

Full Length Research Paper

Hepatoprotective effects of glycine and vitamin E, during the early phase of liver regeneration in the rat

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Our objective was to demonstrate the protective effect of glycine (Gly) and vitamin E (VE) on a model of ethanol-induced acute liver injury during the early phase of liver regeneration after partial hepatectomy (PH) in rats. Fifty male Wistar rats (body weight (b.w.), 240 - 280 g) were divided into four groups (n = 10, each, respectively) as follows: 1) control partial hepatectomy (PH), 70%; 2) PH + ethanol (EtOH) at 1.5 g/kg b.w.; 3) PH + Gly (0.6 g/kg b.w) + EtOH, and; 4) PH + VE (400 International units [IU]) + EtOH. Twenty four h after surgery, animals were killed and liver damage and oxidative stress parameters were measured. Ethanol caused a decrease in serum albumin (2.27 vs 3.12 g/dL; p < 0.05), cholesterol (31.4 vs 48.0 mg/dL; p < 0.05), Aspartate aminotransferase (AST, 70 vs 380 UI; p < 0.05), and alanine aminotransferase (ALT, 110 vs 170 UI; p < 0.05) in comparison with the PH control group, but these decreases were reverted with either Gly or VE administration. Furthermore, Gly and VE administration decreased (p < 0.05) Thiobarbituric acid reactive (TBARS) levels, stimulated superoxide dismutase (SOD) activity, and a significant restitution of liver weight was observed. Our results suggested a protective effect against liver injury with glycine and VE supplementation. Treatment with either Gly or VE causes an elevation in total SOD activity and a decrease in TBARS levels, showing a protective effect in liver regeneration on a model of ethanol-induced acute liver injury after PH in rats.

Key words: Antioxidants, ethanol-induced liver injury, free radicals, glycine, liver regeneration, vitamin E.

INTRODUCTION

The liver is an organ with regenerative capacity (Khan and Mudan, 2007). Hepatocytes exhibit good regenerative response to several stimuli, including massive destruction of hepatic tissue by toxins, viral agents, or surgical extraction (Rabelo et al., 2006). On the other hand, regenerative response inhibition after partial hepatectomy (PH) has been described when a single dose of ethanol is applied to the regenerative subject (Morales-González et al., 2004).

Experimental evidence indicates that ethanol-induced

liver- regeneration inhibition is caused by reactive oxygen species (ROS) increase during ethanol metabolism (Koch et al., 2004; Nishitani and Matsumoto, 2006). It has been suggested that ROS-production increase affects the early-phase of hepatic regeneration (HR); thus, ROS increase plus deficiencies of dietetic antioxidants such as vitamin E (VE) could be an important risk factor in ethanol-associated hepatic disease (Koch et al., 2004; Nishitani and Matsumoto, 2006). On the other hand, it is known that a hypermetabolic state in the liver is generating during ethanol consumption. An increase in reduced Nicotinamide adenine dinucleotide (NADH) production by cytosolic enzyme alcohol dehydrogenase action-related ethanol oxidation exerts stimuli on the mitochondrial respiratory pathway, the main ROS source (Adachi and Ishii, 2000).

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Moreover, the microsomal ethanol oxidation system (MEOS) carries out redox reactions that produce ROS, such as the superoxide radical ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2), and the hydroxyl radical ($\cdot OH$) (Nanji and French, 2003; Masalkar and Abhang, 2005). Previous reports have demonstrated the beneficial effects of glycine (Gly) in the prevention of several forms of liver damage, such as necrosis and inflammation caused in initial phases of chronic ethanol ingestion (Yin et al., 1998; Ishizaki et al., 2004). A protective role has been suggested for Gly action against anoxic and oxidative stress produced by toxic agents such as ethanol (Qu et al., 2002). On the other hand, VE has two important functions in the membrane: as a liposoluble antioxidant that prevents ROS damage in polyunsaturated fatty acids, and also as a membrane stabilizer agent acting against damage caused to phospholipids (Bradford et al., 2003). VE acts by means of breaking the antioxidant chain that prevents ROS-produced cell membrane damage (Brigelius and Traber, 1999). Recent studies have demonstrated that VE can directly reduce ROS production by interfering in the union between the membrane and the NADPH oxidase complex (Factor et al., 2000). Recently, we reported that short-term (7-day) antioxidant supplementation attenuates lipid peroxidation and protects against liver injury and dysfunction in an ethanol intoxication model during partial hepatectomy (PH)-induced liver regeneration (Ramírez-Farías et al., 2008). In this study, we investigated the effects of VE and glycine (Gly) in rats with acute ethanol administration-induced liver injury in early liver regeneration. We sought to evaluate whether antioxidant supplementation can attenuate ethanol-induced hepatic injury and dysfunction during liver regeneration.

MATERIALS AND METHODS

Animals and chemicals

Male Wistar rats (body weight [b.w.], 240 - 280 g; n = 50) were obtained from Harlam-Laboratory (Harlam de México, S. A. de C.V.). Animals were housed individually in plastic boxes at the Instituto de Ciencias de la Salud Bioterium of the Universidad Autónoma del Estado de Hidalgo (UAEH). These were maintained at 22°C with a 12 h light/dark cycle and allowed to consume standard rat pellet chow (Rodent Laboratory, México, S.A. de C.V.) and water *ad libitum* prior to treatments. After 10 days of adaptation, the experiment was initiated. All procedures involving experimental animals were performed according to Federal Regulations for Animal Experimentation and Care (Ministry of Agriculture; SAGAR, Mexico). Gly, VE, and thiobarbituric acid were obtained from Sigma Chemical Co., St. Louis, MO, USA, and all other chemicals were of the best quality available.

Surgical procedures

Two thirds partial hepatectomy (PH) was performed according to the technique of Higgins and Anderson (1931) and Ramírez-Farías et al. (2008). Surgeries were performed between 08:00 and 10:00 h under light diethyl ether anesthesia and consisted of removal of the

median and left lateral lobes of liver. As controls, sham-operated rats were subjected to the same surgical procedure without remission of liver mass.

Experimental designs

After surgery, animals were grouped (10 subjects per group) according to treatment as follows: 1) PH rats received an intra-gastric saline solution (PH control group); 2) PH treated rats with intra-gastric doses of ethanol (1.5 g/kg bw; PH-EtOH group); 3) PH-ethanol treated rats with intra-gastric doses of glycine (0.6 g/kg b.w., PH-EtOH-Gly group), and 4) PH-ethanol treated rats with intra-peritoneal doses of vitamin E (400 International Units [IU], PH-EtOH-VE group). Sham-operated rats receiving intra-gastric administration of saline solution (0.9% NaCl) were used as controls. All the treatments (ethanol solution, glycine and vitamin E) were given only doses after surgery (Morales-González et al., 1998, 1999, 2006; Senthikumar et al., 2004; Senthikumar and Nalini, 2004).

Post treatment, animals were killed by decapitation after 24 h as previously described using anesthesia with sodium pentobarbital (40 mg/kg b.w.) (Ramírez-Farías et al., 2008).

Serum and liver samples

After sacrifice, blood samples were taken and serum was isolated by centrifugation at 1000 rpm according to the technique of Ramírez-Farías et al. (2008) and maintaining these frozen at -70°C until use.

Liver was isolated, weighed, and placed immediately in an ice-cold buffer (0.25 M sucrose, 10 mM TRIS, and 0.3 mM EDTA; pH 7.4) and was washed until bloodless. Liver was homogenized using 8 - 10 strokes of a Teflon homogenizer. Homogenate was centrifuged for 15 min at 1500 rpm. Total supernatant was aliquoted and frozen at -70°C until its later use as total liver homogenate sample.

Enzymes and metabolite assay from serum

Alanine aminotransferase (ALT; EC 2.6.1.2) and aspartate aminotransferase (AST; EC 2.6.1.1) activities were measured in serum samples as liver-injury indicators. ALT and AST were measured colorimetrically utilizing a diagnostic kit from Spinreact (Spinreact de México, S.A de C.V.) following the manufacturer's instructions and reported in units/L.

Principle of the method: Aspartate aminotransferase (AST) formerly called glutamate oxaloacetate (GOT) catalyses the reversible transfer of an amino group from aspartate to α -ketoglutarate forming glutamate and oxalacetate. The oxalacetate produced is reduced to malate by malate dehydrogenase (MDH) and NADH. The rate of decrease in concentration of NADH, measured photometrically, is proportional to the catalytic concentration of AST present in the sample. Alanine aminotransferase (ALT) o glutamate pyruvate transaminase (GPT) catalyses the reversible transfer of an amino group from alanine to α -ketoglutarate forming glutamate and pyruvate. The pyruvate produced is reduced to lactate by lactate dehydrogenase (LDH) and NADH. The rate of decrease in concentration of NADH, measured photometrically, is proportional to the catalytic concentration of ALT present in the sample.

Serum samples were employed to determine albumin, total bilirubin, and cholesterol levels. All three of the latter metabolites were determined by spectrophotometric techniques using commercial kits (Spinreact de México, S.A. de C.V.) following manufacturer instructions. Albumin is reported as g/dL, and both total bilirubin and cholesterol are reported as mg/dL.

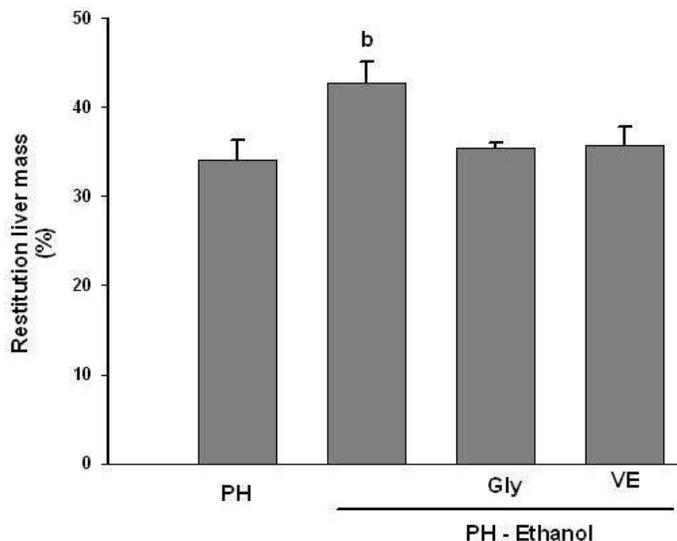


Figure 1. Effect of glycine and vitamin E on the percentage of liver-weight restitution of in rats from all ethanol-treated groups treated after partial hepatectomy. Values are means \pm standard error of mean (SE) ($n = 10$). Restitution liver mass was expressed as %. ^b $P < 0.05$ vs. the hepatectomized group (PH). PH: partial hepatectomy; Gly: glycine; VE: vitamin E.

Thiobarbituric acid reactive substance assay

Thiobarbituric acid reactive substances (TBARS) were determined in total liver homogenate according to the method reported by Song et al. (2003) and slightly modified by Ramírez-Farías et al. (2008). Briefly, 0.2 mL of liver sample was mixed with 0.2 mL of sodium dodecyl sulfate (8.1% w/v), 1.5 mL of acetic acid (5% v/v), 1.5 mL of thiobarbituric acid (0.8% w/v), and 0.6 mL of distilled water. The reaction mixture was placed in a water bath at 90°C for 60 min. After cooling, 1 mL of distilled water and 3 mL of butanol/pyridine mixture (1:10, v/v), were added and vortexed. After centrifugation at 3,000 rpm for 10 min, the resulting upper phase was determined at 532 nm. TBARS concentration was calculated using 1,1,5,3-tetraethoxypropane as standard and reported in nmol/mg (Ramírez-Farías et al., 2008; Song et al., 2003).

Superoxide dismutase assay

Superoxide dismutase (SOD) (EC 1.15.1.1) was assayed in total homogenate liver samples according to Misra and Fridovich (1972). Briefly, liver homogenate was incubated at 37°C placed in 10 volumes of ice-cold solution Tris-HCl buffer (150 mM Tris-HCl; pH 8.0) with horse Cytochrome C (0.5 mM) and NAD⁺ (10 mM) was added to start the reaction, followed by 3 min in spectrophotometer (UV/vis spectrophotometer Shimadzu 1240)- SOD activity was expressed as U/mg protein. Total protein concentration in all samples was determined according to the Lowry method employing bovine serum albumin (BSA) as standard (Lowry et al., 1951).

Liver-weight restitution

Hepatic regeneration was determined by calculation of liver-weight restitution. The extent to which the liver mass had been reconstituted was also evaluated. Resected livers were weighed at time of PH. This weight was divided by 0.7 to derive initial (pre-PH)

Table 1. Albumin, bilirubin and cholesterol levels on serum from rats after of partial hepatectomy (PH) and a single administration of ethanol and application of vitamin E or Glycine.

Group	Albumin (g/dL)	Bilirubin (mg/dL)	Cholesterol (mg/dL)
Sham	3.46 \pm 0.2	0.29 \pm 0.2	51.8 \pm 4.3
PH	3.12 \pm 0.5	0.28 \pm 0.1	48.0 \pm 6.3
PH-EtOH	2.27 \pm 0.11 ^a	0.19 \pm 0.09	31.4 \pm 2.1 ^a
PH-EtOH-Gly	3.56 \pm 0.11 ^c	0.08 \pm 0.04	51.1 \pm 3.7 ^c
PH-EtOH-VE	3.39 \pm 0.04 ^c	0.08 \pm 0 ^c	59.1 \pm 8.1 ^c

Values are the mean \pm SE for each experimental group ($n = 10$) as it corresponds. PH: partial hepatectomy; Gly: glycine; VE: vitamin E; Sham: controls group. ^a $P < 0.05$ vs the sham group; ^c $P < 0.05$ vs hepatectomized + ethanol group

weight of each liver. Each post-PH liver remnant was also weighed at time of death. For each rat, weight of remnant liver was normalized to the weight of this rat's entire liver at time of PH and expressed as percentage of rat's initial liver weight according to the following formula: (wt of liver remnant/initial liver wt) \times 100 (Orrego et al., 1981). Data from all PH rats per treatment group were used to calculate mean \pm Standard error of mean (SE) at end of experimental time.

Statistics

All data are expressed as means \pm SE. Statistical analysis was performed utilizing student t-test and ANOVA where appropriate. Differences between groups were considered statistically significant at $p < 0.05$.

RESULTS

Ethanol and antioxidant effect on liver regeneration

Figure 1 shows the results obtained for liver-mass restitution as a liver-regeneration indicator. Liver-mass restitution was increased in the PH-EtOH compared with PH group at 24 h (34.05 vs. 42.74%; $p < 0.05$). The effects of a single Gly or VE dose on liver-mass restitution during liver regeneration are shown in Figure 1. Both Gly and VE regulated liver-mass restitution was altered by ethanol consumption.

Effects of antioxidant treatment on serum metabolite levels

Table 1 shows serum-level changes of albumin, bilirubin, and cholesterol, which reflect liver function integrity. Animals subjected to PH without ethanol treatment do not present modifications in these metabolites in comparison with sham group. However, when ethanol was administered to PH rats, these presented a decrease in serum albumin (2.27 vs. 3.46 g/dL; $p < 0.05$) and cholesterol (31.4 vs. 51.8 mg/dL; $p < 0.05$) levels in comparison with sham group. When Gly was administered to PH-EtOH

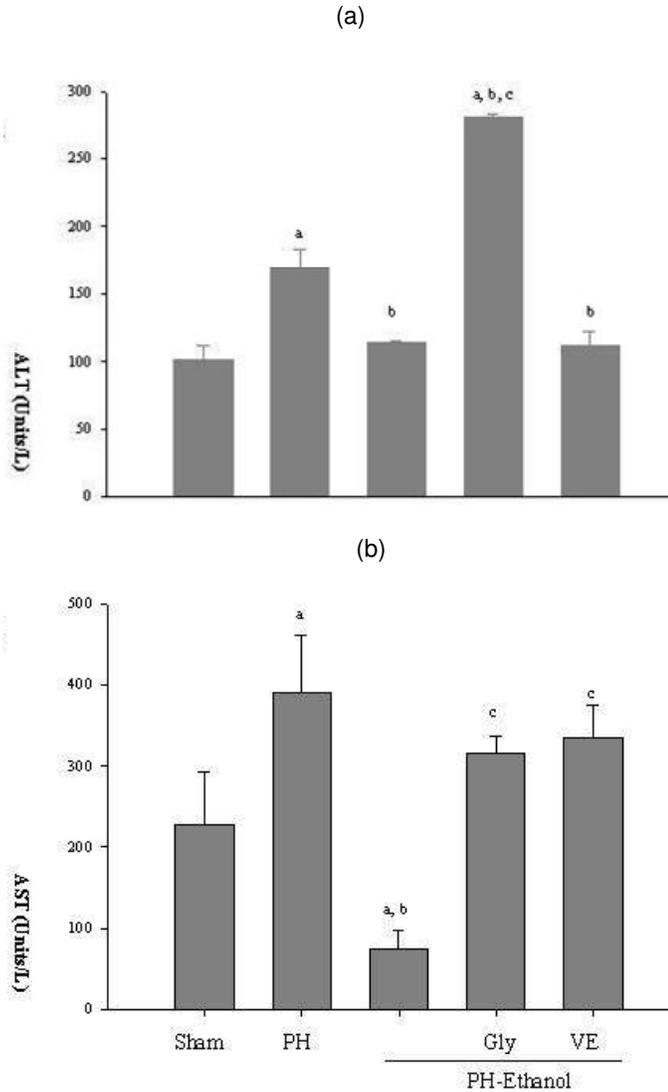


Figure 2. Effect of glycine and vitamin E on specific activities of serum alanine aminotransferase (ALT, panel A) and aspartate aminotransferase (AST, panel B) during liver regeneration and ethanol intoxication. Values are means ± Standard error of mean (SE) (n = 10). The activity is reported in Units/L. ^aP < 0.05 vs. the sham group (sham); ^bP < 0.05 vs. the hepatectomized group (PH); ^cP < 0.05 vs. hepatectomized + ethanol group (PH-EtOH). PH: Partial hepatectomy; Gly: Glycine; VE: Vitamin E.

group, serum albumin (3.56 vs. 3.46 g/dL) and cholesterol (51.1 vs. 51.8 mg/dL) content was scarcely normalized in comparison with sham group. A similar situation was found in the VE-treated group (Table 1). Bilirubin serum levels did not change significantly in experimental groups.

Effects of antioxidant treatment on serum enzyme activity

The effect of Gly and VE supplementation combined with

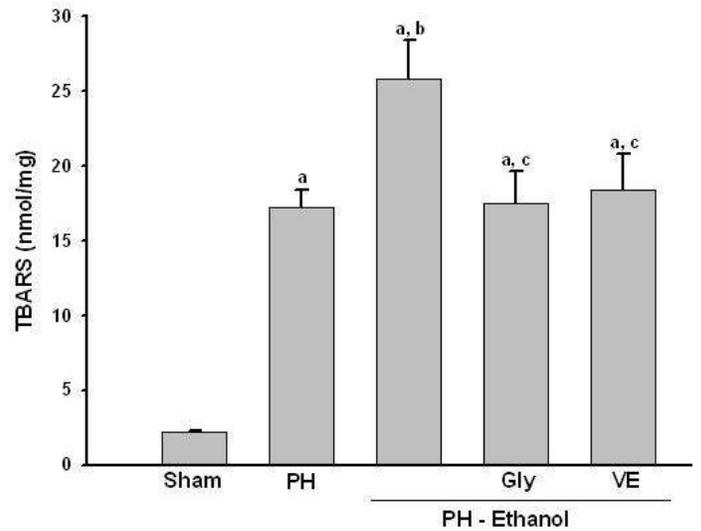


Figure 3. Effect of glycine and vitamin E on hepatic lipid peroxidation (determined as Thiobarbituric Acid Reactive Substances (TBARS) content) in all ethanol-treated groups during liver regeneration. Values are means ± Standard error of means (SE) (n = 10). TBARS content is reported in nmol/mg. ^aP < 0.05 vs. the sham group (sham); ^bP < 0.05 vs. the hepatectomized group (PH); ^cP < 0.05 vs. the hepatectomized + ethanol group (PH-EtOH). PH: Partial hepatectomy; Gly: Glycine; VE: Vitamin E.

ethanol on the enzymes' ALT and AST activity is indicated in Figure 2. Enzyme activity increased significantly post-surgery (1.5 and 1.7 times that of sham group, respectively). On the other hand, animals subjected to PH and receiving ethanol elicited unexpected ALT- and AST-lowering activities (32 and 80%, as compared with PH group, respectively). In the case of Gly treatment, two peaks of increased ALT activity were found at 24 h as compared with PH and sham groups. On the other hand, AST activity in Gly and VE treatment was hardly normalized in comparison with PH group (Figure 2).

Effect of antioxidants on thiobarbituric acid reactive substances (TBARS)-induced by ethanol treatment in PH rats

TBARS were evaluated in the homogenized of all experimental groups to assess ROS-produced damage (Figure 3). PH group exerts a significant increase on TBARS concentration compared with sham group (17.2 vs. 2.19 nmol/mg; p < 0.05).

Nonetheless, animals submitted to PH and that received ethanol exhibited a significant increase in TBARS concentrations (25.8 vs. 2.19 nmol/mg, and 25.8 vs. 17.2 nmol/mg; p < 0.05) in comparison with sham and PH groups, respectively. A different result was observed in Gly-treated groups (17.5 nmol/mg), and VE (18.4 nmol/mg), in which both antioxidants normalized this

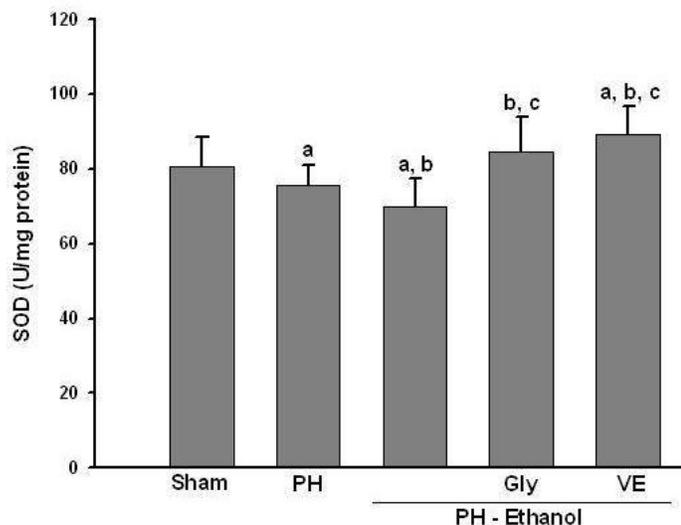


Figure 4. Effect of glycine and vitamin E on (SOD) activity in liver from all ethanol-treated groups during liver regeneration. Values are means \pm Standard error of mean (SE) ($n = 10$). SOD activity was expressed as U/mg protein. ^aP < 0.05 vs. the sham group (sham); ^bP < 0.05 vs. the hepatectomized group (PH); ^cP < 0.05 vs. hepatectomized + ethanol group (PH-EtOH). PH: Partial hepatectomy; Gly: Glycine; VE: Vitamin E.

parameter (Figure 3).

Effect of antioxidant treatment on hepatic SOD levels

Figure 4 demonstrates SOD hepatic activity in all experimental groups. Initially, both PH and PH-EtOH groups showed a SOD-activity decrease in comparison with sham group. The decrease in PH group was statistically more significant (13.5%) in comparison with PH group (5.5%).

This ethanol-induced SOD-activity decrease was greatly modified by Gly and VE administrations: Gly administration significantly increased SOD activity compared with ethanol-treatment group (84.58 vs. 69.73 U/mg; $p < 0.05$), and VE administration increased SOD activity (89.22 vs. 69.73 U/mg; $p < 0.05$) compared with ethanol-treatment group.

DISCUSSION

Liver regeneration is the organized and controlled response of the liver toward tissue damage induced by trauma, infections, toxic agents, or post-surgery resection. These stimuli originate sequential changes on gene expression and molecular structure; thus, morphological, biochemical, and histological changes are matters of study. Some research studies have demonstrated that both acute and chronic ethanol administration inhibit DNA synthesis and produce certain physiological alterations

that are reflected in the organism, such as altered serum metabolites or modifications in enzymes activity, which reflect liver integrity (Morales-González et al., 1999, 2001; Michalopoulos and DeFrances 1997).

Surprisingly, 24 h after surgery, liver-mass restitution in PH-EtOH group presented an increase in this parameter compared with PH group (Figure 1), but weight gain does not necessarily indicate that active proliferation is taking place in this group. Contrariwise, regenerative-liver weight gain could be explained by the inflammatory process and by lipid and fat accumulation produced by ethanol consumption (Morales-González et al., 1999; Orrego et al., 19981). This is supported by results found in albumin and cholesterol levels, in which can observe that PH-EtOH group has a significant decrease (Table 1) on serum albumin concentration compared with sham group. This can probably be explained by deficiency in ethanol administration-associated liver function ethanol administration due to that albumin-concentration decrease is directly related with the decrease in normal ATP levels generated in liver (Jikko et al., 1984).

On the other hand, Orrego et al. (1981) and Morales-González et al. (1999) reported that ethanol consumption increased lipid deposits in liver and decreased protein and DNA synthesis. An inflammatory process in regenerative liver takes place, producing a weight increase in the regenerative liver not produced by an increase in protein or DNA. Our results show that ethanol increases fat deposits in liver and decreases protein synthesis; thus, this can be associated with the decrease in serum cholesterol present in this group in comparison with sham group (Table 1), suggesting a reduction in cholesterol transport through the organism because of the decrease in the proteins required for this (Orrego et al., 1981).

The previous study and this one agrees with the suggestions of other authors to consider the PH as the good *in vivo* model most adequate to study cellular proliferation because it is a relatively simple technique and with the advantage that after surgery, only a short time is required to evaluate hepatic regeneration and physiological status (Higgins and Anderson, 1931; Morales-González et al., 1999; Ramírez-Farías et al. 2008). Data obtained in this research suggest a protective effect with both Gly and VE supplementation during cellular proliferation after ethanol administration. These groups presented a significant increase in serum albumin and cholesterol in comparison with PH-EtOH group, and were similar to sham group (Table 1) and exhibited a similar weight gain as PH group (Figure 1). This confirms the protective effect of this Gly and VE over the free radicals generated by the ethanol, as well as its efficiency in the cellular regeneration of the liver and its physiological status (Morales-González et al., 2006; Ramírez-Farías et al., 2008).

However, both bilirubin and the aminotransferase enzymes (ALT and AST) are parameters found in screening tests for liver-disease diagnosis; both ALT and AST are liberated into the blood stream in high concentrations in

which there is a membrane alteration in the hepatocyte; nevertheless, hepatocellular necrosis is not a requirement for liberation of these, which causes a low correlation between level of aminotransferases and hepatic damage (Morales-González et al., 1999). Results of this study agree with reports of other authors (Morales-González et al., 1999, 2004), who observed a significant increase in AST and ALT serum concentrations in PH compared with sham group (Figure 2). This could be explained by early liberation of hepatocytes, which initiate a proliferative process such as a sign, due to that it has been reported that liver regeneration is linked by selective liberation of enzymes (Morales-González et al., 1999). This is backed by decrease in the activity of both ALT and AST caused by ethanol ingestion during liver proliferation induced by partial hepatectomy (the PH-EtOH group) (Figure 2). Notwithstanding this, both antioxidants (Gly and VE) revert aminotransferase decrease; therefore, liver-weight gain is normalized at 24 h post-surgery (Figure 1).

During the liver regeneration process, the ethanol-intoxicated experimental group shows an evident increase in total bilirubin concentrations in comparison with PH and control groups, and these results are in agreement with those reported by Morales-González et al. (1999) in which ethanol administration does not significantly modify serum albumin concentrations.

It is known that lipids comprise the most susceptible biomolecules against ROS damage and that lipoperoxidation produces formation of malondialdehyde (MDA), the final product of fatty-acid peroxidation and an indicator of lipid oxidative damage (Masalkar and Abhang 2005).

Furthermore, an increase TBARS increase during liver regeneration has been reported. The fact that ROS can be produced by regenerative hepatocytes and can act as potential early-phase regenerative-process mediators has been suggested to explain this TBARS increase (Guerrieri et al., 1999).

MDA has been used as primary oxidative-stress indicator. Other indicators, such as SOD, have been used as secondary oxidative-stress markers and correlated significantly with MDA.

It is well known that the superoxide is one of the main reactive oxygen species in the cell; as such, SOD has a key antioxidant role (Tsai et al., 1992). The PH group exhibited a TBARS-concentration increase 24 h after the PH in comparison with sham group (Figure 3), which agrees with previous reports. In other studies (Masalkar and Abhang 2005, Song et al., 2003) it was found that acute intoxication with ethanol produces an oxidative-stress increase due to increased formation of ROS as products of metabolism of ethanol and its metabolite—acetaldehyde—that in the same fashion decreases antioxidant systems. In addition, an acute dose of ethanol (1.5 g/kg) has shown to be capable of inhibiting the complex process of hepatic regeneration (Morales-

González et al., 1999, 2001). This effect was found as well in our study when we ascertained an increase in TBARS concentration when ethanol was administered at a 1.5 g/kg dose in PH-EtOH (Figure 3), this resulting 50% greater than that of animals with PH, as well as a 13.5% decrease in SOD activity in comparison with sham group (Figure 4). Liver regeneration requires greater energy demands and control of oxidative stress. Prolonged exposure to H₂O₂ induces cell cycle inhibitors, such as the cyclin-dependent kinase inhibitor p21, which arrest cells in G-1 and prevent these from entering S phase in which DNA is replicated. Therefore, chronic exposure to H₂O₂ promotes senescence (Tsai et al., 1992). Our results strongly sustain that Gly and VE protect the proliferating liver in its early stages from oxidative damage produced by acute ethanol administration, significantly decreasing TBARS concentrations and presenting levels similar to PH group (Figure 3). Thus, these antioxidants revert the effect of ethanol by decreasing SOD activity during liver regeneration by means of generating a significant increase in levels of this antioxidant enzyme (Figure 4), consequently conferring a hepatoprotective effect against ethanol during liver regeneration. In addition to this, and as other studies have demonstrated, the protective effect of Gly and VE aid against necrosis, inflammation (Yin et al., 1998; Ishizaki et al., 2004), and oxidative stress by different toxic agents such as ethanol (Qu et al., 2002). Therefore, VE, as a stabilizer of biological membranes, can act as a liposoluble antioxidant that acts in preventing ROS damage of polyunsaturated fatty acids (Bradford et al., 2003).

Isolated administration of antioxidants does not only modify the proliferation process, but also changes the serum concentration of quantified metabolites and enzyme activity, presenting similar concentrations to sham group (data not shown); nonetheless, these antioxidants prevent ethanol-produced oxidative damage in regenerative liver, opening a window for a future use of antioxidants involving low cost and easy acquisition in the treatment of liver diseases associated with alcohol consumption.

In conclusion, results showed that one dose of Gly or VE applied to animals submitted to partial hepatectomy and treated with ethanol presented normal, safe and constant cellular proliferation in liver in its early stages (24 h) induced by surgical hepatic-mass elimination. Administration of VE is more efficient than Gly use in liver-regeneration protection against acute ethanol-produced damage. This effect is probably caused by stabilization of biological membranes produced by this vitamin, which is in agreement with recent reports (Ramírez-Farías et al., 2008; Kirimlioglu et al., 2006).

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