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Extract of *Coelatura aegyptiaca*, a freshwater clam, ameliorates hepatic oxidative stress induced by monosodium glutamate in rats

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The purpose of the present work was to evaluate the possible ameliorative effect of *Coelatura aegyptiaca* extract (CE) and vitamin C on oxidative stress induced by monosodium glutamate (MSG) in rat liver. Male rats were randomly divided into four main groups: control group, received distilled water, group 2 (100 mg/kg) and 3 (250 mg/kg) received CE for seven consecutive days. Group animals (24 rats) were administered MSG (4 mg/g body weight) daily for 10 days. This group was then subdivided into 4 subgroups. All subgroups treated orally for 7 days with, distilled water (subgroup I), CE at a dose of 100 and 250 mg/kg body weight (subgroups II and III), and 250 mg vitamin C/kg body weight (subgroup IV). Furthermore, the antioxidant activity of the CE was determined using a DPPH (2,2-diphenyl-1-picrylhydrazyl) test system. The CE and vitamin C caused significant ameliorative effects by decreasing the activities of serum aminotransaminases (AST and ALT), alkaline phosphatase (ALP), levels of serum glucose, lipid profile parameters, liver malondialdehyde level (MDA), nitric oxide (NO), superoxide dismutase activity (SOD), as well as the activity of liver catalase (CAT), and increasing the level of liver glutathione reduced (GSH). The results of the present study elucidate that CE could ameliorate the liver oxidative stress induced by MSG administration, since CE was strongly scavenged DPPH radicals and showed antioxidant activity.

Key words: Monosodium glutamate, freshwater extracts, liver oxidative stress, lipid profile parameters, 2,2-diphenyl-1-picrylhydrazyl.

INTRODUCTION

Monosodium glutamate (MSG), the sodium salt of glutamic acid (GA), is one of the most frequently applied additives in the developed world (Pavlovic et al., 2009). Modern nutrition enables a continuous intake of the flavor enhancer, with resulting rise and accumulations of GA in blood (Walker and Lupien, 2000). This amino acid acts at multiple receptor types, divided into two main groups: ionotropic glutamate receptors (iGluR) and metatropic glutamate receptors (mGluR) (Hinoi et al., 2004). In addition to the central nervous system (CNS), glutamate receptors (GluR) are also found on non – neuronal cells. However, several studies showed toxic effects of MSG in various regions of the CNS (Park et al., 2000), liver and kidney (Farombi and Onyema et al., 2006), mainly by generation of reactive oxygen species (ROS) and resulting oxidative stress. In MSG sensitive individuals, the adverse effects are observed even at doses recommended in food (Schaumburg et al., 1969).

Furthermore, disruption in the levels of biochemical parameters such as carbohydrates, lipids and proteins in MSG treated rats has also been well documented (Ahluwalia and Malik, 1989). Chronic administration of MSG (4 mg/g body weight and above) was found to induce oxidative stress in experimental animals (Singh et al., 2003; Diniz et al., 2004). Oxidative stress is a biochemical disequilibrium propitiated by excessive production of free radicals (FR) and ROS, which provoke oxidative damage to biomolecules that cannot be counteracted by antioxidative systems (Foyer et al., 2008; Minibayeva et al., 2009). MSG intake had been related to increased palatability and food consumption (Diniz et al., 2005), several studies have found that the mechanism by which over feeding exerts deleterious effects to health is its remarkable ability to induce oxidative damage (Diniz et al., 2002, 2004). The use of oxygen to fuel oxidative metabolism results in production

Table 1. The amino acid contents of freshwater extract from *C. aegyptiaca* clam (CE).

Amino acids	CE (mg/100 g)
Aspartic	258.28
Glutamic acid	291.55
Serine	160.75
Glycine	193.65
Glutamine	504.55
Histadine	235.75
Arginine	310.12
Therionine	504.55
Alanine	417.65
Proline	502.51
Tyrosine	419.59
Valine	341.62
Methionine	291.03
Cysteine	318.61
Isoleucine	439.45
Leucine	312.28
Phenylalanine	351.92
Lysine	283.50
Taurine	171.29

of free radicals FR or ROS (Feuers, 1998). Because this is vital for oxidative phosphorylation by the electron transport system, increased intake of a food may be related to oxidative stress, which is an imbalance between oxidant and antioxidant systems (Esposito et al., 1999).

Liver plays a major role against damage by free radicals by virtue of having a variety of antioxidants, free radical scavenging molecules and enzymes like catalase and superoxide dismutase, which under normal circumstances, help the hepatic cells to maintain a reducing environment, preventing the potentially deleterious effects of FR on cell membranes and organelles (Baskaran et al., 1999).

The liver contains considerable amounts of poly-unsaturated fatty acids that are prone to damage by FR through oxidative stress (Onyema et al., 2006). It has been reported previously that administration of MSG caused increased lipid peroxidation in hepatic tissue (Choudhary et al., 1996) along with significant increase in blood glutamate and glutamine levels (Ahluwalia and Malik, 1989) and hence induced hyper-lipidemia and hyperglycemia, which are important factors for the onset of oxidative stress (Malik and Ahluwalia, 1994; Ahluwalia et al., 1996). Vitamin C (Ascorbic acid) is an essential water soluble nutrient that primarily exerts its effect on host defense mechanisms and immune homeostasis by being the most important physiological antioxidant (Hartel et al., 2004). In line with the previous results of Farombi and Onyema (2006), they showed a protective role of vitamin C in rat liver, brain and kidney.

Unfortunately, conventional or synthetic drugs used in the treatment of liver diseases are not enough and mostly can cause serious side effects. This is one of the reasons for many people worldwide, including those in developed countries, to turn to complementary or alternative medicine. Products from marine water source have recently become attractive as nutraceutical and functional foods and as a source material for the development of drugs and specific health foods (Koyama et al., 2006). Recent attention has been focused upon supplements derived from freshwater foods and their utilization as hepatoprotective agents (Chijimatsu et al., 2008; Chijimatsu et al., 2009; Peng et al., 2008).

Freshwater bivalves occurring in Egypt represent a neglected animal group and little is known about them or their diversity; perhaps due to the fact that they have no apparent economic or medical importance, although they are eaten in Asia as a supplemental protein source (Sleem and Ali, 2008). Egyptian freshwater clams (*Coelatura aegyptiaca*) are a Molluscan Bivalve belonging to Unionoidae common in the Egyptian Nile River. Consumption of these clams as food by humans may significantly influence their health status. Therefore, it is important to know the pathological effects and mechanisms of action of these foods. Thus in the present study, hot water extract of the freshwater clam extract (CE) from *C. aegyptiaca* was used with a goal of determining its potential as antioxidant. Initially, the rationale for this work based on the taurine content found in this extract (Table 1). Taurine is sulfur containing amino acids which has been previously found to exhibit antioxidant properties (Das et al., 2009; Li et al., 2009).

The present study aimed to evaluate the possible effect of *C. aegyptiaca* extract for the first time in Egypt, in order to establish whether it would exacerbate or ameliorate the adverse effects of MSG on some serum parameters and oxidative markers in the liver of rats using vitamin C as a positive control group.

MATERIALS AND METHODS

Experimental Animals

Male albino rats, *Rattus norvegicus*, (100 to 120 g) were used in all experiments. Animals were grouped and housed in polyacrylic cages (six animals per cage) in the well ventilated animal house of the Department of Zoology, Faculty of Science, Cairo University. Animals were fed on standard pellet and given water *ad libitum*. Rats were acclimatized to laboratory conditions for 7 days before commencement of the experiment. All the animals received human care in accordance with the guidelines of the National Institute of Health, USA for ethical treatment of laboratory animals.

Chemicals

2,2-diphenyl-1-picrylhydrazyl (DPPH) were purchased from Sigma-Aldrich (St. Louis, MO, USA). MSG was obtained from Win Lab UK. Vitamin C was purchased from SAS chemicals Co. Mumbai, India. Kits for aspartate aminotransaminase (AST), alanine

aminotransaminase (ALT), alkaline phosphatase (ALP), glucose, total lipids, triglycerides (TG), total cholesterol (TC), high density lipoprotein (HDL), low density lipoprotein (LDH), malondialdehyde (MDA), glutathione reduced (GSH), nitric oxide (NO), superoxide dismutase (SOD), and catalase (CAT) estimations were purchased from Biodiagnostic Company (Dokki, Giza, Egypt).

Preparation of crude freshwater extract (CE)

Freshwater clam *C. aegyptiaca* were collected from the River Nile at Giza Governorate, Egypt. The crude extract was prepared as follows: fresh clam 1 kg was extracted in a boiler with 1 L of distilled water for 30 min. 3 times. After filtration, the filtrate obtained was then concentrated and dried using a lyophilizer (LABCONCO lyophilizer, shell freeze system, USA). The extract was analyzed for amino acids content using Atomic Absorption Hitachi L8900-Tokyo, Japan by using the method of Paula et al. (2006), and taurine content using HPLC- Varian 940, USA by using method described by Sakai and Nagaswa (1992).

Determination of antioxidant activity (scavenging activity of DPPH radical)

The DPPH free radical scavenging assay was carried out for the evaluation of the antioxidant activity of the freshwater clam *C. aegyptiaca* extract according to the method of Brand et al. (1995). Different concentrations (5, 10, 15, 20, 25, 30 and 35 mg/ml) of the CE and ascorbic acid (positive control) were placed in different test tubes and mixed with 1 ml of 250 mM DPPH (dissolved in methanol). The reaction mixture was shaken vigorously and incubated at 28°C in a dark room for 40 min. The control was prepared as above without any extract, and methanol was used for the baseline correction. The changes in absorbance (Abs) was measured at $\lambda = 517$ nm using a spectrophotometer (U-2001, model 121-0032 Hitachi, Tokyo, Japan). The inhibition of DPPH radicals was calculated from the equation:

$$\% \text{ of radical scavenging activity} = \frac{[\text{Abs (control)} - \text{Abs (sample)}] / \text{Abs (control)}}{1} \times 100$$

Experimental design

Forty two rats were randomly divided into four main groups: (1) control group, in which six rats were given orally distilled water daily for 17 days using gastric tube; extract groups (2 and 3), in which rats were given orally distilled water for 10 days and then administered CE for seven consecutive days at a doses of 100 mg/kg body weight (six rats) (group 2) or 250 mg/kg body weight (six rats) (group 3). The 4th group of animals (24 rats) were administered MSG (4 mg/g body weight) daily for 10 days. The animals were then randomly divided into 4 subgroups (six rats per each subgroup). The subgroups orally administered distilled water (subgroup I), CE at a dose of 100 and 250 mg/kg body weight (subgroups II and III), and 250 mg vitamin C/kg body weight (subgroup IV) for seven days. At the end of each experiment rats were sacrificed after being fasted over night; blood was collected in centrifuge tubes centrifuged at 3000 rpm for 20 min. Liver was quickly removed, cleaned with physiological saline. Both serum and liver tissues immediately stored at -20°C until their use for biochemical assays.

Biochemical assessment

The appropriate kits (Bio-Diagnostic, Dokki, Giza, Egypt) was used

for the determination of serum aminotransferase enzymes activities (AST and ALT) according to Reitman and Frankel (1957), alkaline phosphatase (ALP) activity (Young et al., 1975), glucose (Trinder, 1969), total lipids (Knight et al., 1972), triglycerides (TG) level (Fossati and Prencipe, 1982), total cholesterol concentration (Allain et al., 1974), high density lipoprotein (HDL) level (Lopez- Virella et al., 1977), low density lipoprotein (LDH) level (Wieland and Seidel, 1983).

Determination of oxidative stress parameters

Liver tissues were homogenized (10% w/v) in 0.15 M Tris-HCl buffer (pH 7.4) and centrifuged at 9000 rpm for 15 min at 4°C. The supernatant obtained was used for lipid peroxidation evaluation which was measured by the formation of malondialdehyde (MDA) (Ohkawa et al., 1979), glutathione reduced (GSH) (Aykac et al., 1985), nitric oxide (NO) (Montgomery and Dymock, 1961), superoxide dismutase (SOD) (Nishikimi et al., 1972), and catalase (CAT) (Aebi, 1984) determinations, using test kit (Bio-Diagnostic-Egypt).

Statistical analysis

Values were expressed as mean \pm SE. To evaluate differences between the groups studied, one way analysis of variance (ANOVA) with LSD post hoc test was used to compare the group means and $P < 0.05$ was considered statistically significant. SPSS, for Windows (version 15.0, Chicago, IL, USA) was used for statistical analysis.

RESULTS

Radical scavenging activity

In DPPH radical scavenging assay, antioxidants react with DPPH (deep violet color), and convert it to yellow colour α, α -diphenyl- β -picryl hydrazine. The degree of discoloration indicates the radical-scavenging potential of the antioxidant (Huang et al., 2005). The crude freshwater CE showed a high effective free radical scavenging in the DPPH assay. The DPPH radical scavenging activity of CE increased as concentration increased (Figure 1). Meanwhile, the concentrations that are more than 20 mg/ml show a decrease in the antioxidant activity of CE, as compared to ascorbic acid (Vitamin C, the reference standard). Again, CE and ascorbic acid show the greatest antioxidant effect at 5 mg/ml which represents 66.54 and 70.32% of DPPH inhibition, respectively.

Effects of freshwater CE and vitamin C on serum AST, ALT and ALP activities

The activities of serum AST, ALT and ALP in the control and different treatment groups are shown in Table 2. Administration of freshwater extract of *C. aegyptiaca* CE at the two selected doses (100 and 250 mg/kg body weight) for seven days was found to have non-significantly changed the activities of AST and ALT of

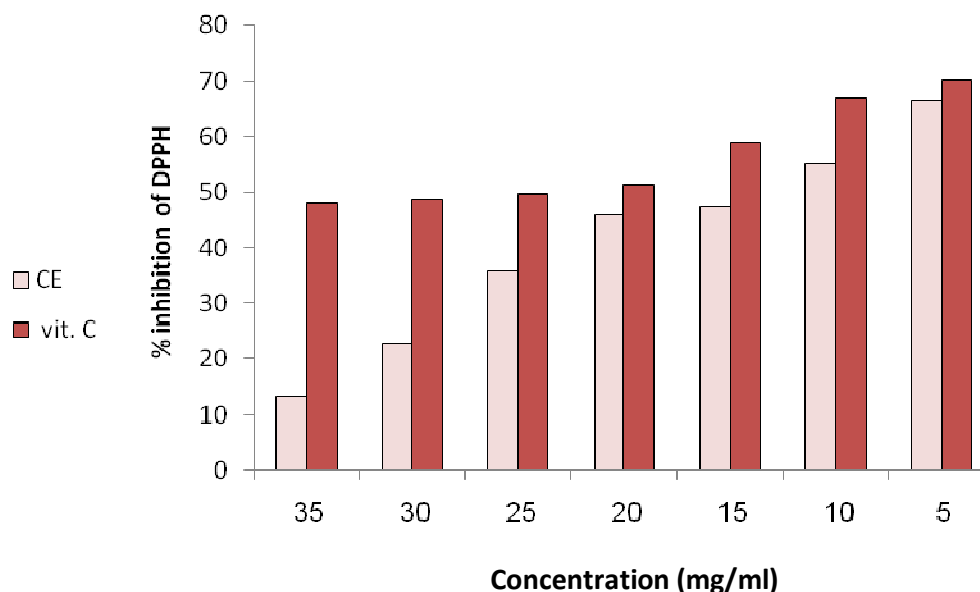


Figure 1. Inhibition of DPPH by freshwater CE from *C. aegyptiaca*.

Table 2. Effect of CE and vitamin C on some serum, enzymes (AST, ALT, ALP) and glucose following MSG- induced oxidative stress in male rats.

Groups	Parameters			
	AST (IU/ml)	ALT (IU/ml)	ALP (IU/L)	Glucose (mg/100 ml)
Control	126.15 ± 2.83	52.74 ± 0.92	128.57 ± 4.21	80.90 ± 3.51
MSG	176.07 ± 8.42 ^a	91.77 ± 2.56 ^a	210.67 ± 4.26 ^a	114.73 ± 2.91 ^a
CE				
100 mg/kg	126.67 ± 5.02	47.96 ± 1.39	128.20 ± 4.28	75.24 ± 2.75
250 mg/kg	129.51 ± 6.83	53.94 ± 2.61	125.64 ± 2.5	78.05 ± 1.66
MSG + CE				
100 mg/kg	121.63 ± 2.05 ^b	51.20 ± 2.38 ^b	182.38 ± 2.83 ^b	88.62 ± 1.23 ^b
250 mg/kg	121.72 ± 5.99 ^b	51.26 ± 2.58 ^b	182.18 ± 2.58 ^b	85.50 ± 4.48 ^b
MSG + vitamin C (250 mg/kg)	119.27 ± 5.21 ^b	52.12 ± 4.89 ^b	187.44 ± 1.87 ^b	93.39 ± 1.05 ^b

All data are means of six rats ± SE. ^aSignificant as compared to control group, ^bSignificant as compared to MSG group at P < 0.05.

rats, as compared to control group (Table 2). However, significant increase (P<0.05) was noticed in the activities of AST and ALT of rats subsequent to administration of MSG (4 mg/g body weight) as compared to control rats (Table 2). On the other hand, post treatment with CE at the two selected doses or vitamin C (250 mg/ kg body weight) for seven days after MSG administration for ten consecutive days was found ameliorated the deleterious effect of MSG by significantly (P<0.05) decreasing the AST and ALT activities of rats, as compared to MSG group (Table 2).

ALP insignificantly changed after oral administration of CE (100 and 250 mg/ kg body weight) for seven days, as compared to the control rats. However, administration of MSG for ten days significantly increased (P<0.05) the ALP activity of rats, as compared to control group (Table 2). On the other hand, oral post treatment with the CE or vitamin C for seven days caused significant decrease (P<0.05) in the ALP activity of rats, as compared to MSG group (Table 2). Although, CE or vitamin C post treatment decreases the activity of ALP, it was still higher than that of the control rats (Table 2).

Table 3. Effect of CE and vitamin C on serum lipid profile (total lipids, triglycerides, total cholesterol, LDL-ch, HDL- ch) following MSG- induced oxidative stress in male rats.

Groups	Parameters				
	Total lipids (g/100 ml)	Triglycerides (mg/100 ml)	Total cholesterol (mg/100 ml)	LDL- ch (mg/100 ml)	HDL- ch (mg/100 ml)
Control	1.42 ± 0.25	105.78 ± 2.26	103.56 ± 4.32	56.82 ± 4.37	63.50 ± 0.75
MSG	2.12 ± 0.05 ^a	144.07 ± 8.79 ^a	134.43 ± 5.35 ^a	100.60 ± 8.36 ^a	69.22 ± 4.12
CE					
100 mg/kg	1.30 ± 0.02	97.34 ± 2.50	94.20 ± 1.49	51.67 ± 2.50	64.86 ± 2.40
250 mg/kg	1.38 ± 0.11	90.14 ± 3.57 ^a	88.95 ± 4.5 ^a	45.46 ± 4.85	63.71 ± 2.01
MSG + CE					
100 mg/kg	1.44 ± 0.08 ^b	117.34 ± 5.67 ^b	101.13 ± 3.47 ^b	57.56 ± 2.66 ^b	59.24 ± 1.46 ^b
250 mg/kg	1.77 ± 0.06 ^b	104.05 ± 1.52 ^b	110.48 ± 1.54 ^b	78.03 ± 4.47 ^b	72.04 ± 3.47
MSG+vitamin C (250 mg/kg)	1.79 ± 0.08 ^b	107.28 ± 3.79 ^b	126.09 ± 1.27 ^b	85.06 ± 4.31 ^b	68.37 ± 2.78

All data are means of six rats ± SE. ^aSignificant as compared to control group, ^bSignificant as compared to MSG group at P < 0.05.

Effects of freshwater CE and vitamin C on serum glucose level

Table 2 shows that oral administration of CE (100 and 250 mg/ kg body weight) for seven days caused a non significant change in the level of serum glucose of rats, as compared to control rats. However, significant increase (P<0.05) in the serum glucose level of rats was observed after ten days of MSG administration, as compared to control rats. Meanwhile, post treatment with CE (100 and 250 mg/ kg body weight) or vitamin C (250 mg/ kg body weight) for seven days was found alleviating the hyperglycemic effect of MSG by decreasing the glucose level significantly (P<0.05), as compared to MSG group (Table 3).

Effects of freshwater CE and vitamin C on the lipid profile levels

Table 3 shows that, oral administration of CE (100 and 250 mg/ kg body weight) for seven days, non significantly changed the level of TG, TC, high density lipoprotein – cholesterol (HDL – ch) and low density lipoprotein – cholesterol (LDL – ch), as compared to the control group. However, serum TG and TC were found significantly decreased (P<0.05), as compared to control rats after seven days of CE (250 mg/kg body weight p.o) administration. MSG administration for ten days significantly increased (P<0.05) serum levels of total lipids, TG, TC and LDL – ch, as compared to control rats (Table 3).

The present study reveals that post treatment of CE at the low dose (100 mg/kg body weight p.o) for seven days caused significant decrease (P<0.05) in the levels of serum total lipids, TG, TC, HDL – ch and LDL – ch of

rats, as compared to MSG group (Table 3). Meanwhile, serum total lipids, TG, TC and LDL – ch levels decreased significantly (P<0.05) subsequent to post treatment with CE (250 mg/kg body weight) for seven days, as compared to MSG group. However, oral post treatment with vitamin C for seven days significantly decreased (P<0.05) the serum levels of total lipids, TG and LDL – ch, as compared to MSG group (Table 3).

Effects of freshwater CE and vitamin C on liver lipid peroxidation

MDA levels were assessed as indicator of lipid peroxidation. Administration of CE at the two selected doses (100 or 250 mg/kg body weight) for seven days caused a non significant change in the MDA level of rats, as compared to control rats (Table 4). However, MSG administration for ten days (4 mg/g body weight p.o) was found significantly increased (P<0.05) the level of liver MDA, as compared to the control rats. Treatment either with CE at the two selected doses or vitamin C for seven days was found ameliorating the effect of MSG on liver MDA level by decreasing it significantly (P<0.05), as compared to the MSG group (Table 4).

Effects of freshwater CE and vitamin C on liver reduced GSH level

Table 4 shows that oral administration of CE (100 or 250 mg/kg body weight) for seven days caused significant increase (P<0.05) in the level of liver GSH of rats. However, significant decrease (P<0.05) was noticed in the GSH level of rats after ten days of MSG administration, as compared to control rats. On the other hand, the level

Table 4. Effect of CE and vitamin C on liver oxidative stress markers (MDA – GSH- NO – SOD - CAT) following MSG- induced oxidative stress in male rats.

Groups	Parameters				
	MDA (nmol/g tissue)	GSH (mg/g tissue)	NO (μ mol/g tissue)	SOD (IU/g tissue)	CAT (μ mol/g tissue)
Control	88.13 \pm 3.66	6.678 \pm 0.51	471.93 \pm 21.41	15.27 \pm 0.89	328.39 \pm 2.36
MSG	163.06 \pm 8.66 ^a	1.64 \pm 0.20 ^a	561.41 \pm 10.12 ^a	35.34 \pm 2.99 ^a	399.26 \pm 2.06 ^a
CE					
100 mg/kg	90.25 \pm 3.87	9.73 \pm 0.34 ^a	412.48 \pm 31.35 ^a	14.08 \pm 0.94	332.71 \pm 2.89
250 mg/kg	82.90 \pm 3.46	9.09 \pm 0.66 ^a	363.82 \pm 13.18 ^a	14.89 \pm 1.0	386.82 \pm 7.95 ^a
MSG + CE					
100 mg/kg	84.53 \pm 1.58 ^b	4.48 \pm 0.40 ^b	422.17 \pm 12.81 ^b	18.28 \pm 0.99 ^b	360.99 \pm 11.74 ^b
250 mg/kg	104.51 \pm 8.08 ^b	4.78 \pm 0.52 ^b	453.10 \pm 15.97 ^b	13.64 \pm 0.95 ^b	348.11 \pm 17.17 ^b
MSG+vitamin C (250 mg/kg)	83.66 \pm 2.63 ^b	4.98 \pm 0.97 ^b	412.04 \pm 10.81	14.42 \pm 1.71 ^b	325.62 \pm 3.63 ^b

All data are means of six rats \pm SE. ^aSignificant as compared to control group, ^bSignificant as compared to MSG group at P < 0.05.

of liver GSH of rats post treated with CE or vitamin C for seven days increased significantly (P < 0.05), as compared to MSG group (Table 4).

Effects of freshwater CE and vitamin C on liver NO level

Liver nitric oxide level was found significantly (P < 0.05) increased subsequent to ten days of MSG administration. However, oral administration with CE (100 or 250 mg/ kg body weight) for seven days for ten days was found significantly decreased (P < 0.05) the level of liver NO, as compared to control group (Table 4). Meanwhile, significant decrease (P < 0.05) was noticed in the liver NO level of rats post treated either with CE at the two selected doses or vitamin C, as compared to MSG group (Table 4).

Effects of freshwater CE and vitamin C on liver SOD activity

Liver superoxide dismutase activity of rats was insignificantly changed after seven days of CE administration (100 or 250 mg/ kg body weight), as compared to control rats (Table 4). However, significant increase (P < 0.05) in the SOD activity was noticed after ten days of MSG administration, as compared to control group.

On the other hand, post treatments either with CE (100 or 250 mg/ kg body weight) or vitamin C (250 mg/ kg body weight p.o) for seven days were found significantly decreased (P < 0.05) the activity of liver SOD of rats, as compared to MSG group (Table 4).

Effects of freshwater CE and vitamin C on liver CAT activity

Administration of CE at the two selected doses for seven days caused general increase in the activity of liver CAT. But, from the statistical point of view, this increase was found significant (P < 0.05) only in case of the administration of CE at the high dose (250 mg/ kg body weight), as compared to the control group (Table 4). Meanwhile, oral MSG administration caused significant increase (P < 0.05) in the liver CAT activity of rats, as compared to control rats. Rats orally administered either CE (100 or 250 mg/ kg body weight) or vitamin C (250 mg/ kg body weight) for seven days after MSG administration for ten days showed significant decrease (P < 0.05) in their liver CAT activities, as compared to MSG group (Table 4).

DISCUSSION

Throughout the world, dietary MSG has been consumed in large amount to induce preferences for food in the meal (Bellisle et al., 1996). Because MSG administration induces hyperphagia and increases the energy intake (Bergen et al., 1998). MSG treatment might induce hepatic metabolic shifting, which result in further injury (Diniz et al., 2004). The extent of MSG induced hepatotoxic effect was assessed by the levels of released cytoplasmic enzymes such as AST and ALT in circulation, hence any necrosis or membrane damage to the liver leads to leakage of these enzymes into the circulation (Pari and Murugan, 2004). Data from the present study reveals significant increase in the activities of serum AST and ALT after MSG administration which indicates

hepatic damage due to the cytotoxic effect of MSG (Ortiz et al., 2006). In consonance with the present result, several investigators reported increase in the activities of AST and ALT subsequent to MSG administration (Onyema et al., 2006; Thomas et al., 2009). ALP is an enzyme that excreted normally through bile through liver and involves in active transport across the capillary wall. Increased activity of ALP which occurs due to *de novo* synthesis by liver cells is a reliable marker of hepatobiliary dysfunction due to damage (Muriel and Escobar, 2003). The increased ALP activity in the present study after ten days of MSG supplementation may be due to increased synthesis in the presence of increasing biliary pressure (Moss and Butterworth, 1974). Again, Rocek et al. (2001) demonstrated that MSG administration could alter the intestinal function and releases the intestinal ALP. There is a much evidence that important stimulus for the control of food intake and energy balance are produced by the circulating energy pool that consists mainly of glucose and lipids (Scharrer, 1999). MSG administration at a high dose (4 mg/g body weight) has been used experimentally to induce a variety of toxic effects mainly in the liver (as mentioned above), since, liver is regarded as one of the central metabolic organs, regulating and maintaining homeostasis. It performs most of the reactions involved in the synthesis and utilization of glucose.

In accordance with the studies of Diniz et al. (2004, 2005) the present study showed that MSG administration induced hyperglycemia which may be due to the inhibitory effect of MSG on growth hormones, thereby decreasing glycogenesis in the liver and inactivating the gluconeogenesis from amino acids (Ahluwalia and Malik, 1989). Several studies have shown that impaired glucose uptake by tissues and imposed hyperinsulinemia are related to changes in glucose transporter (Seraphim et al., 2001) and that food restriction promotes increased glucose transporter content in all insulin – sensitive tissues (Papa et al., 1979). Therefore, the increased glucose levels seen in the MSG group was a consequence of the increased food intake as a result of MSG administration.

There are other consequences that were found to be related to MSG administration in the present study such as alterations in the serum lipid profile markers that assessed by the significant increase in the levels of total lipids, triglycerides, total cholesterol and low density lipoprotein – cholesterol. Several investigators showed that MSG administration at a dose levels above 4 mg/g body weight induced hyperlipidemia (Malik and Ahluwalia, 1994; Ahluwalia et al., 1996). They added that these disturbances in the lipid profile markers were due to the destruction of arcuate nucleus in the hypothalamus as a result of MSG administration which could function in the regulatory manner towards fat metabolism. Moreover, it was found that MSG administered animals had increased triacylglycerol levels and hypertriglyceridemia which identified a high risk dysmetabolic situation

(Blackburn et al., 2003).

Farombi and Onyema (2006) reported that the toxic effects of MSG in the liver were caused mainly by generation of ROS and resulting oxidative stress. The oxidative stress in MSG rats was associated with increased food intake. Overfeeding increases obstruction of electron flow and enhances degenerative diseases by free radical generation (Esposito et al., 1999). Increased caloric intake is an important factor to decrease mitochondrial membrane fluidity and to generate free radicals (Diniz et al., 2005). Emerging data on the antioxidant hypothesis have indicated the ROS generated during oxidative stress have specific roles in the modulation of cellular events (Berner and Stern, 2004). ROS react with protein thiol moieties to produce a variety of sulfur oxidations, thus diminishing the insulin receptor signal and inhibiting cellular uptake of triacylglycerol from the blood (Chen et al., 2003). These observations explain how MSG induce hyperglycemia and hyperlipidemia through increased food intake that leads to oxidative stress causing metabolic syndrome. The results of Thomas et al. (2009) and Ding et al. (2001) showed that, the MSG rats developed increased plasma triglycerides, cholesterol and free fatty acid contents.

The metabolism of xenobiotics to a large extent takes place in the liver. The by-products of such metabolism sometimes become more toxic than the initial product. This could lead to liver injury and the emergence of liver diseases (Ishak et al., 1991). In the present study, elevation in the level of the end products of lipid peroxidation, MDA in the liver of MSG treated rats was observed. This result may be due to the increases in the blood glutamate and glutamine which are reported to favor lipogenesis (Ceresosima et al., 1986). Glutamate is poorly transported across cell membranes and could accumulate intracellularly, altering the redox state of the cell. The more reduced redox state of the cell favors lipid synthesis and tends to shut down lipolysis (Sips et al., 1982). Hence, the increased level of glutamine could initiate lipid peroxidation by changing the redox potential of the cell.

Increased glucose level that is mentioned above as a result of MSG administration could be another mechanism by which MSG causing lipid peroxidation (LPO). It is well known that hyperglycemia can lead to autooxidation of an aldose and ketose to a more reactive dicarbonyl sugar (glucosone), which would then react with proteins to form a ketoimine adduct (Baynes, 1991). The reduced oxygen products formed in the autooxidation reaction include superoxide anion and H₂O₂ which, in the presence of metal ions, would cause oxidative damage to neighboring molecules. Therefore, autooxidative glycosylation is one of the mechanisms for the production of free radicals leading to fragmentation of proteins and oxidation of associated lipids during glycation reactions.

GSH is an intracellular reductant and plays a major role

in catalysis, metabolism and transport. It protects cells against free radicals peroxides and other toxic compounds (Harlan et al., 1984; Hiraishi et al., 1994). GSH can diminish oxidative stress either by protecting the detoxifying enzymes by increasing the efficacy of nicotinic amide dinucleated phosphate (NADPH), or by helping in the elimination of compounds which produce peroxidation in the cell membranes (Machlin and Bandich, 1987). This could be one of the reasons for the decreased level of hepatic GSH in the present study. Younes and Seigers (1981) have reported that once the GSH concentration is depleted to 20% of its original content, lipid peroxidation is initiated and an inverse relationship exists between GSH and lipid peroxidation. Again, the present study confirmed the result of Onyema et al. (2006) who suggested that MSG-induced lipid peroxidation contributed to the depletion of tissue level of GSH.

Nitric oxide (NO) is a molecule that easily passes through the cell membrane, and it is synthesized from the amino acid L-arginine in many cells of the body. Nitric oxide synthase (NOS) is involved in the formation of NO, and it is a deoxygenase that is dependent on NADPH (Rodeberg et al., 1995). NO exerts various influences on the pathogenesis of tissue. It has a double edged knife in pathophysiology, since, both the abundance and paucity of NO causes diseases (Kim et al., 2002). The direct toxicity of NO is enhanced by reacting with superoxide radical such as peroxynitrite which is capable of oxidizing cellular structure and causes lipid peroxidation, a process leading to membrane damage (Weinstein et al., 2000). This may be another explanation for lipid peroxidation induced by MSG in the present result, since, the level of NO in the liver tissue was found increased significantly subsequent to MSG administration in the present work. Nasser et al. (2003) revealed that several mediators of systemic vasodilatation such as NO has been reported in the liver cirrhosis. Inducible NO synthase (iNOS) was seen mainly in inflammatory cell infiltrating portal tracts, blood monocytes like cells, hepatocytes, sinusoidal cells, and endothelial cells (Nasser et al., 2003). Therefore, it is clear that NO is augmented in the cirrhotic patients and it is mainly produced by induction of iNOS.

SOD are a class of closely related enzymes that catalyze the breakdown of the superoxide anion into oxygen and hydrogen peroxide (Zelko et al., 2002). The amount of SOD is organ specific and it is abundant in hepatic tissue (Singh and Ahluwalia, 2002). CAT is a key component of the antioxidant defense system. It catalyzes the conversion of hydrogen peroxide to water and oxygen, using an iron or manganese cofactor (Chelikani et al., 2004). The increased activities of SOD and CAT enzymes after MSG administration in the present study was in accord with the reports of Farombi and Onyema (2006) and Onyema et al. (2006). These increases could be attributed to their increased synthesis resulting from the induction, since, antioxidant enzymes are induced in response to oxidative stress (Limaye et al., 2003). Thus, this increase in the activities of SOD and

CAT could be a compensation for GSH depletion.

Glutamate toxicity involves an imbalance in the homeostasis of cysteine, the precursor of GSH, leading to depletion of intracellular GSH levels and reduced ability to protect against oxidative injury in the cell and, ultimately cell damage. Moreover, LPO may eliminate the active sulfhydryl group of GSH and other enzymes. Thus oxidative stress and accumulation of free radicals seems to be responsible for MSG toxicity (Farombi and Onyema, 2006; Diniz et al., 2005; Onyema et al., 2006).

There is an initial interest in the freshwater CE from *C. aegyptiaca* was promised on DPPH assay (Figure 1), showing its radical scavenging activity and analytical analysis (Table 1), showing high levels of taurine in oyster meat, a characteristic maintained in the process of concentrating the extract. It has proposed that taurine has intrinsic potential to protect against free radical damage (Schurr and Rigor, 1987). Perhaps more importantly, the metabolic precursors of taurine, which include hypotaurine, cysteamine, cysteine – sulphinic acid and cysteic acid, have been found to have significantly greater potential to scavenge and detoxify hydroxyl radicals, and superoxide radicals, hydrogen peroxide and hypochloride (Arnoma et al., 1988). Because the CE is high in taurine, it is likely to be a rich source of these antioxidant taurine precursors. In addition, high levels of the precursor amino acids of GSH, glycine, glutamine and cysteine are also present. Data from the present study revealed that CE administration alone for seven days at the two selected doses causes significant increase in the liver GSH level, liver CAT activity and decrease in the levels of serum triglycerides, total cholesterol and liver NO levels. Previous investigations revealed the antioxidant effect of different extracts from *Ostrea gingas* oyster (Kimura et al., 1998), lipped mussel *Perna canaliculus* (Halpern, 2000), and *Crassostrea gigas* oyster (Tanka et al., 2003).

In the present study post treatment with CE at the two selected doses as well as vitamin C for seven days after MSG administration caused significant recovery of the serum activities of AST, ALT and ALP towards nearly normal conditions. These findings were indicating the stabilization of plasma membranes as well as the protecting of the hepatic tissue from damaging by MSG. This observation correlates with the finding of Koyama et al. (2006) who had reported the hepatoprotective effect of a hot water extract from the edible thorny oyster *Spondylus varius* against liver injury induced by carbon tetrachloride. Similarly, Peng et al. (2008) had shown the ameliorative effect of freshwater CE from *Corbicula fluminea* against liver injury induced by hemorrhage in rats.

Data from the present study showed that post treatment with CE at the two selected doses as well as vitamin C for seven days nearly ameliorated the changes occurs in the levels of glucose and lipid profile parameters (especially HDL- ch level) as a result of MSG administration. This observed decrease in the glucose level may be due to post treatment with CE was stimulating

the lipogenesis from glucose (Kimura et al., 1998). Meanwhile, from the results of clam analysis, there is a high taurine content which speculated to be the cause of decreased level of cholesterol (Murakami et al., 1996 a, b). In accord with the findings of Chijimatsu et al. (2008) and Tanaka et al. (2006), the present study revealed significant decrease in the levels of triglycerides and total cholesterol of rats after the treatment with CE alone at the dose of (250 mg/kg body weight). Furthermore, the reports of Tanaka et al. (1994, 1998, 2001), stated that non lipid fractions of sea food, short necked clam, squid shrimp, and octopus, yield decreased triglycerides and cholesterol concentrations of the serum and liver in mice and rats.

In the present study, post treatment either with CE at the two selected doses and vitamin C could restore the alterations in the levels of liver, MDA, GSH, NO, SOD, or CAT due to MSG administration towards the normal levels. These findings indicated that administration of CE decreased lipid peroxidation, improved antioxidant status and thereby prevent the damaging to the liver and leakage of its enzymes (AST and ALT). This indicated that the CE possesses not only the capacity to scavenge the small number of ROS that are inevitably generated due to the incomplete reduction of oxygen in electron transfer reactions in normal aerobic metabolism, but also the capacity to block the MSG induced massive ROS production. This effect is confirmed by the increased level of GSH and CAT when CE was administered only.

In conclusion, the present study revealed that CE has ameliorative effects against the MSG induced hepatic oxidative stress as it alleviates the alterations in AST, ALT, ALP, glucose, lipid profile parameters (total lipids, triglycerides, total cholesterol, HDL- ch, LDL- ch), and oxidative stress markers in the liver (MDA, GSH, NO, SOD, CAT). However, further studies on this Egyptian freshwater CE from a Bivalve *C. aegyptiaca* must be carried out to provide the opportunity to develop a new food adducts from this clam for the prevention and treatment of oxidative stress induced ailments.

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REFERENCES

- Aebi H (1984). Catalase *in vitro*. Methods Enzymol., 105: 121-126.
- Ahluwalia P, Malik VBT (1989). Effects on monosodium glutamate on (MSG) on serum lipids, blood glucose and cholesterol in adult male mice. Toxicol. Lett., 45: 195- 198.
- Ahluwalia P, Tewari K, Choudhary P (1996). Studies on the effect of monosodium glutamate (MSG) on oxidative stress in erythrocyte of adult male mice. Toxicol. Lett., 84:161-165.
- Allain CC, Poon LS, Chan CS, Richmond W, Fu PC (1974). Enzymatic determination of total serum cholesterol. Clin. Chem., 20: 470-475.
- Arnoma OI, Halliwell B, Honey BM, Butler J (1988). The antioxidant action of taurine, hypotaurine and their metabolic precursors. Biochem. J., 256: 251-255.
- Aykac M, Uysal M, Yalcin AS, Kocak – Toker N, Sivas NA, Oz H (1985). The effect of chronic ethanol ingestion on hepatic lipid peroxide, glutathione peroxidase, and glutathione transferase in rats. Toxicol., 36: 71-76.
- Baskaran S, Lakshmi S, Prasad PR (1999). Effect of cigarette smoke on lipid peroxidation and antioxidant enzymes in albino rats. Ind. J. Exp. Biol., 37: 1196-1200.
- Baynes JW (1991). Role of oxidative stress in development of complications in diabetes. Diabetes, 40: 405-412.
- Bellisle F, Dalix AM, Chappuis AS, Rossi F, Fiquet P (1996). Monosodium glutamate affects mealtime food selection in diabetic patients. Appetite, 26: 267-276.
- Bergen HT, Mizuno TM, Taylor J (1998). Hyperphagia and weight gain after gold- thioglucose and monosodium glutamate: relation to hypothalamic neuropeptide-Y. Endocrinol., 139: 4483-4488.
- Berner YN, Stern F (2004). Energy restriction controls aging through neuroendocrine signal transduction. Aging. Res. Rev., 3: 189-198.
- Blackburn P, Lamarche B, Couillard C, Pascot A, Bergeron N (2003). Post- prandial hyperlipidemia: another correlate of the "hypertriglyceride- mice waist" phenotype in men. Atherosclerosis, 171: 327-336.
- Brand WW, Cuvelier HE, Berset C (1995). Use of a free radical method to evaluate antioxidant activity. Food Sci. Technol., 82: 25-30.
- Ceresosima E, Williams P, Hoxworth B, Lacy W, Abumrad N (1986). Glutamine blocked lipolysis and ketogenesis of fasting rats. Am. J. Physiol., 250: E248-E252.
- Chelikani P, Fita I, Loewen P (2004). "Diversity of structures and properties among catalases". Cell Mol. Life Sci., 61(2): 192-208.
- Chen K, Thomas SR, Keaney JF (2003). Beyond LDL oxidation: ROS in vascular signal transduction. Free Radic. Biol. Med., 35: 117-132.
- Chijimatsu T, Tatsuguchi I, Abe K, Oda H, Mochizuki S (2008). A freshwater clam (*Corbicula fluminea*) extract improves cholesterol metabolism in rats fed on a high- cholesterol diet. Biosci. Biotechnol. Biochem., 72: 2566-2571.
- Chijimatsu T, Tatsuguchi I, Oda H, Mochizuki S (2009). A freshwater clam (*Corbicula fluminea*) extract reduces cholesterol level and hepatic lipids in normal rats and xenobiotics- induced hypercholesterolemic rats. J. Agric. Food Chem., 57(8): 3108-3112.
- Choudhary P, Malik VBT, Puri S, Ahluwalia P (1996). Studies on the effect of monosodium glutamate on hepatic microsomal lipid peroxidation, calcium, ascorbic acid and glutathione and its dependent enzymes in adult male mice. Toxicol. Lett., 89: 71-76.
- Das J, Ghosh J, Manna P, Sinha M, Sill PC (2009). Taurine protects rat testes against NaAsO₂ – induced oxidative stress and apoptosis via mitochondrial dependent and independent pathways. Toxicol. Lett., 187(3): 201-210.
- Ding SY, Shen ZF, Xie MZ (2001). Preliminary study of insulin resistance induced by neonatal monosodium glutamate treatment in normal Wistar rats. Chinese Pharmacol. Bull., 17: 181-185.
- Diniz YS, Faine LA, Galhardi CM, Rodrigues HG, Ebaid GX et al (2005). Monosodium glutamate in standard and high- fiber diets: metabolic syndrome and oxidative stress in rats. Nutrition, 21: 749-755.
- Diniz YS, Fernandes AA, Campos KE, Mani F, Ribas BO, Novelli EL (2004). Toxicity of hypercaloric diet and monosodium glutamate: oxidative stress and metabolic shifting in hepatic tissue. Food Chem. Toxicol., 42: 319-325.
- Diniz YZ, Faine LA, Almeida JA, Silva MDP, Ribas BO, Novelli ELB (2002). Toxicity of dietary restriction of fat enriched diets on cardiac tissue. Food Chem. Toxicol., 40: 1892-1899.
- Esposito LA, Melov S, Panov A, Cottrell B, Wallace B (1999). Mitochondrial disease in mouse results in increased oxidative stress. Proc. Natl. Acad. Sci., 96: 4820-4825.
- Farombi EO, Onyema OO (2006). Monosodium glutamate – induced oxidative damage and genotoxicity in the rat: modulatory role of vitamin C, vitamin E and quercetin. Hum. Exp. Toxicol., 25: 251-259.
- Feuers RJ (1998). The effects of dietary restriction on mitochondrial dysfunction in aging. Ann. NY Acad. Sci., 854: 192-200.
- Fossati P, Prencipe L (1982). Serum triglycerides determined

- colorimetrically with an enzyme that produced hydrogen peroxide. *Clin. Chem.*, 28(10): 2077-2080.
- Foyer CH, Pellny TK, Locato V, De Gara L (2008). Analysis of redox relationships in the plant cell cycle: determinations of ascorbate, glutathione and poly (ADP-ribose) polymerase (PARP) in plant cell cultures. *Methods Mol. Biol.*, 476: 199-215.
- Halpern GM (2000). Anti-inflammatory effects of a stabilized lipid extract of *Perna canaliculus* (Lyprinol). *Allerg. Immunol.*, 32(7): 272-278.
- Harlan JM, Levine JD, Callahan KS, Schwartz BR, Harker LA (1984). Glutathione redox cycle protects cultured endothelial cells against lysis by extracellularly generated hydrogen peroxide. *J. Clin. Invest.*, 73: 706-713.
- Hartel C, Strunk T, Bucsky P, Schultz C (2004). Effects of vitamin C on intracytoplasmic cytokine production in human whole blood monocytes and lymphocytes. *Cytokine*, 24: 101-106.
- Hinoi E, Takarada T, Ueshima T, Tsuchihashi Y, Yoneda Y (2004). Glutamate signaling in peripheral tissues. *Eur. J. Biochem.*, 271: 1-13.
- Hiraishi H, Terano A, Ota S, Mutoh H, Sugimoto T, Harada T, Razandi M, Ivey KJ (1994). Protection of cultured rat gastric cells against oxidant-induced damage by exogenous glutathione. *Gastroenterology*, 106: 1199-1207.
- Huang D, Boxin OU, Prior R L (2005). The chemistry behind antioxidant capacity assays. *J. Agri. Food Chem.*, 53(6): 1841-1856.
- Ishak KG, Zimmerman HJ, Ray MB (1991). Alcoholic liver disease: pathologic, pathogenetic and clinical aspects. *Alcohol Clin. Exp. Res.*, 15: 45-66.
- Kim WJ, Wookang K, Oh GT, Song J, Dookim N, Pak YK (2002). Induction of hepatic inducible nitric oxide synthase by cholesterol *in vivo* and *in vitro*. *Exp. Mol. Med.*, 34:137-144.
- Kimura Y, Ohminami H, Okuda H (1998). Effects of extract of oyster on lipid metabolism in rats. *J. Ethnopharmacol.*, 59(3):117-123.
- Knight TA, Anderson S, James MR (1972). Chemical basis of the sulphophospho – vanilline reaction for estimating total serum lipids. *Clin. Chem.*, 18(3):199-202.
- Koyama T, Chounan R, Uemura D, Yamaguchi K, Yazawa K (2006). Hepatoprotective effect of a hot - water extract from the edible thorny oyster *Spondylus varius* on carbon tetrachloride – induced liver injury in mice. *Biosci. Biotechnol. Biochem.*, 70: 729-731.
- Li CY, Deng YL, Sun BH (2009). Taurine protected kidney from oxidative injury through mitochondrial – linked pathway in a rat model of nephrolithiasis. *Urol. Res.*, 37(4): 211-220.
- Limaye PV, Raghuram N, Sivakami S (2003). Oxidative stress and gene expression and antioxidant enzymes in het renal cortex in streptozotocin- induced diabetic rats. *Mol. Cell Biochem.*, 243: 147-152.
- Lopez – Virella MF, Stone P, Ellis S, Colwell JA (1977). Cholesterol determination in high density lipoproteins separated by three different methods. *Clin. Chem.*, 23(5): 882-884.
- Machlin L, Bandich A (1987). Free radical tissue damage: Protective role of antioxidant nutrients. *FASEB. J.*, 1: 441-445.
- Malik VBT, Ahluwalia P (1994). Studies on the effect of monosodium glutamate (MSG) on various fractions of lipids and carbohydrate metabolic enzyme in liver and blood of adult male mice. *Toxicol. Lett.*, 74: 69-77.
- Minibayeva F, Kolesnikov O, Chasov A, Beckett RP, Lüthje S, Vylegzhanina N, Buck F, Böttger M (2009). Wound-induced apoptotic peroxidase activities: their roles in the production and detoxification of reactive oxygen species. *Plant Cell Environ.*, 32(5): 497-508.
- Montgomery HAC, Dymock JF (1961). The determination of nitrite in water. *Analyst*, 86: 414-416.
- Moss DW, Butterworth PJ (1974). *Enzymology and Medicine*. Pitman Medical, London, 139.
- Murakami S, Nara Y, Yamori Y (1996a). Taurine accelerates the regression of hypercholesterolemia in stroke-prone spontaneously hypertensive rats. *Life Sci.*, 58: 1643-1651.
- Murakami S, Yamagishi I, Asami Y, Ohta Y, Nara Y, Yamori Y (1996b). Hypolipidemic effect of taurine in stroke-prone spontaneously hypertensive rats. *Pharmacol.*, 52: 303-313.
- Muriel P, Escobar Y (2003). Kupffer cells are responsible for liver cirrhosis induced by carbon tetrachloride. *J. App. Toxicol.*, 23(2):103-108.
- Nasser A, Seham A, Ian A, Madiha M, Mahmud S, Raymond FT (2003). Expression of nitric oxide synthase isoforms in human liver cirrhosis. *J. Pathol.*, 200(5): 647-655.
- Nishikimi M, Appaji N, Yagi K (1972). The occurrence of superoxide anion in the reaction of reduced phenazine methosulfate and molecular oxygen. *Biochem. Biophys. Res. Common.*, 46: 849-854.
- Ohkawa H, Ohishi N, Yagi K (1979). Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal. Biochem.*, 95: 351-358.
- Onyema OO, Farombi EO, Emerole GO, Ukoha AI, Onyeze GO (2006). Effect of vitamin E on monosodium glutamate induced hepatotoxicity and oxidative stress in rats. *Ind. J. Biochem. Biophys.*, 43: 20-24.
- Ortiz GG, Bitzer- Quintero OK, Zarate CB, Rodriguez- Reynoso S, Larios- Arceo F et al (2006). Monosodium glutamate- induced damage in liver and kidney: a morphological and biochemical approach. *Biomed. Pharmacol.*, 60: 86-91.
- Papa PC, Seraphim PM, Machado UF (1997). Loss of weight restores GLUT-4 content in insulin- sensitive tissues of monosodium glutamate- treated obese mice. *Int. J. Obesity*, 21: 1065-1070.
- Pari L, Murugan P (2004). Protective role of tetrahydrocurcumin against erythromycin estolate- induced hepatotoxicity. *Pharmacol. Res.*, 49(5): 481-486.
- Park CH, Choi SH, Piao Y et al (2000). Glutamate and aspartate impair memory retention and damage hypothalamic neurons in adult mice. *Toxicol. Lett.*, 115: 117-125.
- Paula C, Martino R, Trugo L (2006). Amino acid composition of Brazilian surubim fish (*Pseudoplatystoma coruscans*) fed diet with different levels and sources of fat. *Food Chem.*, 96(1): 126-130.
- Pavlovic V, Pavlovic D, Kocic G, Sokolovic D, Sarac M, Jovic Z (2009). Ascorbic acid modulates monosodium glutamate induced cytotoxicity in rat thymus. *Bratisl. Lek. Listy*, 110(4): 205-209.
- Peng TC, Subeq YM, Lee CJ, Lee CC, Tsai CJ, Chang FM, Lee RP (2008). Freshwater clam extracts ameliorates acute liver injury induced by hemorrhage in rats. *Am. J. Chin. Med.*, 36(6):1121-1133.
- Reitman S, Frankel S (1957). A colorimetric method for the determination of serum glutamic oxaloacetic and glutamic pyruvic transaminases. *Am. J. Clin. Pathol.*, 2: 56-60.
- Rocek L, Lenharalt L, Mozes S (2001). Effect of feeding and refeeding on duodenal alkaline phosphatase activity in monosodium glutamate obese rats. *Physiol. Res.*, 50: 365-372.
- Rodeberg DA, Chet MS, Bass RC, Arkovitz SM, Garcia VF (1995). Nitric oxide: An review. *Am. J. Surg.*, 170: 292-303.
- Sakai T, Nagaswa T (1992). Simple, rapid and sensitive determination of plasma taurine by high- performance liquid chromatography using precolumn derivative formation with fluorecamine. *J. Chromatography*, 576(1): 155-157.
- Scharrer E (1999). Control of food intake by fatty acid oxidation and ketogenesis. *Nutrition*, 15: 704-714.
- Schaumburg HH, Byck R, Gerstl R, Mashman JH (1969). Monosodium L- glutamate: its pharmacology and role in the Chinese restaurant syndrome. *Sciences*, 163: 826-828.
- Schurr A, Rigor BM (1987). The mechanism of neuronal resistance and adaptation to hypoxia. *FEBS Lett.*, 224: 4-8.
- Seraphim PM, Nunes MT, Machado UF (2001). GLUT-4 protein expression in obese and lean 12- month- old rats. *Braz. J. Med. Biol. Res.*, 34: 1353-1362.
- Singh K, Ahluwalia P (2002). Studies of the effect of monosodium glutamate (MSG) administration on the activity of xanthine oxidase, superoxide dismutase and catalase in hepatic tissue of adult male mice. *Ind. J. Clin. Biochem.*, 17(1): 29-33.
- Singh P, Mann KA, Mangat HK, Kaur G (2003). Prolonged glutamate excitotoxicity: effects on mitochondrial antioxidants and antioxidant enzymes. *Mol. Cell Biochem.*, 243: 139-145.
- Sips HJ, DeGraaf PA, VanDam K (1982). Transport of L- aspartate and L- glutamate in plasma membrane vesicles from rat liver. *Eur. J. Biochem.*, 122: 259-264.
- Sleem SH, Ali TG (2008). Application of RADP-PCR in taxonomy of certain freshwater Bivalves of Genus *Coelatura*. *Global J. Mol. Sci.*, 3(1): 27-31.
- Tanaka K, Fukuda M, Ikeda I, Sugano M (1994). Effects of dietary short-necked clam, *Tapes japonica*, on serum and liver cholesterol

- levels in mice. *J. Nutr. Sci. Vitaminol.*, 40: 325-333.
- Tanaka K, Ikeda I, Yoshida H, Imaizumi K (2001). Effects of dietary defatted squid on cholesterol metabolism and hepatic lipogenesis in rats. *Lipids*, 36: 461-466.
- Tanaka K, Nishizono S, Kugino K, Tamari M, Kurumiya M, Abe N, Ikeda I (2006). Effects of dietary oyster extract on lipid metabolism, blood pressure, and blood glucose in SD rats, hypertensive rats, and diabetic rats. *Biosci. Biotechnol. Biochem.*, 70(2): 462-470.
- Tanaka K, Sakai T, Ikeda I, Imaizumi K, Sugano M (1998). Effects of dietary shrimp, squid and octopus on serum and liver lipid levels in mice. *Biosci. Biotechnol. Biochem.*, 62: 1369-1375.
- Tanka K, Ikeda I, Kase A, Kob K, Nishizono S, Aoyama T, Imaizumi K (2003). Effects of feeding oyster, *Crassostrea gigas*, on serum and liver lipid levels in rats. *J. Nutr. Sci. Vitaminol.*, 49(2): 100-106.
- Thomas M, Sujatha KS, George S (2009). Protective effect of *Piper longum Linn.* on monosodium glutamate induced oxidative stress in rats. *Ind. J. Exp. Biol.*, 47(3): 186-192.
- Trinder P (1969). Determination of glucose in blood using glucose oxidase with an alternative oxygen acceptor. *Ann. Clin. Biochem.*, 6: 24-25.
- Walker R, Lupien JR (2000). The safety evaluation of monosodium glutamate. *J. Nutr.*, 130: 1049S-1052S.
- Weinstein DM, Mihm MJ, Bauer JA (2000). Cardiac peroxy-nitrite formation and left ventricle dysfunction following doxorubicin treatment in mice. *J. Pharmacol. Exp. Ther.*, 294: 396-401.
- Wieland H, Seidel D (1983). A simple specific method for precipitation of low density lipoproteins. *J. lipid Res.*, 24(7): 904-909.
- Younes M, Seigers CP (1981). Mechanistic aspects of enhanced lipid peroxidation following glutathione depletion *in vivo*. *Chem. Biol. Interact.*, 34: 257-266.
- Young DS, Pestner LC, Gibberman V (1975). Effects of drugs on clinical laboratory tests. *Clin. Chem.*, 21: 246D.
- Zelko I, Mariani T, Folz R (2002). "Superoxide dismutase multigene family: a comparison of the CuZn-SOD (SOD 1), Mn-SOD (SOD 2), and EC-SOD (SOD3) gene structures, evolution, and expression". *Free Radic. Biol. Med.*, 33(3): 337-349.