



Journal of Medical Sciences

ISSN 1682-4474

science
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Hepatoprotective Effect of Carnosine on Liver Biochemical Parameters in Chronic Ethanol Intoxicated Rat

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The aim of the present study is to investigate the effect of ethanol and the action of carnosine treatment on certain liver parameters; enzymes, total proteins and glycogen in rats. These enzymes including Krebs cycle enzyme: succinate dehydrogenase (SDH); glycolytic enzymes: lactate dehydrogenase (LDH) and its isoenzymes; glycogenolytic metabolic machineries: glycogen phosphorylase, glucose-6-phosphatase (G-6-Pase) and glycogen; hydrolytic enzymes: acid phosphatase (AP) and alkaline phosphatase (ALP); amino acid metabolic enzymes: aspartate aminotransferase (AST) and alanine aminotransferase (ALT) and nucleic acid catabolic enzyme: 5'-nucleotidase. The chronic ethanol-intoxicated rats showed a marked disturbance in all the measured parameters, indicating the toxic effect of ethanol by inducing oxidative stress on liver tissue. Administration of carnosine ameliorated the toxic effect of ethanol by enhancement all of the measured parameters.

Key words: Ethanol toxicity, carnosine, enzymes, glycogen

INTRODUCTION

Chronic alcoholism produces a wide spectrum of liver and other organ diseases depending on the amount and duration of alcohol intake. The liver affection ranges from fatty change to hepatitis and cirrhosis. Protein deficiency and enzyme activity depression were proved to be associated with alcoholic liver disease. Chronic liver disease in human is characterized by a fall in serum albumin concentration and enzyme activities (Devaki *et al.*, 1992; Ozaras *et al.*, 2003). Oxidative stress is well recognized to be a key step in the pathogenesis of tissue injury by ethanol generating reactive oxygen species (ROS) in many tissues and decreasing in the endogenous antioxidants as well as enhancement of lipid peroxidation process (Sadrzadeh *et al.*, 1994).

L-carnosine (beta-alanyl-L-histidine) is an active physiological dipeptide that is distributed naturally in several human tissues especially the skeletal muscle, cardiac muscle and the brain (Jackson and Lenney, 1996). Carnosine exhibits antioxidant actions (Kang *et al.*, 2002a) and inhibition of lipid peroxidation (Nagasawa *et al.*, 2001; Soliman *et al.*, 2001; Chen and Ho, 2002). In addition, it exerts anti-inflammatory activity presumably due to its antioxidant and antiglycation properties (Soliman *et al.*, 2001, 2002).

The present research has been directed to study the effect of hepatoprotective effect of carnosine administration in rat liver against ethanol toxicity through estimation of lactate dehydrogenase (LDH; E.C.1.1.1.27) and its isoenzymes, succinate dehydrogenase (SDH; E.C. 1.3.99.1), glucose-6-phosphatase, (G-6-pase; E.C.3.1.3.9), glycogen phosphorylase (E.C.2.4.1.1), acid phosphatase (AP; E.C.3.1.3.2), alkaline phosphatase (ALP; E.C.3.1.3.1), aspartate aminotransferase (AST; E.C.2.6.1.1), alanine aminotransferase (ALT; E.C. 2.6.1.2) and 5'-nucleotidase (E.C.3.1.3.5), total protein and glycogen.

MATERIALS AND METHODS

Chemicals: The chemicals used were of analytical quality, products of Merck, Germany, Sigma, USA and El Nasr Pharmaceutical Chemical Company, Egypt.

Doses and route of administration

- Ethyl alcohol was administered orally at a dose of 12 g kg⁻¹ body weight/day (Gil-Martin *et al.*, 1998), where the ethyl alcohol (99.8%) was diluted using bidistilled water to 20% and the dose was equivalent to 1.3 ml/kg/day.

- Dipeptide carnosine was supplemented orally at a dose of 5 mg/kg body weight/day, dissolving in bidistilled water (Soliman *et al.*, 2001).

Animals: Seventy male albino rats (Rattus Norvegicus strain) of similar age and weight (120 g) were selected for this study. They were obtained from Animal House, Ophthalmic Institute, Giza, Egypt. Animals were kept in a controlled environment and were allowed free access of diet and water during the treatment period at Animal House, Medical Biochemistry, Faculty of Medicine, Cairo University. Biological determinations were carried out at Medicinal Chemistry Department, National Research Center, Dokki, Cairo, Egypt in August 2005.

Experimental design: Animals were divided into seven groups, each of ten animals. Group 1: Normal healthy control. Group 2: Ethanol intoxicated for 12 weeks. Group 3: Withdrawal group (ethanol ingested for 12 weeks then left abstinent 2 weeks free). Group 4: L-carnosine-ethanol treated (during as that of ethanol ingestion) for 12 weeks. Group 5: L-carnosine-ethanol treated (received prophylactic L-carnosine for two weeks before starting ethanol ingestion for 12 weeks). Group 6: Ethanol-carnosine treated (received L-carnosine for two weeks after 12 weeks of ethanol intoxication). Group 7: Healthy carnosine supplemented.

Preparation of tissue homogenates

- Liver tissue was homogenized in normal physiological saline solution (0.9N NaCl) by a ratio 1:9 w/v. The homogenate was centrifuged for 5 min at 3000×g at 4°C and the supernatant was used for estimation of succinate dehydrogenase, lactate dehydrogenase, glucose-6-phosphatase, acid phosphatase, 5' nucleotidase, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase and total protein content.
- Liver tissue was homogenized in 1:2 w/v of 100 mM maleate - NaOH buffer (pH 6.6) containing 20 mM NaF, 1 mM EDTA, 0.5 mg/ml bovine serum albumin and 10 mM DL-Dithiothreitol. The supernatant obtained after centrifugation at 3000×g at 4°C was used for estimation of glycogen phosphorylase.
- Liver tissue was homogenized in (0.01M) tris-glycine buffer pH (8.3) by a ratio 1:9 w/v. The supernatant obtained after centrifugation at 3000×g at 4°C for 5 min was used for the determination of LDH-isoenzymes, where 100 µg protein was applied to each gel.
- One gram of liver tissue was boiled in 5 ml 30% KOH for estimation of glycogen.

Parameter assays: Enzyme activities were evaluated using end point assay method. Succinate dehydrogenase: reduction of FAD is coupled with a reduction of tetrazolium salt as INT, the produced formazan of INT is measured colorimetrically at 490 nm (Shelton and Rice, 1957). Lactate dehydrogenase: the reduction of NAD coupled with the reduction of tetrazolium salt with PMS serving as an intermediate electron carrier, the resulted formazan of INT was measured colorimetrically at 503 nm (Babson and Babson, 1973). Lactate dehydrogenase isoenzymes (Dietz and Lubrano, 1967). The four enzymes, G-6-Pase, Glycogen phosphorylase, acid phosphatase and 5'-nucleotidase were measured colorimetrically at 660 nm (Swanson, 1955; Hedrick and Fischer, 1965; Wattiaux and De Duve, 1956; Bodansiky and Schwartz, 1963, respectively). Alkaline phosphatase ALP as a liberated phenol in the presence of amino-4-antipyrine and sod-arsenate as a blocking agent and potassium ferricyanide as a color reagent. The developed color measured at 510 nm (Kind and King, 1954). AST and ALT through measuring oxaloacetate and pyruvate produced respectively (Reitman and Frankel, 1957). Total protein (Bradford, 1976); glycogen (Nicholas *et al.*, 1956).

Statistical analysis: Data are expressed as mean±SD of ten rats in each group and statistically analyzed using analysis of variance (ANOVA) and least significant difference (LSD) post-hoc (SPSS Computer Program).

RESULTS

Table 1 showed that ethanol ingestion recorded significant decrease in activities of SDH, LDH, ALP, AST and ALT. Ethanol intoxication activated two enzymes; AP

and 5'-nucleotidase. The ethanol withdrawal (group 3), caused promotion changes in all these parameters to normal levels.

Carnosine-treatment for 12 weeks in conjunction with ethanol intoxication (group 4) presented lowered activities of all enzymes except two enzymes; AP and 5'-nucleotidase that were above the normal levels. Prophylactic 2 weeks-carnosine administration (group 5) presented promotion to normal level for SDH, while the others are decreased and 5'-nucleotidase activity was increased. The 2 weeks-carnosine administration after ethanol withdrawal (group 6) caused promotion changes in all these parameters to normal levels except 2 enzymes LDH and ALT, which remained low. The healthy carnosine (group 7) presented normal levels for all parameters.

Table 2 and Fig. 1 showed that liver LDH-isoenzymes in the control animals exhibited variation in its subunits, where LDH₅ was the most dominating showing the highest contribution value followed by LDH₄. Ethanol administration caused significant reduction value of LDH₅. In the withdrawal ethanol group, LDH₅ was still decreased, while LDH₄ was normalized. The 12 weeks-carnosine-ethanol groups recorded significant activation of LDH₅ compared to the ethanol group yet, it was still below the normal value. The 2 weeks-carnosine-ethanol groups, whether carnosine given before or after, presented increased activity of LDH₅ and decreased that of LDH₄. However, the healthy carnosine group showed much higher activity of LDH₅ and decreased those of LDH₁, LDH₂, LDH₃ and LDH₄ at the expense of LDH₅.

Table 3 showed significant reduction in protein concentration of ethanol ingested rats and L-carnosine-ethanol treated rats (during the same period as that of

Table 1: Effect of ethanol and carnosine on liver enzymes in rats

Enzymes	Control	Ethanol		Ethanol + Carnosine			Carnosine	Improvement %		
	Group (1)	Group (2)	Group (3)	Group (4)	Group (5)	Group (6)	Group (7)	a	b	c
Succinate dehydrogenase	0.51±0.03 (2,4)	0.37±0.03 (1,3,5,6,7)	0.50±0.01 (2,4)	0.39±0.03 (1,3,5,6,7)	0.47±0.03 (2,4)	0.48±0.03 (2,4)	0.49±0.03 (2,4)	3.92	19.60	21.56
Lactate dehydrogenase	83.88±1.65 (2,4,5,6)	66.74±0.62 (1,3,5,6,7)	81.77±3.79 (2,4,5)	69.72±1.65 (1,3,6,7)	73.48±5.33 (1,2,3,6,7)	78.99±4.97 (1,2,4,5)	80.64±5.97 (2,4,5)	3.55	8.03	14.60
Aspartate aminotransferase	2.45±0.22 (2,4,5)	1.38±0.09 (1,3,5,6,7)	2.31±0.14 (2,4,5)	1.60±0.05 (1,3,5,6,7)	1.90±0.43 (1,2,3,4,6,7)	2.21±0.24 (2,4,5)	2.30±0.20 (2,4,5)	8.97	21.22	33.8s
Alanine aminotransferase	1.80±0.11 (2,3,4,5,6)	1.27±0.11 (1,3,5,6,7)	1.59±0.22 (1,2)	1.45±0.07 (1,6,7)	1.61±0.18 (1,2)	1.65±0.19 (1,2,4)	1.64±0.08 (2,4)	10.0	18.88	21.11
Alkaline phosphatase	9.71±0.76 (2,4,5)	6.02±0.71 (1,3,4,5,6,7)	9.33±0.37 (2,4)	7.63±0.45 (1,2,3,6,7)	8.34±1.24 (1,2)	9.26±0.65 (2,4)	9.00±1.29 (2,4)	16.58	23.89	33.36
Acid phosphatase	0.42±0.02 (2,4,5)	0.47±0.03 (1,3,5,6)	0.42±0.01 (2,4,5)	0.46±0.02 (1,3,6)	0.45±0.02 (1,2,3,6)	0.43±0.02 (2,4,5)	0.44±0.06 (-)	2.38	4.76	9.52
5'-nucleotidase	0.63±0.03 (2,4,5)	0.70±0.03 (1,3,6,7)	0.64±0.01 (2,4)	0.69±0.02 (1,3)	0.68±0.01 (1)	0.65±0.02 (2)	0.65±0.06 (-)	1.58	3.17	7.93

Enzymes units: μ mol/min/mg protein, Data are means±SD. of ten rats in each group, Group 1: Normal healthy control, Group 2: 12 weeks ethanol, Group 3: 12 weeks ethanol+2 weeks withdrawal, Group 4: 12 weeks ethanol + carnosine, Group 5: 2 weeks carnosine +12 weeks ethanol, Group 6: 12 weeks ethanol + 2 weeks carnosine, Group 7: 12 weeks carnosine, a, b and c are improvement percentages of groups 4, 5 and 6, respectively, Significant level at p<0.0001 Analysis of data is carried out by one-way (ANOVA) (analysis of variance) accompanied by post-hoc (SPSS Computer Program)

Table 2: Effect of ethanol and carnosine on lactate dehydrogenase isoenzymes in liver of rats

Enzymes	Control	Ethanol		Ethanol + Carnosine			Carnosine	Improvement (%)		
	Group (1)	Group (2)	Group (3)	Group (4)	Group (5)	Group (6)	Group (7)	a	b	c
LDH ₁	1.15±0.10 (2,3,4,5)	2.54±0.08 (1,3,4,5,6,7)	3.12±0.37 (1,,2,4,5,6,7)	1.45±0.06 (1,2,,3,7)	1.40±0.09 (1,2,3,7)	1.30±0.05 (2,3,7)	1.01±0.01 (2,3,4,5,6)	94.78	99.13	107.82
LDH ₂	2.56±0.54 (2,3,7)	6.01±0.41 (1,4,6,7)	5.71±0.95 (1,4,5,6,7)	5.00±0.41 (1,2,3,5,6,7)	3.06±0.16 (2,3,4,6,7)	2.44±0.53 (2,3,4,5)	2.02±0.04 (2,3,4,5)	39.45	115.23	139.45
LDH ₃	6.39±0.99 (2,3,4,5,6,7)	13.17±0.80 (1,3,4,5,6,7)	8.62±0.50 (1,2,4,5,6,7)	9.64±1.04 (1,2,3,5,6,7)	4.85±0.40 (1,2,3,4,6,7)	3.48±0.50 (1,2,3,4,5)	3.02±0.39 (1,3,4,5,7)	55.24	130.20	151.64
LDH ₄	17.83±3.89 (2,5,6,7)	17.93±1.55 (5,6,7)	17.06±0.86 (6,7)	17.36±2.03 (5,6,7)	14.86±3.03 (1,2,4,7)	13.30±0.98 (1,2,3,4,7)	4.06±1.37 (1,2,3,4,5,6)	3.19	17.21	25.96
LDH ₅	72.34±2.79 (2,3,4,5,6,7)	60.35±2.23 (1,3,4,5,6,7)	62.22±1.16 (1,3,5,6,7)	65.97±3.24 (1,2,5,6,7)	75.71±2.93 (1,2,3,4,6,7)	79.75±1.42 (1,2,3,4,5,7)	89.77±1.64 (1,2,3,4,5,6)	7.76	21.23	26.81

Data are means±SD of ten rats in each group, Group 1: Normal healthy control, Group 2: 12 weeks ethanol, Group 3: 12 weeks ethanol +2 weeks withdrawal, Group 4: 12 weeks ethanol + carnosine, Group 5: 2 weeks carnosine +12 weeks ethanol, Group 6: 12 weeks ethanol + 2 weeks carnosine, Group 7: 12 weeks carnosine, a, b and c are improvement percentages of groups 4, 5 and 6, respectively, significant level at p<0.0001, Analysis of data is carried out by one-way (ANOVA) (analysis of variance) accompanied by post-hoc (SPSS Computer Program)

Table 3: Effect of ethanol and carnosine on liver glycogen, glycogen phosphorylase, glucose-6-phosphatase and total protein in rats

Enzymes	Control	Ethanol		Ethanol + Carnosine			Carnosine	Improvement (%)		
	Group (1)	Group (2)	Group (3)	Group (4)	Group (5)	Group (6)	Group (7)	a	b	c
Glycogen	3.33±0.31 (2,6,7)	2.61±0.21 (1,4,5,6,7)	2.85±0.27 (4,5,6,7)	3.65±0.27 (2,3,5,6,7)	4.15±0.25 (1,2,3,4)	4.33±1.32 (1,2,3,4)	4.81±0.39 (1,2,3,4,6)	31.23	46.24	51.56
Glycogen phosphorylase	0.83±0.10 (2,3,4,5,6,7)	0.55±0.06 (1,7)	0.48±0.04 (1,4,5,6)	0.64±0.04 (1,3,7)	0.66±0.05 (1,2,3,7)	0.68±0.09 (1,2,3,7)	0.47±0.06 (1,2,4,5,6)	12.04	13.25	15.62
Glucose-6-phosphatase	18.11±0.87 (2,4)	13.45±1.42 (1,3,4,5,6,7)	18.03±0.57 (2,4)	15.11±0.69 (1,2,5,6,7)	17.08±0.93 (2,4)	17.76±1.26 (2,4)	17.38±1.88 (2,4)	9.16	20.04	23.89
Total protein	106.84±11.88 (2)	87.32±11.22 (1,3,5,6,7)	103.33±7.31 (2)	95.54±10.87 (7)	103.03±12.11 (2)	103.65±10.65 (2)	109.54±5.41 (2,4)	7.693	14.70	15.28

Enzyme unit: μ mol/min/mg protein. Glycogen content: mg/g tissue, Data are means±SD of ten rats in each group, Group 1: Normal healthy control, Group 2: 12 weeks ethanol, Group 3: 12 weeks ethanol +2 weeks withdrawal, Group 4: 12 weeks ethanol + carnosine, Group 5: 2 weeks carnosine +12 weeks ethanol, Group 6: 12 weeks ethanol + 2 weeks carnosine, Group 7: 12 weeks carnosine, a, b and c are improvement percentages of groups 4, 5 and 6, respectively, Significant level at p<0.0001, Analysis of data is carried out by one-way (ANOVA) (analysis of variance) accompanied by post-hoc (SPSS Computer Program)

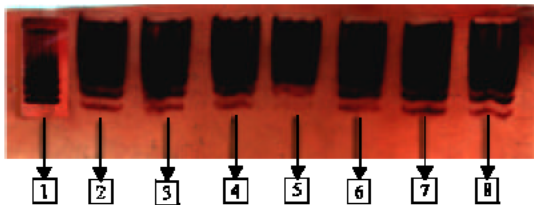


Fig. 1: Electrophoretic separation pattern of LDH isoenzymes in liver of rats, 1; Standard (heart), 2; Normal healthy control, 3; 12 weeks ethanol, 4; 12 weeks ethanol +2 weeks withdrawal, 5; 12 weeks ethanol + carnosine, 6; 2 weeks carnosine +12 weeks ethanol, 7; 12 weeks ethanol + 2 weeks carnosine, 8; 12 weeks carnosine

ethanol ingestion), while it recorded high levels in groups received carnosine either before or after ethanol intoxication as well as ethanol withdrawal group. Glycogen phosphorylase recorded variable decreased activities of in all tested groups. G-6-Pase activity was inhibited in two groups: ethanol-intoxicated and intoxicated-12 weeks-carnosine animals. Liver glycogen concentrations were low in the two ethanol groups

(intoxicated and intoxicated-2 weeks withdrawal) while it presented high levels in all groups receiving carnosine.

The % of improvement in the three carnosine-treated-intoxicated groups: 12 weeks, 2 weeks prophylactic and 2 weeks after withdrawal from the intoxicated animals presented unidirectional gradual step-wise rise in a successive manner (Table 1-3).

DISCUSSION

The present chronic ethanol toxicity study recorded significant decrease in hepatic SDH, LDH, ALP, AST, ALT (Table 1), glycogen phosphorylase and G-6-Pase (Table 3) activities. These decreased activities were in accordance with previous researches (Devaki *et al.*, 1992; Cahill *et al.*, 1997; Addolorato *et al.*, 2001; Ozaras *et al.*, 2003). These lowered enzyme activities could principally be explained by the toxic actions of ethanol on proteins and on hepatocytes cellular organelles in general (Iles and Nagy, 1995). Ethanol itself forms a toxic environment favorable to oxidative stress such as hypoxia, endotoxaemia and cytokines release (Bautisa and Spitzer, 1999). Ethanol also interpolating the biomembranes and expanding them increases membrane fluidity and enzyme

release (Strubelt *et al.*, 1999). The toxic metabolic effects of ethanol oxidation are mainly due to increased liberation of ROS, production of deleterious active acetaldehyde, increased NADH/NAD ratio and disturbed intracellular calcium stores (Gasbarrini *et al.*, 1996; Lieber, 2000).

These toxic agents result from ethanol oxidation by alcohol and aldehyde dehydrogenases (Mantle and Preedy, 1999) and three ethanol-inducible enzymes. These include cytochrome P450 isoenzyme, CYP2E1 (Zima *et al.*, 2001), which plays the major role in toxicity and other P450 isoenzymes that share in liberating ROS although they are not involved in ethanol oxidation (Niemela, 2001). The third inducible enzyme, xanthin oxidase is responsible for acetaldehyde metabolism and leads to more liberation of ROS, namely superoxide free radicals (Zima *et al.*, 2001). Elevation of free cytosolic calcium by ethanol was previously proved to additionally activate xanthin oxidoreductase to xanthine oxidase direction (McCord, 1985).

Thus increased hepatocyte liberation of ROS causes increased lipid peroxidation and oxidative inactivation of both membranous and soluble proteins (Mantle and Preedy, 1999). The active acetaldehyde as well, could further inactivate both types of cellular proteins by forming protein-adducts (French, 2001; Hipkiss *et al.*, 2002). Additionally, oxidative reactions of ethanol and its product acetaldehyde increase NADH/NAD ratio causing shift to the left in the equilibrium of a number of oxidoreductive couples e.g., lactate-pyruvate and malate-oxaloacetate that resulted in hyperlactacidaemia and depression of citric acid cycle respectively (Volpi *et al.*, 1997). The structural and functional integrity of hepatic organelles, in general (Van Noorden and Frederiks, 1992) and mitochondrial function in particular (Marcinkeviciute *et al.*, 2000) would suffer. The increased membranous lipid peroxidation (Cahill *et al.*, 1997 and Lieber, 1997) would allow redistribution of intracellular calcium (Gasbarrini *et al.*, 1996) leading to leakage of calcium from mitochondrial stores and increasing its cytosolic level (Farber, 1981; Gasbarrini *et al.*, 1996). These facts were in accordance with Addolorato *et al.* (2001) stating that ethanol ingestion caused dose-dependant cell injury accompanied with decrease in ATP content and increase in free cytosolic calcium and leading to disturbed plasma membrane transport (Gasbarrini *et al.*, 1996). Membrane damage was partly attributed to disturbed cellular energy (Kristensen, 1990).

The reflection of these derangements on different subcellular fractions was investigated by many workers. Glycolysis key enzymes and LDH activities were reported to decrease (Addolorato *et al.*, 1998). Many metabolic mitochondrial insults were as well recorded; citric acid

cycle pathways, NAD dehydrogenase, cytochrome b and cytochrome oxidase leading to decreased availability of ATP (Cahill *et al.*, 1997). Mitochondrial DNA is also affected by deletions and DNA-protein cross links (Cahill *et al.*, 1997). Nuclear DNA showed increased frequency of single band break (Navasumrit *et al.*, 2000) that supported a previous report stating that decreased protein synthesis was one of the factors in lowered enzyme activities (De Feo *et al.*, 1995). That chronic ethanol intoxication has an inhibitory effect on DNA replication and RNA transcription was previously proved (Devaki *et al.*, 1992). All these factors more or less participate in the lowered activities of the above mentioned enzymes. Individual causative mechanisms were specifically proved for each of these enzymes.

Thus the decreased SDH activity was specifically explained by the disturbed structural and functional integrity of hepatic organelles. SDH enzyme being closely linked to the inner mitochondrial membrane would suffer (Van Noorden and Frederiks, 1992). In addition, the increased NADH/NAD ratio causing shift to the left in the equilibrium of the oxidoreductive couple malate-oxaloacetate resulted in depression of citric acid cycle fairly contribute to SDH lowered activity (Volpi *et al.*, 1997).

The specified factors leading to LDH inhibition in chronic alcoholism were the increased NADH/NAD ratio causing shift to the left in the equilibrium of the oxidoreductive couple lactate-pyruvate resulting in hyperlactacidaemia (Volpi *et al.*, 1997). The extracellular release of LDH (Mantle and Preedy, 1999) could lead to liver LDH lowering. This found support the statement that chronic alcoholism caused extracellular release of hepatic LDH raising its activity in plasma (Gasbarrini *et al.*, 1996; Yang *et al.*, 2005).

Regarding to LDH isoenzymes (Table 2), LDH₅ presenting the main dominating subunit in the control found support in the statement of Varley *et al.* (1980) that LDH₅ was, normally, the dominant one in rat and Man. That LDH₅ contribution was decreased in the ethanol intoxicated group also matched the finding that LDH₅ was increased in serum, in case of liver disease, even the serum total LDH activity remained within normal limits (Varley *et al.*, 1980). The relative increased contribution of LDH₁, LDH₂ and LDH₃ should be considered a false rise being at the expense of the decreased LDH₅, although in some other mammalian species, e.g., cattle, they could be primarily increased in liver stressful conditions (Maly and Toranelli, 1993).

For both the decreased levels of AST, ALT and ALP two factors were more specified, extracellular release and decreased protein synthesis. The extracellular release for the transaminases lead to their increase in serum (Ozcan

and Mengil, 1998; Huang *et al.*, 2005). As regards ALP, the extracellular discharge was also recorded (Ishii *et al.*, 1997; Yue *et al.*, 2006). Decreased protein synthesis as a cause of lowered activities of the two transaminases had long been noticed (Waldrof and Harper, 1963). The authors stated that AST and ALT activities were decreased even relative to the lowered liver protein. ALT and AST release to the blood stream was recently reported in addition to decreased synthesis (Ozaras *et al.*, 2003).

The present decrease in liver glycogen, glycogen phosphorylase and G-6-Pase was in accordance with the recorded decreased liver glycogen, phosphorylase activity (Van Horn *et al.*, 2001). Reduced gluconeogenesis (Brecher and Lehti, 1996) might be an additional factor causing decreased glycogen. Although Winston and Reitz (1980) recorded increased absolute values of phosphorylase, yet they proved more relative increase in the inactive b form than its active a form that could support the present reduced activity. However, the present lowered phosphorylase activity might point more to decreased glycogen synthesis as a cause of decreased glycogen content. The decrease in G-6-Pase enzyme activity was also supported by an inhibitory action of liberated acetaldehyde, the inhibition of the G-6-Pase transport protein (translocase, T₁) and elevated cytosolic calcium structurally affecting the microsomes (Brecher and Lehti 1996; Scott and Burchell, 1991; Exton, 1982, respectively).

The increased activity of the hydrolytic enzyme acid phosphatase in the ethanol group was attributed to the damage in lysosomal membrane (Mc Cord, 1985). This was in agreement with Salah *et al.* (1976) who noticed that all lysosomal enzymes are activated in conditions characterized by increased tissue catabolism. The increase of cytosolic calcium could contribute to more breakdown and damage of lysosomal and other cellular membranous structures (Farber, 1981).

The increased 5'-nucleotidase enzyme activity in the chronic alcohol group found support in a previous record that increased nucleic acid catabolism in chronic alcohol toxicity lead to its elevation as the enzyme is related to the break down of nucleic acid, especially messenger RNA (Deaciuc *et al.*, 1992). In spite of the records that 5'-nucleotidase in canalicular membranes was not affected by ethanol feeding (Iles and Nagy, 1995) and that stating a significant inhibition of 5'-nucleotidase in liver plasma membrane after ethanol toxicity (Srivastava, 1990), yet one can state that the enzyme in these sites might have been affected by extracellular discharge through the damaged cell membranes.

Concerning protein concentration, Doyle *et al.* (1994) supported the present decreased result. They proved it to inhibition of hepatic protein secretion by ethanol that was associated with accelerated catabolism of unsecreted plasma proteins suggesting hepatocellular degradative processes that are usually responsive to the change in the levels of prosecretory protein and/or perturbation of the secretory process. The same harmful lowering effect of ethanol ingestion was proved to be reflected on other liver parameters; on urea (Donohue *et al.*, 1991 and Yoshiro *et al.*, 1991a, b), on protein and albumin/globulin ratio (Devaki *et al.*, 1992). Wickramasinghe and Hasan (1994) also found reduction in serum albumin in alcohol toxicity. The lowering of serum albumin level had also been supported by Troitskii *et al.* (1991) demonstrating the modification of rat serum albumin that was attributed to switch to α -fetoprotein during chronic and acute alcohol toxicity. Donohue *et al.* (1991) reported that there was 50% degradation in total plasma protein and 46% increase in albumin catabolism where the reduction was more severe with malnutrition. In addition, Dinarello (2000) stated that the acute phase response caused decreased hepatic albumin synthesis.

Carnosine administration either with, before or after ethanol ingestion showed amelioration in all the measured parameters according to the way and the period of administration. However administration of carnosine after intoxication with ethanol recorded the more potent effect. This amelioration might be due to the proved universal buffer action of carnosine combating many stressful conditions. The dipeptide scavenged ROS and protected protein modification mediated by peroxy radicals generated in the lipid peroxidation (Kang *et al.*, 2002b). Carnosine administration also stimulated glycolysis during both anoxia and hyperthermic stress (Churchill *et al.*, 1995; Deev *et al.*, 1997, respectively). Moreover, carnosine normalized adenylate energy charge and increased hepatocyte glycogen (Soliman *et al.*, 2001; 2002) and blood glucose level in stressed animals (Yamano *et al.*, 2001). Le Blanc and Soucy (1994) recorded significant increase in serum insulin concentration after carnosine administration, a fact that could promote glucose utilization and glycogen storage. In addition, carnosine improving the present tested liver parameters may be comparable to carnosine protective effect on the parenchymatous gastric glands in chronic alcoholism (Cho *et al.*, 1992).

The stimulation of protein synthesis in healthy carnosine group may be related to a protein promoting action of carnosine. Hipkiss and Chana (1998) proved the ability of carnosine to protect the already formed proteins against inactivation. Kang *et al.* (2002a, b) suggested that

carnosine acts as a peroxy radical scavenger to protect protein modification. Carnosine also plays a role in the disposal of glycated protein in the affected tissue and decreases protein denaturation (Yeorgans and Seidler, 2003).

In conclusion, carnosine treatment successfully ameliorated the toxic effect of ethanol on the glycolytic, citric acid, glycogenolytic as well as the enzymes responsible for amino acid, hydrolytic enzymes and nucleic acid catabolic enzyme by variable degrees according to the way and the period of administration.

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