results agreed with [7,24,25], were reported that serum ALT and AST were significantly increased in MSG induced liver damage. The ability of MSG to cause alteration in the activity of these enzymes could be a secondary event following MSG induced liver damage with the consequent leakage from hepatocytes [25, 27]. There was a significant ($P< 0.01$) restoration of these enzymes levels on administration of the Ginko biloba leaf extract.

The reversal of increased serum enzymes in MSG induced liver damage by the Ginko biloba leaf extract may be due to the prevention of the leakage of intracellular enzymes by its membrane stabilizing activity. This is in agreement with the commonly accepted view that serum levels of transaminases return to normal with the healing of hepatic parenchyma and the regeneration of hepatocytes [26,27].

In the liver tissues, the oxidative stress will be present in case of an imbalance between the production of ROS and the antioxidant defences.

The elevation of urea, creatinine and uric acids in MSG group when compared with control this elevation decreased in treated group with GLE. This results are in harmony with previous studies which reported that MSG increased significantly ($P<0.01$) urea and creatinine activities [28-30).

Oxidative stress in an indicator of the damage that results from a change in the balance between oxidants and antioxidants in favour of oxidants. Oxidative cellular damage with its dual of free radical generation and profound lipid peroxidation are hallmarks of MSG toxicity.

A significant increased ($P< 0.05$) in lipid peroxidation levels and total protein in co-treated group MSG with GLE in rat liver and kidney when compared to the MSG group. These results agree with Poirier et al., [28-31].

Monosodium glutamate led to oxidative stress in the rat liver and kidney, white GLE significantly prevented monosodium glutamate-induced oxidative stress. So, the administration of GLE after MSG challenge may have beneficial effects that could possibly be ascribed, in the part, to its regulation of the oxidant/antioxidant, balance. So, it is therefore possible that GLE could scavenge free radicals and produce beneficial effects against MSG damage in liver and kidney.

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