

Monosodium Glutamate induced hepatotoxicity and the possible mitigating effect of Vitamin C and Propolis

Abstract

Monosodium Glutamate has been recognized as flavor enhancer that adversely affects liver function's parameters. The aim of this study was to evaluate the effects of Monosodium Glutamate on the liver enzyme functions, lipid profile and antioxidant system and also assessed the ameliorating effects of Vitamin E and propolis in the rat liver tissues. Mature male wistar rats (weighing 150-200 g and each group of seven animals) were given Monosodium Glutamate (60 mg/Kg bw) and/or Vitamin C (200 mg/Kg) and/or Propolis (200 mg/Kg) extract daily via gavage for 4 weeks. In the present study, Monosodium Glutamate exposure resulted in an increase in the TBARS level and a decrease in the SOD, CAT, GPX activities in liver homogenates, with respect to the control. Supplementations of Vitamin E and/or propolis to Monosodium Glutamate-induced groups declined lipid peroxidation, increased SOD, CAT, GPX activities. As a result, Monosodium Glutamate induced hepatic toxicity is reduced by Vitamin E and/or selenium to a great extent by the entire restoration of the liver function markers and the antioxidant status.

Key words: Monosodium Glutamate, Vitamin C, propolis, Oxidative stress, Liver functions.

Abbreviations: MSG: Monosodium Glutamate, Vit.E: Vitamin E, SOD: Superoxide dismutase, CAT: Catalase, MDA: Malondialdehyde, GPX: Glutathione peroxidase.

1-Introduction

Eating behavior plays a vital role in metabolism and diet-related disorders. Some mechanisms for food cravings may benefit mammals from nutrient deficiency, otherwise overfeeding of palatable food may cause an imbalanced intake of nutrients. Monosodium glutamate (MSG) is one of the most abundant naturally occurring non-essential amino acids and MSG-treatment is able to produce metabolic changes, which can further result in severe bodily disturbances [1].

Glutamate is one of the most abundant amino acids in nature. Glutamate which is a derivative of l-glutamic acid is reported to be a natural nutrient in many foods. Today it is increasingly used (in the form of its salt MSG (monosodium glutamate) in food processing and home cooking all over the world aims to improve food palatability for humans. It gives a particular taste named "umami" is now recognized as the fifth basic taste. Recently it was recognized as an excitatory neurotransmitter in mammalian nervous system [2].

During intestinal absorption, a large amount of glutamic acid is transaminated and consequently alanine levels in portal blood are elevated. If large amounts of glutamate are ingested, portal glutamate levels will increase, this elevation results in an increase hepatic

metabolism of glutamate, leading to the release of glucose, lactate, glutamine, and other amino acids, into systemic circulation [3].

Antioxidants have been reported to play a significant role in the protection against lipid peroxidation [4]. Some investigators reported that antioxidants inhibit chemical carcinogenesis when the antioxidants are administered either prior or during carcinogenic changes [5].

Antioxidants as vitamins, can prevent the uncontrolled formation of free radicals, or inhibit their reaction with biological sites, also the destruction of most free radicals rely on the oxidation of endogenous antioxidants mainly by scavenging and reducing molecules [6]. Vitamin C, as a water soluble antioxidant is reported to neutralize ROS and reduce oxidative DNA damage and hence genetic mutations [7].

Propolis has attracted the attention of researchers because of its various biological activities and therapeutic properties. Propolis is a sticky, resinous product that is produced by honeybees for the construction and maintenance of their hives. It is made by mixing the honeybees own waxes with the resins that are collected from various plants. Propolis contains a variety of chemical compounds, such as polyphenols (flavonoid aglycones, phenolic acids and their esters, and phenolic aldehydes), alcohols and ketones, sesquiterpene quinones, coumarins, steroids, amino acids, and inorganic compounds [8].

One of the biological activities of propolis, its antibacterial and antifungal activities of propolis is the most extensively investigated [9]. Its pharmacological activities, such as anticancer [10], anti-inflammatory [11], antibiotic [12], antioxidative [13], antiviral [14] anaesthetic and immunostimulant [13] have been described to the ethanolic extracts of propolis.

In the last few years, fear had increased due to the adverse reactions and toxicity of MSG, with few and limited literature regarding the biochemical and histological studies of the damage in liver treatment of animals with MSG. So, the present study was designed to investigate the effects of MSG on the testicular tissue of mature male rats. In addition, to the best of our knowledge, no comprehensive study concerning the protective effect of vit C and propolis on MSG-induced hepatic dysfunction. The main objective of the present work is to study the effect of propolis or vit C and their role in the amelioration of the hepatic toxicity induced by MSG.

2-Material and methods:

2.1. Chemicals:

Monosodium glutamate ($C_5H_9NO_4 \cdot Na$) Purity 99% NT, it was sold in most open market under the license of Ajinomoto Co. INC. Tokyo, Japan. A stock solution was prepared by dissolving 60 g of MSG crystals in 1000 ml of distilled water. The dose schedule was so adjusted that the amount of MSG administration per animal was as per their respective weight. The applied doses were selected according to [15]. Vitamin (C) was supplied by

Merck (Germany) and it was dissolved in dist.water and administered orally for 30 successive days at a dose 200 mg/Kg [16].

Commercially available diagnostic UV kits were used to determine alanine transferase (ALT), lactate dehydrogenase (LDH) and total proteins (Human diagnostic worldwide,Germany). Moreover, total cholesterol, high density lipoprotein-cholesterol (HDL-c) and ascorbic acid (AA), were bought from Bio-diagnostic, Egypt (Stanbio Co.,Spain).

2.2. Propolis preparation:

Fifty grams of the resinous material of Egyptian propolis were cut into small pieces and extracted with 600 ml of 80% (v/v) ethanol at 60°C for 30 min. After extraction, the mixture was centrifuged and the supernatant was evaporated to complete dryness under vacuum at 40°C [17]. It was kept at 4 °C for further use. An aqueous suspension of propolis was prepared in 1% gum acacia suspension, and orally administered to the animals for 3 weeks at a dose of 200 mg/kg [18].

2.1. Animals and experimental design:

Seventy male Wistar rats (Weighing 220-250 g), were used in all experiments of this study. They were obtained from the Animal House of the National Research Center (Dokki, Giza, Egypt). The animals were maintained on solid-bottom shoe box, type polycarbonate cages with stainless steel wire-bar lids, using a wooden dust free litter as a bedding material. Animals were allowed free access to diet and water in good air conditioned room and were allowed free access and tap water for two weeks before starting the experiment. We have followed the European community Directive (86/609/EEC) and national rules on animal care. One group served as control. Animals were weighed and randomly allocated into 7 groups (10 rats each) as following:

Group 1 – control rats treated with 1 mg/kgBW corn oil per day; Group 2 – MSG– treatedrats (60 mg/Kg BW per day in distilled water) [15]; Group 3– vit C treated rats (200 mg/kg BW per day in corn oil)[16]; Group Group 4 – Propolis treated rats (200 mg/kg BW per day in distilled water) [18]; Group5– MSG plus Vit.C-treated rats. Group 6 – MSG plus propolis.Group 7 – MSG treatedrats followed by MSG and propolis daily for successive 30 days.The doses were administered in the morning (between 9.30 and 10.30 h) to non-fasted rats.The first day, when the animals were treated was considered experimental day 0. At the end of the 30 days of treatment, all animals were scarified and dissected. The testis tissues were quickly processed for light microscopie investigations and biochemical examinations.

2.4. Biochemical Analyses

2.4.1. Lipid profile

Triglycerides, cholesterol and high density lipoprotein-cholesterol (HDL-c) were determined using the commercial kits. Low density lipoprotein-cholesterol (LDL-c) levels were calculated by using the following formula of Muruganandan et al.[19] $LDL-c = \text{total cholesterol} - (\text{HDL-c} + \text{triglycerides})/5$. Volatile low density lipoprotein-cholesterol (VLDL-c) levels were calculated by using the following formula : $VLDL-c = \text{triglyceride}/5$.

2.4.2. Hepatic markers

Serum aspartate transaminase (AST), alanine transaminase (ALT) and lactate dehydrogenase (LDH) enzyme activities were determined with kits from Human diagnostic worldwide, Germany.

2.5. Tissue homogenates preparation and estimation of antioxidant capacities parameters:

The excised liver tissue was washed with distilled water for the removal of blood, and later the fatty parts were removed. Tissues were homogenized in ice-cold 50 mM sodium phosphate buffer (pH 7.4) containing 0.1 mM ethylenediaminetetraacetic acid (EDTA). The supernatant was separated by means of centrifugation at 5000 rpm for 20 min at 4°C. The supernatant was used for the analyses of all biochemical parameters.

2.5.1. Lipid peroxidation assay

TBARS content was evaluated by using the thiobarbituric acid (TBA) test as described by Ohkawa et al. [20]. After incubation of testis homogenates with TBA at 95°C, TBARS reacts to form a colored complex. Absorbance was measured spectrophotometrically at 532 nm to determine the TBARS content. The level is expressed as nmol/mg protein.

2.5.2. Measurement of superoxide dismutase (SOD)

SOD activity was measured according to the method described according to Misra and Fridovich [21] by assaying the auto oxidation of pyrogallol at 440 nm for 3 min. One unit of SOD activity was calculated as the amount of protein that caused 50% pyrogallol autooxidation inhibition. A blank without homogenate was used as a control for non-enzymatic oxidation of pyrogallol in Tris-EDTA buffer (50 mM Tris, 10 mM EDTA, pH 8.2). The SOD activity is expressed as U/mg protein.

2.5.3. Measurement of catalase (CAT)

CAT activity was measured according to the method described by Aebi [22] by assaying the hydrolysis of H₂O₂ and the resulting decrease in absorbance at 240 nm over a 3 min period at 25°C. Before determination of the CAT activity, samples were diluted 1:9 with 1% (v/v) Triton X-100. CAT activity is expressed as mmol/mg protein.

2.5.4. Measurement of glutathione peroxidase (GPx)

GPx activity was measured using H₂O₂ as substrate according to the method described by Paglia and Valentini [23]. The reaction was monitored indirectly as the oxidation rate of NADPH at 240 nm for 3 min. A blank without homogenate was used as a control for non-enzymatic oxidation of NADPH upon addition of hydrogen peroxide in 0.1 M Tris buffer, pH 8.0. Enzyme activity was expressed as nmol/mg protein.

2.6. Statistical analysis:

Data were collected, arranged and reported as mean $\pm$ standard error of mean (SEM) of twelve groups, and then analyzed using the computer program (SPSS/version 15.0). The statistical method was one way analysis of variance ANOVA test, and if significant differences between means were found, Duncan's multiple range test (whose significant level was defined as $P < 0.05$) according to [24] to estimate the effect of different treated groups.

3. Results:

3.1. Biomarkers of liver and lipogram assessment:

All the parameters of the lipid profile (TG, TC, LDL-c and VLDL-c) were increased except the HDL-c was decreased when the rats exposed to the dose of MSG (Table.1). Administration of Vit C or/and propolis to the MSG-treated group restored most normal values of the parameters cited above (Fig. 1). Serum ALT activity of Vit C and propolis groups elicited non-significant changes when compared with the control group. In the MSG-treated group, the ALT was increased by 78 fold when compared to control (Fig. 2). Treating the rats with 60 mg/Kg of MSG with the combination of Vit C and propolis decreased the activity of ALT more than combination with each compound separately. The same observation has been noticed in the AST activity that increased by administration the dose of MSG and decreased by the treatment of each antioxidant separately and the most increment was recorded in the combination of both antioxidants (Vit.C and propolis) with MSG (Fig. 2). LDH enzyme highly increased in MSG-treated group by 1890 fold when compared with the control group, but increased by 543 and 545 fold in the groups treated with the MSG and Vit C or MSG and propolis, respectively (Fig. 2). The best combination group that highly decreased LDH value was recorded in the group treated with a combination of Vit.C and propolis with MSG as LDH was increased by 340 fold as compared to a normal control group.

3.2. Assessment of enzymatic antioxidants and non-enzymatic lipid peroxidation as oxidative biomarkers:

As shown in Table 3, the data showed that treatment with MSG caused a significant ($p < 0.05$) decrease in the activity of SOD level in MSG treated group as compared to control group. Meanwhile the administration of Vit.C alone and propolis alone to rats elicited non-significant changes as compared to normal control group. Administration of Vit.C in combination with propolis exhibited slight significant decrease in SOD activity with respect to control group by 27.35 %. In addition, a significant recovery relating to CAT and GPX was observed in response to the presence of Vit.C with propolis in treated rats with MSG. Treatment of the rats with MSG alone decreased the CAT and GPX activities. However, Co administration of Vit.C and propolis with MSG increased the CAT and GPX activities as compared with the MSG group.

The treatment of the rats with MSG alone induced a highly significant increase in MDA level (non-enzymatic antioxidant) as MDA is the final product marker of lipid peroxidation. The treatment of rats with Vit.C and propolis in combination with MSG afforded a significant increase in MDA but with the significant reduction in MDA as compared to SG treated group only by 53.2%.

4. Discussion:

The present study was conducted to elucidate the protective role of Vit.C or propolis separately or in combination against MSG-induced hepatic damage and oxidative stress

204 in liver tissues of rats. There is no literature report available on the efficacy of Vit.C and
205 propolis in combination in cases of MSG exposure in rats. In this respect, the present
206 study bears originality.

207 Variation in total cholesterol levels were significantly within the groups; however, Group
208 treated with MSG had shown elevated cholesterol levels more than groups treated with a
209 combination of MSG and VitC and propolis, which suggest a direct correlation between
210 MSG administration and the increment of cholesterol level and affecting on fat
211 metabolism. Inuwa et al.[25] found that the potential explanation for MSG – obesity link
212 lies in the alteration of regulatory mechanisms that affect fat metabolism. Also, another
213 explanation in this study related to increased levels of TG, LDL-c and vLDL-c and
214 decreasing HDL-c level suggests that MSG could be a risk factor or coronary heart
215 disease [26].

216 According to our previous results recorded by Hamza et al.[27] as they reported the
217 role of propolis in decreasing blood glucose levels in diabetic rats and thus enhancing
218 lipid metabolism as it is known that liver is organ involved in glycogenesis,
219 glycogenolysis, gluconeogenesis, and glycolysis and thus affecting by direct way on lipid
220 metabolism and this confirmed our obtained results by decreased the levels of
221 TC, TG, LDL-c and vLDL-c in groups treated by either propolis or combination between
222 propolis and MSG.

223 Results of Elmazoudy et al [28] supported our results as they clearly indicated that
224 previous administration of the propolis induced marked change in lipoproteins and elicited
225 marked fall in the total cholesterol and LDL-c levels in rats induced by CPF in the rat
226 blood serum.

227 The elevated levels of ALT and AST levels could be an indication of hepatocellular
228 damage induced by MSG [25]. Our findings are greatly supported by our previous results
229 obtained by Diab et al., 2012 as they showed that the AST activity determined to be
230 higher in the group that was administered Chlorpyrifos and AST was demonstrated to be
231 decreased in the groups that were administered Propolis in combination with
232 Chlorpyrifos.

233 Ramadan et al., [29] reported that oral administration of Propolis for 70 days; decreased
234 the activities of AST and ALT in plasma. Also, Sforcin et al., [30] reported that treatment
235 of rats with Propolis does not induce any alteration in AST level. Moreover, Mani et al.,
236 [31] found no alteration in AST value in the serum of Propolis treated rats for (30 or 90
237 or 150 days) at doses of (1, 3 and 6 mg/kg/day) and so all these findings supported our
238 obtained results that propolis protect against hepatotoxicity induced by MSG
239 administration.

240 It has been generally accepted that the leakage of LDH enzyme correlates well with
241 cellular viability, thus being a useful indicator of plasmatic membrane damage and/or
242 necrosis of hepatocytes [26].

243 The present significant change in liver enzymes AST and ALT in MSG treated group and , thus,
244 may confirm that the hepatocytes are more affected by MSG exposure.

245 Oxidative stress refers to disrupted redox equilibrium between the production of
246 freeradicals and the ability of cells to protect against damage caused by these species.
247 Defense against oxidative stress are maintained by using several mechanisms which
248 include antioxidant machinery. The main cellular components susceptible to damage by
249 free radicals are lipids (peroxidation of unsaturated fatty acids in cell membranes),
250 proteins (denaturation), carbohydrates and nucleic acids; this in turn can impair cellular
251 structure and function [32].

252 It has been indicated that the LPO is one of the molecular mechanisms involved in
253 pesticide-induced cytotoxicity [33]. These findings support the occurrence of oxidative
254 stress in the present study induced by CPF. The results showed that the LPO level was
255 significantly increased in rat testicular tissue treated with CPF. The toxic manifestations
256 induced by the tested

257 pesticide may be associated with the enhanced production of ROS or the increase in
258 MDA levels which is induced by the pesticide itself (degradation of phospholipids and
259 ultimately result in

260 cellular deterioration) or by a possible increase in free radicals caused by CPF [34].

261 Among the antioxidant enzymes, SOD, CAT and GPx are the first line of defense against
262 oxidative injury. SOD is the primary step of the defense mechanism in the antioxidant
263 system against oxidative stress by catalyzing the dismutation of superoxide radicals (O_2^-)
264 into molecular oxygen (O_2) and hydrogen peroxide (H_2O_2) [35]. H_2O_2 is neutralized by
265 the combined action of CAT and GPx in all vertebrates [36]. These enzymes act in
266 coordination and the cells may be pushed to oxidative stress state if any change occurs in
267 the levels of enzymes.

268 Our obtained data confirmed by the results obtained by Hamza et al., 2014b who
269 reported that MSG caused an elevation in lipid peroxidation level parallel with significant
270 decline in SOD, CAT as well as GPx activities in testis tissues.

271 The current data displayed MSG-induced reduction in the activities of the antioxidant
272 enzymes (SOD, CAT and GPx) and the content of non-enzymatic antioxidant (MDA).
273 This effect might be due to increased H_2O_2 production and ROS generation which in turn
274 induces oxidative stress.

275 In the present study, a significant decrease in the specific activity of SOD in liver tissues
276 was observed in MSG-treated liver tissue, suggests an increased superoxide radical
277 production and other ROS thereby induce oxidative damage [37]. CAT activity of hepatic
278 tissue was decreased in MSG-treated rat.

279 GPx is the general name of an enzyme family with peroxidase activity whose main
280 biological role is to protect the organism from oxidative damage. The biological function
281 of GPx is to reduce lipid hydroperoxides conversion to their corresponding alcohols and
282 to reduce free H_2O_2 reaction [38].

283 In our present study, a significant fall in GPx activity was observed in MSG-treated
284 animals. This observation may be due to enhanced free radical production (as evidence

by increase LPO) and apart from CAT also involved in the removal of H_2O_2 . H_2O_2 generated due to MSG toxicity, .Hence, the GPx level decreases on MSG administration.

It was found that the administration of vit C and/or propolis to MSG-injected rats restored SOD, CAT and GPx activity to nearly control level. However, MDA level was decreased significantly after Vit C administration as compared to MSG. Therefore, the present decrease in GPx activity and restoration of SOD activity to nearly control level, in addition to the decrease in MDA content in the liver tissues, could indicate the antioxidant properties of Vit C. It also increased the antioxidant enzymatic activity. In accordance with our results, the mild oxidative state observed in the hippocampus due to MSG administration may be due to the inherent high content of Vit C in this brain area. The consumption of vit C is useful for the antioxidant effect as it offers an effective and safe way of increasing body immune system against free radicals and, at the same time, keeps the oxidative stress in a state of balance [39].

The results indicate the protective effect of vit C and/or propolis in combating oxidative stress undergone by the rat. In addition, the high antioxidant activity was found in the rat treated with vit C alone and in combination with propolis as reported before by Hashim [40] and Diab et al. 2012 [41]. This result indicates that vit C and propolis are a strong scavenger of free radicals.

In conclusion, the present findings strongly indicate that hepatic damage, hyperlipidemia and oxidative stress resulting from MSG in a dose dependent manner is involved in toxicity mechanisms of liver of rat. MSG increased the enzymes leakage of LDH due to increase of the generation of free radicals as hepatic LPO increased. MSG decreased the enzymatic and non-enzymatic antioxidants. These results suggest that these MSG-induced metabolic disorders associated with oxidative stress while, Vit. C and propolis exhibited strong antioxidant activities. The results emphasize the importance of reassessment of MSG toxicity, a substance widely used in food industry. Protection can be derived from treatment of the rats with antioxidant vit C and propolis that increased the stability of membrane and decreased the liberation of cellular enzymes. Treatment with vit C and propolis after treatment with the MSG restored the same efficiency of the antioxidant enzymes, and they play a preventive role against MSG-induced cellular oxidative stress.

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Tables

Table (1): Effects of treatment of Monosodium Glutamate, Vitamin C, Propolis and their combinations with MSG on Lipid profile parameters.

Groups	Cholesterol (mg/dl)	Triglycerides (mg/dl)	HDL-c (mg/dl)	LDL-c (mg/dl)	VLDL-c (mg/dl)
1- control group	67.22±4.57 ^g	98.99±4.77 ^f	36.78±3.62 ^{ab}	33.24±2.52 ^f	6.64±2.45 ^e
2-Mono sodium Glutamate (MSG)	255.14±22.1 ^a	193.62±9.54 ^a	7.25±2.42 ^g	210.23±12.45 ^a	42.04±4.21 ^a
3-Vitamin C	70.11±2.15 ^{ef}	100.25±10.25 ^{ef}	31.20±5.24 ^c	37.20±5.42 ^{ef}	7.44±3.52 ^{de}
4-Propolis	69.11±2.15 ^{fg}	101.63±8.65 ^{ef}	33.25±3.24 ^{bc}	36.52±3.52 ^{ef}	7.30±5.75 ^{de}
5-MSG+Vit.C group	99.41±6.75 ^{bc}	135.74±6.48 ^c	20.34±3.21 ^e	115.32±4.36 ^c	23.06±6.75 ^b
6-MSG+Propolis	97.52±3.54 ^c	142.03±7.25 ^{bc}	17.28±3.21 ^f	128.98±6.38 ^b	25.79±3.68 ^b
7- MSG+Vit.C + Propolis	81.08±4.12 ^d	129.24±9.45 ^d	27.36±2.75 ^d	69.21±4.12 ^d	13.84±3.96 ^c

Means within the same column in each category carrying different litters are significant at ($P \leq 0.05$) using Duncan's multiple range tests, where the highest mean value has symbol (a) and decreasing in value were assigned alphabetically.

Table (2): Effects of treatment of Monosodium Glutamate, Vitamin C, Propolis and their combinations with MSG on Liver function parameters.

Groups	ALT (U/L)	AST (U/L)	LDH(U/L)
1- control group	13.00±0.56 ^{ef}	12.20±0.54 ^{de}	310.66±20.63 ^d
2-Mono sodium Glutamate (MSG)	91.00±1.18 ^a	177.80±7.39 ^a	2200.40±25.37 ^a
3-Vitamin C	12.35±1.42 ^f	11.98±0.23 ^e	341.30±23.10 ^{cd}
4-Propolis	13.01±1.35 ^{ef}	12.24±1.01 ^{de}	345.69±17.33 ^{cd}
5-MSG+Vit.C group	70.45±1.18 ^c	109.36±5.36 ^b	853.12±23.04
6-MSG+Propolis	78.80±1.82 ^{bc}	107.80±6.02 ^b	855.40±25.37 ^b
7- MSG+Vit.C + Propolis	20.40±0.30 ^d	55.40±1.12 ^c	650.21±18.23

Means within the same column in each category carrying different litters are significant at ($P \leq 0.05$) using Duncan's multiple range tests, where the highest mean value has symbol (a) and decreasing in value were assigned alphabetically.

Table (3): Effects of treatment of Monosodium Glutamate, Vitamin C, Propolis and their combinations with MSG on antioxidant parameters capacities in liver homogenates.

Groups	MDA (nmol/mg protein)	CAT (nmol/mg protein)	SOD (U/mg protein)	GPx (nmol/mg protein)
1- control group	0.24±0.01 ^b	0.86±0.12 ^a	9.65±0.36 ^{ab}	6.67±0.63 ^{ab}
2-Mono sodium Glutamate (MSG)	0.92±0.03 ^a	0.25±0.10 ^e	2.63±0.45 ^e	1.10±0.85 ^f
3-Vitamin C	0.25±0.02 ^b	0.85±0.23 ^a	9.30±0.96 ^b	6.55±0.74 ^b
4-Propolis	0.26±0.02 ^b	0.87±0.15 ^a	9.38±0.42 ^b	6.50±0.36 ^b
5-MSG+Vit.C group	0.63±0.01 ^d	0.43±0.18 ^d	5.55±0.36 ^d	3.79±0.10 ^{cd}
6-MSG+Propolis	0.76±0.04 ^d	0.49±0.20 ^{cd}	5.58±1.20 ^d	3.87±0.57 ^c
7- MSG+Vit.C+ Propolis	0.43±0.03 ^c	0.65±0.35 ^b	7.01±1.11 ^c	4.36±0.85 ^e

Means within the same column in each category carrying different litters are significant at ($P \leq 0.05$) using Duncan's multiple range tests, where the highest mean value has symbol (a) and decreasing in value were assigned alphabetically.

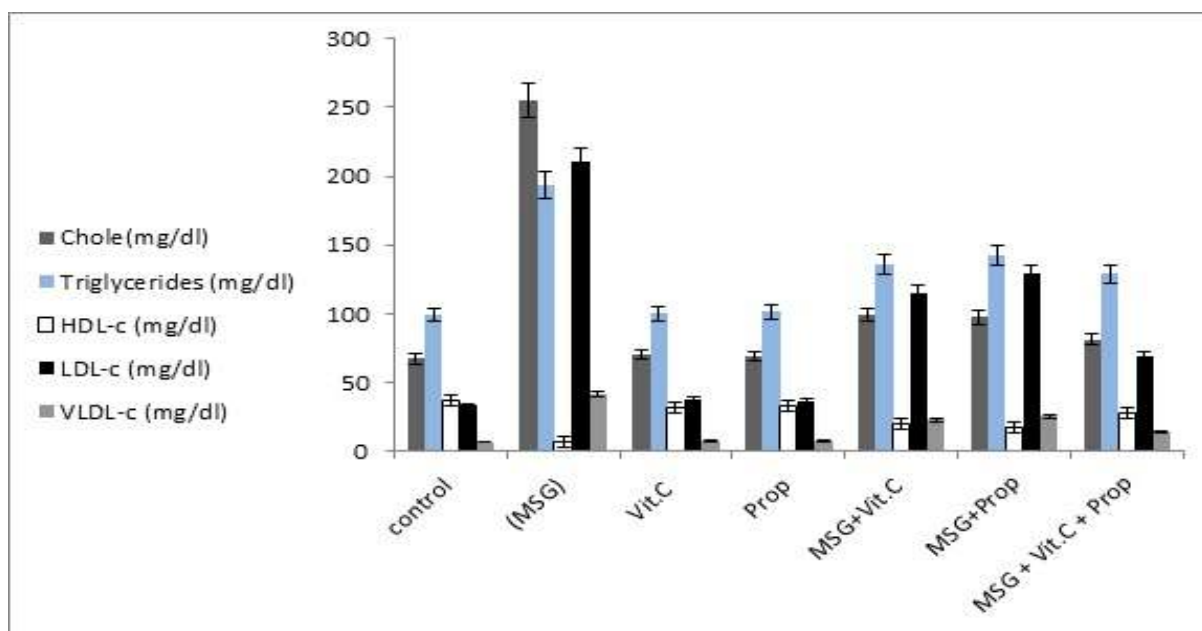
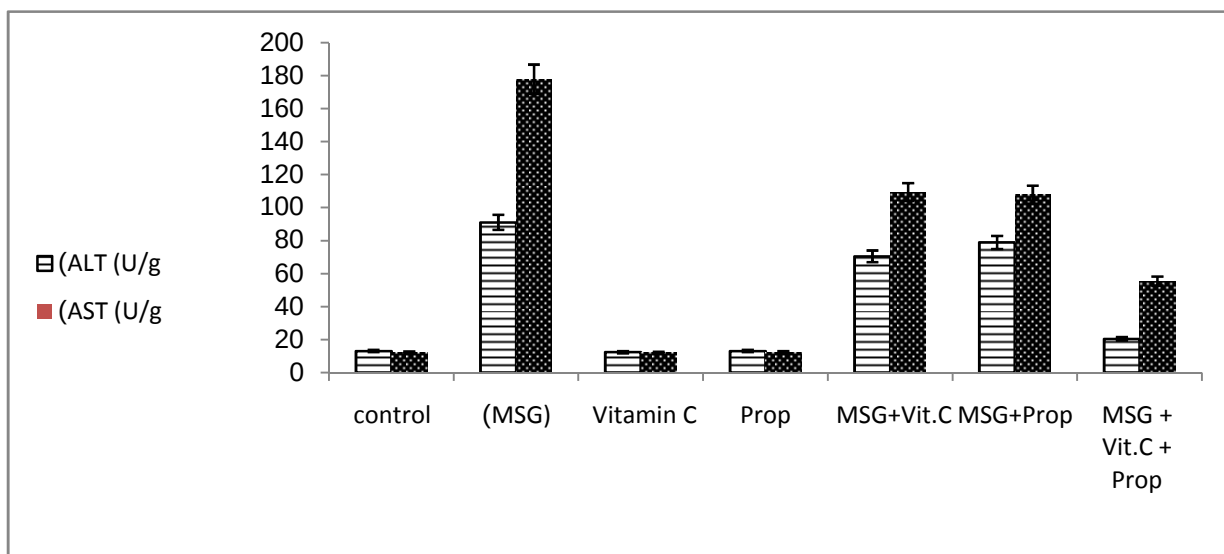


Fig (1):Effects of treatment of Monosodium Glutamate, Vitamin C, Propolis and their combinations with MSG on Lipid profile parameters.



Fig(2):Effects of treatment of Monosodium Glutamate, Vitamin C, Propolis and their combinations with MSG on Liver function biomarkers.

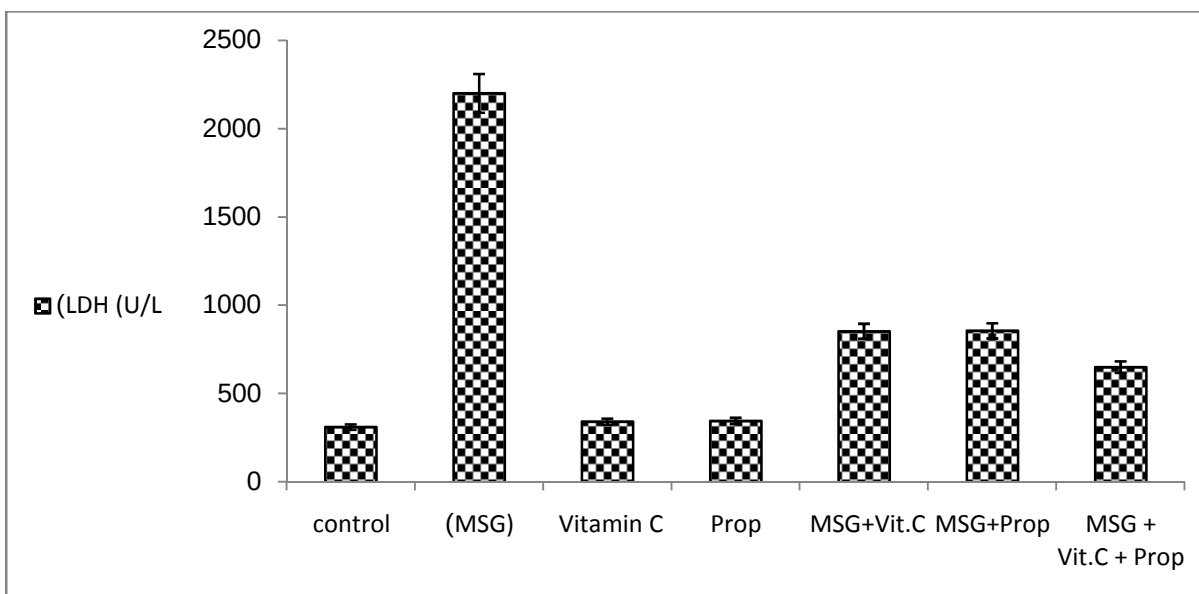


Fig (3):Effects of treatment of Monosodium Glutamate, Vitamin C, Propolis and their combinations with MSG on LDH enzyme level.

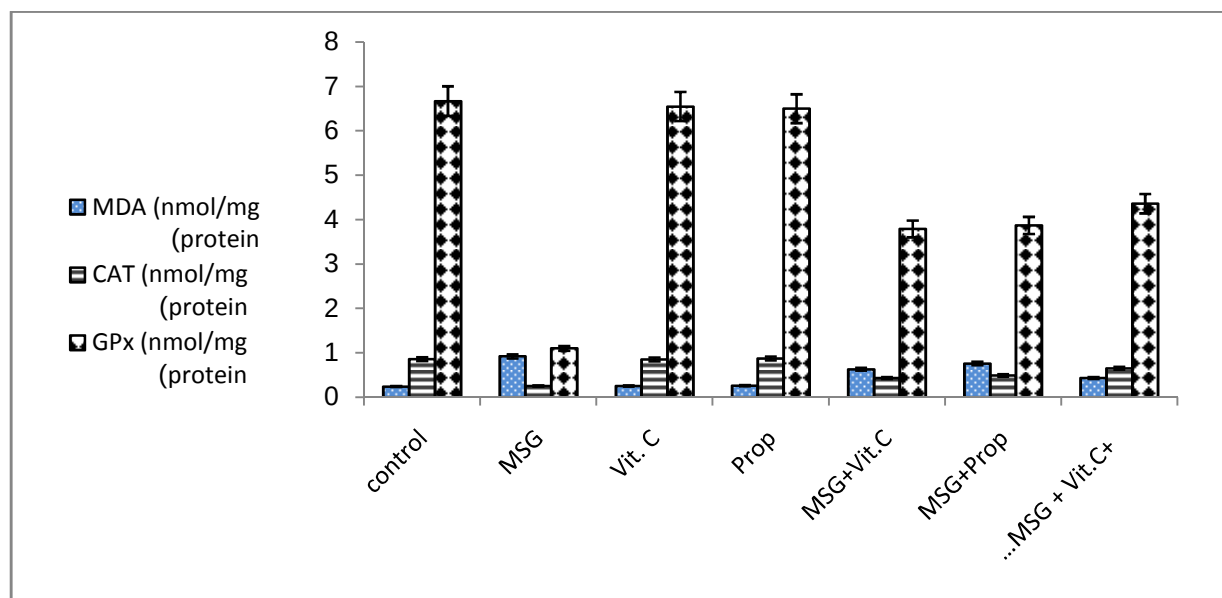


Fig (4):Effects of treatment of Monosodium Glutamate, Vitamin C, Propolis and their combinations with MSG on antioxidant parameters capacities in liver homogenates.