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Toxic Effects of Monosodium Glutamate on the Hepatic Tissue Damage of Rats and the Beneficial Role of Garlic Extract: Involvement of Oxidative Stress, Hyperlipidemia and Hyperglycemia

ASHARF M. ELBESSOUMY[1], DOAA A. GHAREEB[1] AND ASHRAF A. KHALIL[2]

[1]Department of Biochemistry, Faculty of Science, Alexandria University
Department of Protein Technology, Institute of Genetic Engineering and Biotechnology
[2]Mubarak City for Science and Technology, Borg Elarab, Egypt

Background and Aims: monosodium glutamate (MSG) has been claimed to be the cause of a range of harmful reactions in people who had eaten foods containing this additive. This study was carried out to investigate the adverse effects of MSG administration on hepatic oxidative stress and consequently hepatic tissue damage; and the possible effect of garlic extract to ameliorate these adverse changes. Methods: male Wister rats were injected subcutaneously with MSG at dose of 4 mg/g body weight for 45 days, or fed on basal rat diet containing 10g/kg diet of garlic extract (1%) and injected with the same MSG dose for the same period. Results: MSG injection showed a significant increase of plasma glucose and blood lipid profile. Moreover, liver TBARS level and glutathione dependent enzymes GR, GPx and GST activities were significantly increased 3, 2.8, 1.6 and 3 folds, respectively. Furthermore, liver damage take placed and this indicated by the significant increase in AST, ALT and ALP activities. Garlic acid administration significantly attenuated MSG induced liver oxidative stress, liver tissue damage, hyperglycemia and hyperlipidemia. Conclusions: these results emphasized the benefit effect of garlic extract as supplementary nutrients to prevent the MSG hepatotoxicity which arisen from oxidative stress involvement.

***Keywords**: monosodium glutamate, garlic extract, oxidative stress, hyperglycemia, liver damage.*

1. INTRODUCTION

Monosodium glutamate (MSG) is the sodium salt of the non-essential amino acid glutamic acid, one of the most abundant amino acids found in nature. It is one of the main flavor enhancers used as an ingredient in various food products [1]. MSG has been claimed to be the cause of a range of adverse reactions in people who had eaten foods containing this additive [2]. MSG has been implicated as the causative agent in the symptom complex known as Chinese restaurant syndrome and also as a trigger for bronchoconstriction in some asthmatic individuals [1]. MSG administration could produce symptoms such as numbness, weakness, flushing, sweating, dizziness and headaches [3] that commenced between 10 min and 2 h after start of the MSG containing meal and lasted 4 h or less [4]. Chinese and Japanese used MSG as a flavor enhancer and ready served foods like soups, sauces etc, found to be induced hyperlipidemia, hyperglycemia and hence oxidative stress [5]. Alterations in the levels of thiobarbituric acid reactive substances (TBARS) and antioxidants like reduced glutathione, catalase and superoxide dismutase were reported in adult mice during MSG treatment [6,7]. Furthermore, disruption in the levels of biochemical parameters such as carbohydrates, lipids and proteins in MSG-treated rats were also well documented [8].

Many plants extracts used in treatment of human health problems have shown antioxidants and free radical scavenging capacities against external and endogenous agents. Garlic has attracted particular attention of modern medicine because of its widespread health use around the

*Corresponding author: *E-mail: hadiergad@yahoo.com, dhedier@mucsat.sci.eg*

world, and the cherished belief that it helps in maintaining good health warding off illnesses and providing more vigor [9]. To date, many favorable experimental and clinical effects of garlic preparations, including garlic extract, have been reported. These biological responses have been largely attributed to reduction of risk factors for cardiovascular diseases and cancer, stimulation of immune function; enhance detoxification of foreign compound, hepatoprotection, antimicrobial effect and antioxidant effect [9]. Raw garlic homogenate has been the major preparation of garlic subjected to intensive scientific study, as because it is the commonest way of garlic consumption. Garlic extract (*Allium sativum Linn*) have reported the presence of two main classes of antioxidant components, namely flavonoids and sulfur-containing compound, diallyl sulfide and trisulfide and allyl – cysteine [9,10]. Raw garlic homogenate is essentially same as aqueous extract of garlic, which has been used in various scientific studies [9]. Allicin (allyl 2 propenethiosulfinate or diallyl thiosulfinate) is thought to be the principal bioactive compound present in aqueous garlic extract or raw garlic homogenate [10]. When garlic is chopped or crushed, allinase enzyme, present in garlic, is activated and acts on alliin (present in intact garlic) to produce allicin [11]. The antioxidant properties of allicin (dialkyl thiosulfinate), the main component in aqueous extract from raw garlic, may explain the possible role of garlic to protect against MSG toxicity [12]. It has been found that allicin scavenges OH · and inhibits lipid peroxidation [13]. However garlic preparations and related organosulfur compounds have also been reported to protect against certain cytotoxicities [14] through promoting the induction of glutathione-S-transferase (GST) [15]. In addition organosulfurs enhance the synthesis of the cellular reduced glutathione content [16], which is catalyzed by antioxidant enzymes as ã-glutamyl transpeptidase [17]. So, this study aimed to benefit from such acomulative actions of garlic in preventing the MSG intoxication.

2. MATERIALS AND METHODS

2.1. Preparation of Aqueous Garlic Extract: garlic was purchased from local market. The outer husks of garlic were peeled off before slicing the cloves. The sliced garlic was dried in an oven at 60 °C up to dryness. The dried garlic was ground and kept in dry glass package until used.

2.2. Animals and Experimental Design: Male Wistar rats (170±30 g) were obtained from the animal house, Faculty of Medicine, Alexandria University. The animals were randomly divided into four groups, with 8 rats in each.

The first one is **Control group**: Rats fed on basal rat diet. The second one is **MSG group**: Rats fed on basal rat diet and injected subcutaneously 1 ml water containing MSG at dose level of 4 mg/g body weight every day [1]. The third one is **Garlic group**: Rats fed on basal rat diet containing 10 g/ kg diet of garlic extract (1%). Finally the last one is **Garlic plus MSG group**: Rats fed on basal rat diet containing 10 g/ kg diet of garlic extract (1%) and injected subcutaneously everyday 1 ml water containing MSG at dose level of 4 mg/g body weight.

All animals were free access to water, housed in individual cages, on alternative 12 h light and dark cycles, at temperature 24±2 °C, and kept on regular diet during the experimental period (45 days). All animal procedures were performed according to the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health, Alexandria University.

On day 45, the rats were sacrificed then blood collected, centrifuged and sera stored at -20 °C. Liver was obtained and homogenized in phosphate buffer, pH 7.4, 0.1M and then tissue homogenates were collected and stored at -20 °C for the biochemical studies.

2.3. Standardized Procedures: were followed for various estimations of serum total lipids (T. Lipid) [18], cholesterol [19], triglycerides (T. G.) [20], high density lipoprotein (HDL) [21], low density lipoprotein (LDL) [22], glucose [23], alanine aminotransferase (ALT), asparatate aminotransferase (AST) and alkaline phosphatase (ALP) [24] by using commercial kits.

Lipid peroxidation and antioxidant enzymes were measured in liver tissue.

2.4. Estimation of Lipid Peroxidation: Briefly, 0.5 ml of livert supernatant and 0.5 ml of Tris-HCl were incubated at 37 °C for 2 h. After incubation 1

ml of 10% trichloroacetic acid was added and centrifuged at 1000 *xg* for 10 min. after cooling 1 ml double distilled water was added and absorbance was measured at 532 nm. Thiobarbituric acid-reactive substances were quantified using an extinction coefficient of 1.56 x 10 5 M^{-1} cm^{-1} and expressed as nmol of malondialdehyde per mg protein. The brain malondialdehyde content expressed as nmol of malondialdehyde per *g* tissue [25].

2.5. Determination of Glutathione-S-transferase (GST) Activity: 100 µl GSH (5mM), 10 µl *p*-nitrobenzyl chloride (1 mM in ethanol) and 25 µl supernatant were added to 1.365 ml phosphate buffer (0.1 M, pH 6.5) and vortexd then incubated for 20 min at room temperature. The concentration of protein was estimated in 25 µl supernatant (Plummer, 1978). The absorbance of sample was read against air at 310 nm. The activity of GST was calculated with the following equations; For GST activity (µmol/ min/mg protein) = At / (1.9 x time x mg protein) [26].

2.6. Determination of Liver Glutathione Peroxidase (GPx) Activity: 50 µl supernatant was added to 100 µl GSH (GSH, 5 mg was dissolved in 10 ml Tris-HCL buffer, 50 mM, pH 7.6), 100 µl cummen H_2O_2 (cummen H_2O_2, 50 µl was mixed with 10 ml Tris-HCl buffer, 50 mM, pH7 .6) and 750 µl Tris-HCl, pH 7.6 (test) and incubated at 37°C for 10 min. For control, 50 µl diluted supernatant and 100 µl GSH were added to 750 µl Tris-HCl, 50 mM, pH 7.6 then incubated at 37 °C for 10 min. One milliliter TCA (15%) was added to test and control as well as 100 µl cummen H_2O_2 was added to control then both were centrifuged at 3000 r.p.m. for 20 min and then the supernatants were separated off. One ml of supernatants was added to 2 ml Tris-HCl, 0.4 mM, pH 8.9 and 100 µl DTNB (DTNB, 0.0198 g was dissolved in 5 ml methanol) and incubated for 5 min. The absorbance was measured at 412 nm against distilled H_2O. The activity of GPx was calculated according to the following equation; GPx activity (U/g wet tissue) = A x 6.2 x 10 x 10 / 13.1 x 0.05 x 10 [27].

2.7. The Activity of Glutathione Reductase: (GR) was determined spectrophotometrically using UV-visible spectrophotometer at 30 °C according to method of Gutterer *et al.* [28] by using commercial kit. The protein content was determined according to Lowry *et al.* [29], using bovine serum albumin as a standard protein.

2.8. Statistical Analyses: Data are expressed as the mean ± SEM for the number (n = 8) of animals in the group as indicated in table and figures. Repeated measures analysis of variance (ANOVA) was used to analyze the changes in parameters. A p-value < 0.01 was considered statistically significant.

3. RESULTS

Administration of monosodium glutamate (MSG) to adult male rats at dose level of 4 mg/g body weight for 45 days, caused hyperglycemia, hyper-lipidemia, hepatocytes damage and hepatic lipid peroxidation associated with high level of antioxidants enzymes (compensating mechanism). Where, the blood levels of glucose, cholesterol, T.G., HDL, LDL and T.L. were significantly increased after MSG IP injection by 32, 79, 53, 41, 157 and 33%, respectively, at $p \leq 0.001$ (Table 1).

Furthermore, the activities of ALT, AST and ALP (indicators for hepatocytes damage) were increased by 71, 70 and 80%, respectively (Table 2). MSG administration caused an increase in the hepatic lipid peroxidation associated with increase in the hepatic activity of glutathione-dependent enzymes, glutathione reductase (GR), GPX and GST. Where, TBARS and the activities of GR, GPX and GST were increased 3, 2.8, 1.6 and 3 fold, respectively ($p \leq 0.001$) than those of control group (Table 2 and Figure 1).

On the other hand, garlic acid oral administration at dose of 10g/kg acts as hypoglycemic and hypo-lipidemic agent because blood glucose level, T. G., T.L, cholesterol, HDL and LDL were significantly decreased than those of control group at $p \leq 0.01$. In addition, table 2 represented that the plasma activities of liver enzymes were lower than that of control group (Table 2). Furthermore, liver lipid peroxidation and liver antioxidants enzymes were significantly decreased by 10, 23, 28 and 26% after garlic intake.

The co-administration of MSG and garlic extract for 45 days reverses the adverse effects of MSG (figure 1 and table 1 and 2). The Blood Glucose Level was significantly decreased than that of MSG group and return to control level. Lipid profile

(cholesterol, T. G., T. L., HDL and LDL) was significantly decreased than that of MSG group but still slightly higher than control group level, at $p \leq 0.01$.

Rat treated with this combination showed a significant decrease in ALT, AST and ALP by 16, 37, and 54% respectively than MSG group levels at pd"0.01. Moreover, the hepatic lipid peroxidation was decreased by 61% than that of MSG group. Finally, the antioxidant enzymes of rat administrated MSG and garlic were significantly lower than that of MSG group and still higher than that of control group at $p \leq 0.01$.

4. DISCUSSION

Throughout the world, dietary MSG has been consumed in large amounts to induce preference for foods in the meal. Interestingly, little is known about the effects of MSG added to dry food on oxidative stress and metabolic parameters [30]. It is reported that MSG administration to adult male mice for 6 days, at dose levels of 4 and 8 mg/g body weight, caused a significant increase in the levels of glutamate and glutamine in the blood; which induced hyper-lipidemia and hyperglycemia [1, 31]. The present study showed that, MSG administration at dose of 4 mg/g for 45 days caused a significant increase in blood glucose level and blood lipid profile (Table 1). The elevation in the level of glucose results in peroxidation of membrane lipid and promotes formation of tissue TBARS.

In the current work, we also observed an increase in liver lipid peroxidation by 190% after MSG intake with hyperglycemia. It is well known that, MSG rats had an imbalanced oxidant / antioxidant system, which characterized by high lipid peroxidation level, and enhanced oxidative stress which generates reactive oxygen species (ROS). ROS reacts with protein thiol moieties to produce a variety of sulfur oxidations, thus diminishing the insulin receptor signal and inhibiting cellular uptake of T. G. from the blood [30-31]. These observations explain the increase of serum level of T.G and glucose as shown in Table 1.

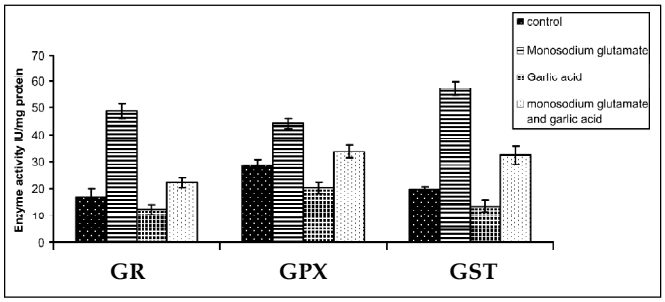

Figure 1: Effect of monosodium glutamate and garlic acid on liver antioxidants enzymes.

The increase in the activities of plasma AST, ALT and ALP (Table 2) indicated that MSG intake may be induced hepatic dysfunction. Supporting our finding it has been found that lipid peroxidation alters the cell membrane permeability, integrity and structure and leads to cell membrane damage [7]. Therefore, the increment of the activities of AST, ALT and ALP in plasma may be mainly due to the leakage of these enzymes from the liver cytosol into the blood stream, which gives an indication on the hepatotoxic effect of MSG.

However, lipid peroxidation produces cell damage by disruption of anatomical integrity of membranes by the formation of compounds which are able to diffuse from the production site like aldehydes. Aldehydes may be inactivated chemically by substances containing [-SH] group. Among the most important thiols is reduce glutathione (GSH) [30-31]. There is glutathione-dependent enzyme (GR, GPX and GST) that controls in the level of GSH to maintain it in the adequate level [8]. In the present work, the activity of these enzymes were increased (Figure 1), this suggest that the system tries to adopt itself to ongoing stress by MSG. GR, a cytosolic NADPH-dependent enzyme, was found to be significantly increased by 150%. The oxidation of GSH to GSSG normally occurs by the reduction of endogenous peroxides by GPX. The GPX was increased by 73% (Figure 1). This suggests that this is not much change in GSH/GSSG level which could inhibit protein biosynthesis [1, 8, 31]. The role of GPX is protective one, as it significantly delays irreversible oxidative degradation of lipids [32]. The activity of GST was increased by 190% (Figure 1). GST is a group of important detoxifying enzyme that conjugates GSH with a large member of electrophiles in order to protect thiol group of protein from electrophilic attack [33]. So we concluded that MSG intake leads to hyperglycemia, hyper-lipidemia, lipid peroxidation, oxidative stress and hepatotoxicity.

Table 1
Effect of Monosodium Glutamate and Garlic Acid Administration on Blood Glucose Level and Lipid Profile

Groups	*Glucose (mg/dl)*	*Cholesterol (mg/dl)*	*TG (mg/dl)*	*HDL (mg/dl)*	*LDL (mg/dl)*	*T.L. (g/dl)*
Control	62±1.5[b]	125 ± 3.1[b]	54.2±2.2[b]	32 ± 4.3[b]	63.4±5.5[b]	409±4.8[b]
MSG	81.4±4.9[a]	224 ± 3.9[a]	83±3.3[a]	45.6±2.6[a]	162.4±4.2[a]	545.4±6.3[a]
Garlic	61.4±2.5[b]	95 ± 3.8[a]	34±1.7[a]	73.4±2.1[a]	44.6±2.4[a]	376.4±5.7[a]
MSG plus Garlic	69±2.1[ab]	156 ± 2.8[ab]	66.2±1.6[ab]	39.8±0.8[ab]	104.4±3.4[ab]	451±4.2[ab]

The results are given as means ± SEM
[a]significant difference was detected between groups when compared to control , $p < 0.01$.
[b]significant difference was detected between groups when compared to MSG group, $p < 0.01$.

Table 2
Effect of Monosodium Glutamate and Garlic Acid Administration on Blood Liver Function Enzymes and Liver Lipid Peroxidation

Groups	*ALT IU/l*	*AST IU/l*	*ALP IU/l*	*TBARS nmol/g*
Control	21.6±2.1[b]	30.6±2.4[b]	15±1.2[b]	3.64±0.17[b]
MSG	36±2.7[a]	51.4±3.1[a]	27.6±2.4[a]	10.58±0.2[a]
Garlic	15.4±1.1[ab]	22±2[ab]	9.6±1.1[ab]	3.27±0.05[ab]
MSG plus Garlic	27.6±1.7[ab]	43±1.5[ab]	18±0.8[ab]	4.02±0.08[ab]

The results are given as means ± SEM

[a]significant difference was detected between groups when compared to control , $p < 0.01$.

[b]significant difference was detected between groups when compared to MSG group, $p < 0.01$.

Garlic (*Allium sativum Linn*) used as a spice and medicinal herb for centuries exhibits a wide range of properties including immuno-modulatory, hepatoprotective, antioxidant, anti-mutagenic and anticancer properties [9, 11, 34]. Several investigators reported that garlic acid intake decreased glucose and lipid profile especially cholesterol [10, 35]. Because garlic can effectively combine with compounds like cysteine and enhances serum insulin so it acts as an anti-diabetic agent by increasing either the pancreatic secretion of insulin from the beta cells or its release from bound insulin [36]. In agreement with these findings, the results showed that garlic extract administration acts as hypoglycemic and hypo-lipidemic agent (Table 1). It was reported that treatment with garlic could restore the activities of liver enzyme because it acts as hepatoprotective [36]. This finding supports our results where garlic acid intake lowers the plasma levels of AST, ALT and ALP (Table 2).

We noticed that garlic extract administration decreased liver lipid peroxidation. In agreement with these findings, several investigators reported the role of garlic as exogenous antioxidants [34-36].

The present study was carried out to assess the benefit effect of garlic extract to detoxify the adverse effects of MSG. After 45 days administration of MSG at dose of 4 mg/g with garlic acid at dose of 10 g/kg, we found a significant decreas in the lipid profile, blood glucose level, and plasma activates of AST, ALT, ALP and glutathione-dependent enzymes as well as TBARS level than those of MSG group but not similar to normal levels. In agreement with the previous mentioned findings, Gorinstein *et al.* (2006) concluded that dietary hypoglycemic garlic was effective in reducing oxidative stress and decrease serum lipid profile in rat model [37]. Several previous studies reported that garlic extract administration inhibit the generation of hydroxyl radical, the most toxic oxy radical [34-36]. Garlic has been found to modulate lipid peroxidation and enhanced the status of antioxidants [9, 11, 34, 35, 38]. In addition, garlic extract used as effective chemopreventive agents [38].

In conclusion, MSG induced oxidative stress associated with liver injury. Furthermore, MSG induced metabolic disorders hyperlipidemia and hyperglycemia so may induc insulin resistance. Garlic extract intake prevented these changes in glucose, lipid profile, prooxidant/antioxidants status seen in MSG group. Because the deleterious effects of MSG inducing were not noticed in rats that ate the garlic acid extract diets, it can be concluded that garlic acid supplementation is beneficial for avoiding hyperglycemia and hyperlipidemia and improving oxidative stress induced by an MSG injection.

Abbreviations

ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, asparatate aminotrasferase; Chol, Cholesterol; MSG, GPx, glutathione peroxidase; GR, glutathione reductase; HDL, high density lipoprotein; LDL, low density lipoprotein; Monosodium glutamate; SOD, superoxide dismutase; TBARS, thiobarbituric acid reactive substances; T.G., Triglycerides; T. L., Total Lipids.

References

[1] Kuldip S., Ahluwalia P. Alteration in Some Antioxidant Enzymes in Cardiac Tissue upon Monosodium Glutamate [MSG] Administration to Adult Male. *Indian J. Clin. Bioch.*, 2005; 20(1): 43-46.

[2] Kwok R. H. Chinese Restaurant Syndrome. *New Eng. J. Med*. 1968; 278: 796-799.

[3] Schamburg H. H., Byck R., Gersti R., Mashman J. H. Monosodium L-glutamate; Its Pharmacology and Role in the Chinese Restaurant Syndrome. *Science*, 1969; 163: 826–828.

[4] Geha R. S., Beiser A., Ren C., Patterson R., Grammar L. C., Ditto A. M., Harris K. E. Review of Allergic Reaction to Monosodium Glutamate and Outcome of a Multicenter Double Blind Placebo-controlled Study. *J. Nutr.*, 2000; 130: 1032S–1038S.

[5] Kuldip S., Ahluwalia P. Studies on the Effect of Monosodium Glutamate [MSG] Administration on Some Antioxidant Enzymes in Arterial Tissue of Adult Male. *J. Nutr. Sci. Vitaminol.*, 2003; 49: 145-148.

[6] Ahluwalia P., Tiwari K., Choudhary P. Studies on the Effects on Monosodium Glutamate (MSG) on Oxidative Stress in Erythrocytes of Adult Male Mice. *Toxicol. Lett.*, 1996; 84: 161–165.

[7] Choudhary P., Malik V. T., Puri S., Ahluwalia P. Studies on the Effects of Monosodium Glutamate on Hepatic Microsomal Lipid Peroxidation, Calcium, Ascorbic Acid and Glutathione and its Dependent Enzymes in Adult Male Mice. *Toxicol. Lett.*, 1996; 89: 71–76.

[8] Ahluwalia P., Malik V. T. Effects of Monosodium Glutamate (MSG) on Serum Lipids, Blood Glucose and Cholesterol in Adult Male Mice. *Toxicol. Lett.*, 1989; 45: 195–198.

[9] Banerjee K. S., Maulik K. S. Effect of Garlic on Cardiovascular Disorders: A Review. *Nutrition J.*, 2002; 1[5]: 1-14.

[10] Amagase H., Petesch B., Matsuura H., Kasuga S., Itakura Y. Intake of Garlic and its Bioactive Components. *J. Nutr.*, 2001; 131: 955S–62S.

[11] Lawson J. D., Wang Z. J. Allicin and Allicin-derived Garlic Compounds Increase Breath Acetone through allyl methyl Sulfide: Use in Measuring Allicin Bioavailability. *J. Agric. Food Chem.*, 2005; 53: 1974–83.

[12] Pedraza-Chaverri J., Medina-Campos ON, Avila-Lombardo R., Zuniga-Bstos AB, Orozco-Ibrra M. Reactive Oxygen Species Scavenging Capacity of Different Cooked Garlic Preparations. *Life Sci.*, 2005; 78: 761-770.

[13] Prasad K., Laxdal V. A., Yu M., Raney B. L. Antioxidant Activity of Allicin, an Active Principle in Garlic. *Molecular Cellular Bioch.*, 1995; 148: 183-189.

[14] Wang B. H., Zuzel K. A., Rahman K., Billington D. Protective Effects of Aged Garlic Extract Against Bromobenzene Toxicity to Precision Cut Rat Liver Slices. *Toxicol.*, 1998; 126: 213-222.

[15] Kim S. G., Chung H. C., Cho J. Y. Molecular Mechanism for alkyl sulfide-modulated carbontetrachloride-induced hepatotoxicity. The Role of CytochromeP450 2E1, P450 2B and Glutathione S Transferase Expression, *J. Pharmacol. Exp. Ther.*, 1996; 277: 1058-1066.

[16] Wu C. C., Sheen L. Y., Chen H. W., Tsai S. J., Li C. K. Effects of Organosulfur Compounds from Garlic Oil on the Antioxidation System in Rat Liver and Red Blood Cells, *Food Chem. Toxicol.*, 2001; 39: 563-569.

[17] Godwin A. K., Meister A., O'Dwyer P. J., Huang C. S., Hamilton T. C., Anderson M. E. High Resistance to Cisplatin in Human Ovarian Cancer Cell Lines is Associated with Marked Increase of Glutathione Synthesis. *Proc. Natl. Acad. Sci.*, 1992; 89: 3070-3074.

[18] Fringes C. S., Dunn R. T. A Colorimetric Method for Determination of Total Serum Lipids Based on the Sulfophosphovanillin Reaction. *Am. J. Clin. Pathol.*, 1970; 53: 89-91.

[19] Allain C. C., Poon L. S., Chan C. S., Richmond W., Fu P. C. Enzymatic Determination of Total Serum Cholesterol. *Clin. Chem.*, 1974; 20: 470-475.

[20] McGowan M. W., Artin J. D., Zak B. A Peroxidase Coupled Method for the Colorimetric Determination of Triglycerides. *Clin. Chem.*, 1983; 29: 538-542.

[21] Lopes-Virella M. F., Stone P., Ellis S., Colwell J. A. Cholesterol Determination in High Density Lipoproteins Separated by three Different Methods. *Clin. Chem.*, 1977; 23: 882-884.

[22] Frieldewald W. T., Levy R. S., Friedricksen D. S. Estimation of Concentration of Low Density Lipoprotein Cholesterol in Plasma without Rise of Preparative Ultracentrifuge. *Clin. Chem.*, 1972; 18: 499-502.

[23] Trinder P. Determination of Glucose in Blood using Glucose Oxidase with an Alternative Oxygen Acceptor. *Ann. Clin. Biol. Chem.*, 1969; 6: 24–27.

[24] Shimidt E. Strategy and Evaluation of Enzymes in Diseases of the Liver and BiliraySystem- A Multifaceted Approach, ed. Demens L. M. and Shaw M., Urbanand Schwarzenberg, Baltimore. 1978.

[25] Hochstein P., Nordenbrand K., Ernster L. Evidence for the Involment of Iron in the ADP-activated Peroxidation of Lipids in Microsomes and Mitochondria. *Biochem. Biophys. Res. Commun.*, 1964; 14: 232-328.

[26] Habig W. H., Pabst M. J., Jakoby W. B. Glutathione-S transferase. *J. Biol. Chem.*, 1974; 249: 7130-7139.

[27] Chiu D., Fredrick H., Tappel A. L. Purification and Properties of Rat Lung Soluble Glutathione Peroxidase. Biochemica. Biophysica. Acta, 1976; 445: 558-566.

[28] Gutterer M. J., Dringen F., Hirrlinger J., Hamprecht B. Purification of Glutathione Reductase from Bovine Brain,

Generation of an Antiserum, and Immunocytochemical Localization of the Enzyme in Neural Cells. J. Neurochem., 1999; 73[5]: 1422-1430.

[29] Lowry O. H., Rosebrough N. J., Farr A. L., Randall R. J. Protein Measurement with the Folin Reagent. *J. Biol. Chem.*, 1951. 193, 265-275.

[30] Diniz Y., Faine L., Galhardi C. Monosodium Glutamate in Standard and High-fiber Diets: Metabolic Syndrome and Oxidative Stress in Rats. *Basic Nutritional Investigation*, 2005; 21: 749-755.

[31] Malik T., Ahluwalia P. Studies on the Effect of Monosodium Glutamate (MSG) on Various Fractions of Lipids and Certain Carbohydrate Metabolism Enzymes in Liver and Adult Male Mice. *Toxicol. Lett.*, 1994; 74: 69-77.

[32] Ursini F., Maiorino M., Valente M., Ferri L., Gregolini C. Purification from Pig Liver of a Protein which Protects Liposomes and Biomembranes from Peroxidative Degradation and Exhibits Glutathione Peroxidase Activity on Phosphatidyl Choline Hydroperoxides. *Biochim. Biophys. Acta*, 1982; 710: 197-211.

[33] Hayer P., Bouchie I., Becketl G. Glutathione-S-transferase in Humans in Health and Disease. Gut, 1991; 32: 813-818.

[34] Leelarungrayab N., Rattanapanone V., Chanarat Z. N., Gebicki J. Quantitative Evaluation of the Antioxidant Properties of Garlic and Shallot Preparations. Nutrition, 2006; 22: 266-274.

[35] Anwar M., Maki A. Oxidative Stress in Streptozotocin-induced Diabetic: Effects of Garlic Oil and Melatonin. C.B.P., 2003; 35 (part A): 539-547.

[36] El-Demerdash F., Yousef M., Abou- ElNaga N. Biochemical Study on the Hypoglycemic Effects of Onion and Garlic in Alloxan- induced Diabetic Rats. *Food Chemical Toxicology*, 2005; 43: 57-63.

[37] Goinstein S., Leontowicz H., Leotowicz M., Drzewiecki J. Raw and Boiled Garlic Enhances Plasma Antioxidants Activity and Improves Plasma Lipid Metabolism in Cholesterol-fed Rats. *Life Science*, 2006; 78: 655-663.

[38] Balasenthil S., Nagini S. Effect of Garlic on Circulatory Oxidant and Antioxidant during 4-nitroquinoline-oxide-induced rat oral carcinogenesis. *Nutrition Research*, 2000; 20 (11): 1581-1589.