Action of vitamin E on experimental severe acute liver failure

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ABSTRACT – Background – Severe Acute Liver Failure (ALF) is a life-threatening clinical syndrome characterized by hepatocyte necrosis, loss of hepatic architecture, and impairment of liver functions. One of the main causes of ALF is hepatotoxicity from chemical agents, which damage hepatocytes and result in increase of reactive oxygen species. The vitamin E isoform is the one with the strongest biological antioxidant activity. Objective – To evaluate the antioxidant effect of vitamin E in this ALF model. Methods – We used 56 rats (mean weight of 300 g) divided into eight groups, four groups assessed at 24 hours and 4 assessed at 48 hours after induction: control group (CO); Vitamin E (Vit. E); Thioacetamide (TAA) and Thioacetamide + Vitamina E (TAA+Vit.E). Rats were submitted to injections of thioacetamide (400 mg/kg i.p.) at baseline and 8 hours later. Vitamin E (100 mg/kg/ip) was administered 30 minutes after the second dose of thioacetamide. The 48-hour group rats received two additional doses of vitamin E (24h and 36h). At 24h or 48h after the administration of the first dose of TAA, rats were weighed and anesthetized and their blood sampled for evaluation of liver integrity through enzymes aspartate aminotransferase (AST) and alanine aminotransferase (ALT). Liver tissue was sampled for assessment of lipid peroxidation (LPO) by the technique TBARS, antioxidant enzymes SOD, CAT, GPx and GST activity, levels of the NO/NO2/NO3 (mmol/L) and histology by H&E in two times. The results were expressed as mean ± standard deviation and statistically analyzed by ANOVA followed by Student-Newman-Keuls, with P<0.05 considered as significant. Results – After treatment with vitamin E, we observed a reduction in liver enzymes AST (U/L) (101.32±19.45 in 24 hours and 97.85±29.65 in 48 hours) related to the TAA group (469.56±0.69 in 24 hours and 598.23±55.45 in 48 hours) and ALT (U/L) (76.59±8.56 in 24 hours and 68.47±6.49 in 48 hours) compared to the TAA group (312.21±10.23 in 24 hours and 359.15±17.58 in 48 hours). There was a reduction of LPO (nmol/mg Prot) in the TAA+Vit.E group (0.77±0.07 in 24 hours and 0.95±0.08 in 48 hours) compared to the TAA group (1.50±0.07 in 24 hours e 1.65±0.16 in 48 hours). SOD decreased in the TAA+Vit.E group (49.48±9.47 in 24 hours and 62.45±18.47 in 48 hours), related to the TAA group (98.46±15.48 in 24 hours and 154.13±21.46 in 48 hours), as well as GST (nmol/min/mg Prot) in the TAA+Vit.E group (350.57±36.93 in 24 hours and 453.29±13.84 in 48 hours) compared to the TAA group (561.57±64.56 in 24 hours and 673.43±38.13 in 48 hours). There was an increase in CAT (pmol/min/mg Prot) in the TAA+Vit.E group (3.40±0.44 in 24 hours and 3.02±0.35 in 48 hours) compared to the TAA group (1.65±0.21 in 24 hours and 1.86±0.42 in 48 hours). The GPx (nmol/min/mg Prot) increased in 24 hours in the TAA+Vit.E group (1.01±0.16) compared to the TAA group (0.41±0.04) and decreased in 48 hours (1.19±0.17) compared to the TAA group (1.76±0.21). There was a reduction in NO2/NO3 (nmol/L) levels in the TAA+Vit.E group (31.47±4.26 in 24 hours and 38.93±5.20 in 48 hours) compared to the TAA group (49.37±5.12 in 24 hours and 53.53±5.97 in 48 hours). The histopathological evaluation showed a decrease in liver injury (necrosis and inflammation) in both studied times. Conclusion – These results suggest that vitamin E was able to protect the liver from lesions caused by thioacetamide.

INTRODUCTION

Severe Acute Liver Failure (SALF) is a syndrome with high morbidity and mortality rates and low prevalence. It is characterized by sudden onset in patients with previously normal liver with rapid progress, leading to hepaticcellular insufficiency, which translates into extensive metabolic disturbances, particular susceptibility to bacterial or fungal infections, collapse of multiple organs, coagulopathy, and central nervous system disorders, with mortality reaching 80% (18,24,28,31). The severe acute attack on the hepatic parenchyma can have different etiologies, such as drugs, xenobiotics and viruses. At present, the treatment of excellence in most cases is liver transplantation. The availability of organs is limited, however, precluding the use of such therapy in all necessary cases. Furthermore, few are the hospitals that have competent surgical teams to effectively perform liver transplantation. The acknowledged therapeutic effectiveness of N-acetylcysteine in cases of SALF triggered by intake of large doses of paracetamol gives us an indication of the possible therapeutic application of other compounds that can act in such situations. So, attentions have been focused on the possibility of restoring liver mass and function through various treatments, in an attempt to delay or arrest the progress of the disease (18,24,28,30).

Research on experimental models of ALT play an extremely important role for the study of its pathogenesis and the many stages...
of its course. Thioacetamide (TAA) is a xenobiotic known as a potent hepatotoxic, carcinogenic and cirrhosis-inducing agent in rats. Its administration causes the death of hepatic cells by both centrilobular necrosis and apoptosis. This process involves reactive oxygen species (ROS), which leads to oxidative stress (OS), with increased damage to DNA, proteins and lipids from the excessive generation of free radicals (FR).

Both ROS and reactive nitrogen species (RNS) as well as other free radicals are critical intermediaries in the physiopathogenesis and physiopathology of hepatocyte lesion. Bioactive products resulting from lipoperoxidation are highly implicated as being key abnormalities responsible for the hepatic injury.

The organism relies on an antioxidant defense system against ROS and RNS, which is divided in two main types: enzymatic, such as enzymes superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase (CAT) and glutathione S-Transferase (GST); and non-enzymatic, such as glutathione (GSH), ascorbic acid (vitamin C), flavonoids, vitamin E, among others. Vitamin E is a component of vegetable oils that is found in nature in four different forms: α, β, γ, and δ-tocopherol. Vitamin E is the main antioxidant vitamin transported in the blood flow by the lipid phase of plasma lipoprotein particles.

The α-tocopherol isomer is the one with the strongest biological antioxidant activity and is widely distributed in tissues and plasma. In the non-hydrophobic portion of α-tocopherol there is the hydroxyl radical (HO), whose atom of hydrogen is easily removable. So, when peroxy and/or alcoxyl radicals are generated during lipid peroxidation, they are likely to combine with fatty acids of the tail of vitamin E, thus stopping to withdraw electrons from membrane fatty acids. Therefore, vitamin E, owing to its structural characteristics, acts as chain breaker, i.e. a scavenger of free radicals, thus precluding lipoperoxidation (LPO).

Given the physiopathogenesis of severe SALF involving the formation of ROS and RNS, the hepatotoxic ability of TAA and the antioxidant effects of Vitamin E, this work was designed to investigate the action of this vitamin on SALF in rats.

**METHODS**

**Ethical considerations**

Animal handling complied with the ethical principles established by Federal Law No. 11.794, which regulates the scientific use of experimental animals in Brazil. This project was approved by the Ethical Research Committee of Universidade Luterana do Brasil (ULBRA) for Animal Use (CEUA- Protocol 2012 – 43P).

**Animals and research design**

Fifty-six male Wistar rats with mean weight of 300 g were used, divided in two experiments according to time of interest, 24h and 48h (28 animals per experiment). Each experiment comprised four groups: control (CO) group, Vitamin E (Vit. E) group, Thioacetamide (TAA) group and Tioacetamide + Vitamin E (TAA+Vit. E). Each experimental group was composed of 7 animals (n=7 based on sampling calculation) obtained from the animal facility of ULBRA. Along the study period the animals were kept in plastic boxes lined with wood shavings on a 12h light/dark cycle and room temperature between 20 and 25°C. They had free access to food and water.

Thioacetamide (Sigma Chemical Co., St. Luis, MO, USA) was diluted in 1 mL of 0.9% NaCl vehicle and administered with intraperitoneal injection (i.p). Vitamin E (α-tocopherol), supplied in gelatinous capsules with oil by Importadora Quimica DELAWARE®, was administered at a dose of 125 mg/kg (i.p.)

**Experimental protocol**

The CO-24h group received three doses of 0.9% NaCl vehicle, with the second dose given 8 hours after the first and the third last dose 30 minutes after the second. Thioacetamide was administered at two doses of 400mg/Kg (i.p) each with an interval of 8 hours, while vitamin E was given at a dose of 125 mg/kg (i.p.) 30 minutes after the second dose of TAA. In the 48-hour experiment, two additional doses of vitamin E were administered, with the second dose given 24 hours after the start of the experiment and the third, 36 hours after it. Doses of vehicle at the same dilution (0.9% NaCl, 1 mL) were administered to the groups in both experiments in order to expose the animals to the same number of administrations. At the end of each experiment, animals were weighed and anesthetized with ketamine 95 mg/kg and xylazine hydrochloride 8 mg/kg (i.p.). Blood samples were collected from the retro-orbital plexus for hepatic integrity assays and livers were dissected out for posterior analyses. At the end of each experiment (24h and 48h), animals were killed by exsanguination under deep anesthesia.

**Plasma analyses**

Liver integrity was determined by evaluation of enzymes aspartate aminotransferase (AST) and alanine aminotransferase (ALT) in plasma using the commercial kit Boehringer Mannheim (Germany). AST (340 nm) and ALT (340 nm) activities were obtained by kinetic assay using the commercial liquiform kit Labtest®.

**Liver homogenates**

Nine mL of phosphate buffer solution (1.15% KCl) per gram of tissue (liver) and phenylmethylsulfonyl fluoride (PMSF) at a concentration of 100 mM in isopropanol (10 µL/mL of KCl) were used. The tissue was homogenized in ULTRA-TURRAX for 40 seconds at 0-2°C and subsequently centrifuged for 10 min at 3000 rpm in refrigerated centrifuge. The precipitate was discarded and the supernatant removed and frozen at -80°C for posterior biochemical analyses.

**Protein**

The Bradford method (1976) was used to quantify proteins, with bovine albumin as standard (SIGMA). The samples were spectrophotometrically measured at 595 nm, and the concentrations expressed in mg/mL and used to calculate thiobarbituric acid reactive substances (TBARS) levels and antioxidant enzyme activity.

**Lipoperoxidation**

The amount of malondialdehyde generated by lipoperoxidation was measured by TBARS, a technique that measures the quantity of substances reacting with thiobarbituric acid. Tissue samples were placed in test tubes with a mixture of 10% thiocloroacetic acid (TCA) and 0.67% thiobarbituric acid (TBA). They were subsequently warmed in bath for 30 min and chilled in ice for about 5 min. After chilling the samples, 1.5 mL of n-butyl alcohol was added to extract the pigment formed. After this procedure they were placed in stirrer for 45 sec and centrifuged for 10 min at 3000 rpm. Finally, the stained product, present in the upper fraction, was read spectrophotometrically at a wavelength of 535 nm. The obtained TBARS concentration was expressed as nmol per milligram of protein.
Analyzes of antioxidant enzymes

Glutathione S-transferase (GST) is based on an enzyme that catalyzes the formation of 1 mmol of DNP-SG per minute at 30°C using 1 mM of the concentration of (reduced) GSH and CDNB, detected spectrophotometrically at 340 nm, values expressed in mmol/min/mg prot(23). The analysis of superoxide dismutase (SOD) activity is defined as its ability to inhibit a detection system that reacts with O₂⁻. The technique of measuring SOD is based on the inhibition of this reaction with adrenalin, detected spectrophotometrically at 480 nm. The data were expressed as units of SOD per milligram of protein (USOD/ mg prot.)(23). The analysis of catalase activity (CAT) is defined by the breakdown of hydrogen peroxide in water and oxygen, being directly proportional to its enzymatic activity, detected spectrophotometrically at 240 nm. The results were expressed in μmoles per milligram of protein (mmoles of H₂O₂)(23). Glutathione peroxidase (GPX) can be studied by measuring the rate of consumption of nicotinamide adenine dinucleotide (NADPH) in the reduction of glutathione oxidase, detected spectrophotometrically at 340 nm and its activity expressed in μmoles per minute per milligram of protein (nmol/ min/mg prot)(23).

Evaluation of nitric oxide metabolites – nitrites e nitrates

Nitric oxide production was measured indirectly through a colorimetric quantitative test by the Griess reaction. It is based on the enzymatic reduction of nitrates to nitrites in the presence of nitrate reductase and NADPH, and posterior reaction of the formed nitrites (or initially present in the samples) with Griess reagent (mixture of sulfanilamide and naphthyl ethylenediamine, specific for NO₂⁻). However, as the excess of NADPH used inhibits the Griess reaction, it is necessary to oxidize all of the NADPH not used in the reduction of nitrates. This is achieved by adding nitrate reductase. The reading was performed in a microplate reader at 540nm and the results expressed in mmol of NO₂⁻/NO₃⁻.

Histological analysis

Histological analyses were performed on liver samples preserved in 10% formaldehyde solution for 24h, which were then embedded in paraffin and cut in 3 mm slices using a rotating microtome. Histological examinations were performed using hematoxylin-eosin staining. A single pathologist, blinded to experimental protocol, analyzed all livers under a binocular Labophot NIKON microscope, at 100X magnification.

Statistical analysis

The results were expressed as mean ± standard error for each experimental group. The software GraphPad Instat, version 3.0 was used for the statistical analysis. For symmetrical data, simple ANOVA was used to compare the differences found in each studied parameter. The complementary Student-Newman-Keuls test was used as well for multiple comparisons. The level of significance for each comparison was at least 5% (P<0.05).

RESULTS

Liver integrity

Table 1 shows the results of AST and ALT evaluation at 24h and 48h. The TAA groups showed a significant increase (P<0.001) as compared to the CO group at both times, while the TAA groups receiving Vit. E (125 mg/kg) reduced these enzymes significantly (P<0.001) at both these times, decreasing and protecting from the damage triggered by TAA. It was thus demonstrated that Vit. E doses contributed to a protective effect on the liver tissue.

**TABLE 1.** Evaluation of enzymes AST and ALT (U/L) in the different experimental groups at the two studied times (24h and 48h)

<table>
<thead>
<tr>
<th>Group</th>
<th>CO</th>
<th>VIT. E</th>
<th>TAA</th>
<th>TAA + Vit. E</th>
</tr>
</thead>
<tbody>
<tr>
<td>24 h</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AST</td>
<td>39.05±6.55</td>
<td>39.99±5.23</td>
<td>469.56±0.69</td>
<td>101.32±19.45</td>
</tr>
<tr>
<td>ALT</td>
<td>22.36±3.45</td>
<td>21.56±2.64</td>
<td>312.21±10.23</td>
<td>76.59±8.56</td>
</tr>
<tr>
<td>48 h</td>
<td>43.12±5.63</td>
<td>41.56±3.45</td>
<td>598.23±55.45</td>
<td>97.85±29.65</td>
</tr>
</tbody>
</table>

Lipoperoxidation measurement

LPO results for the 24h and 48h groups can be seen in Figure 1, where a significant increase (P<0.001) of LPO was found in TAA groups as compared to the others. As Vit. E is administered at 24h, LPO is reduced as compared to the TAA group (P<0.001), and the same was found at 48h. This is clear evidence that Vit. E administration in the TAA group reduces LPO at both studied times.

**FIGURE 1.** Evaluation of lipoperoxidation through TBARS (nmol/mg prot) in the different experimental groups assessed at 24h and 48h.

Antioxidant enzymes

SOD, CAT, GPx and GST activities were assessed at 24h and 48h (Table 2). GST and SOD activities were significantly increased in the TAA groups as compared to control groups at 24h and 48h, and decreased in the TAA+Vit.E groups as compared to the TAA groups at both times. Cat activity was significantly reduced in the TAA groups as compared to controls at 24h and 48h, and increased in the TAA+Vit.E groups as compared to TAA at both studied times. GPx showed a different pattern across the studied times. At 24h GPx activity was reduced in the TAA as compared to controls and was reduced in the TAA+Vit.E group as compared to TAA.

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>CO</th>
<th>VIT. E</th>
<th>TAA</th>
<th>TAA + Vit. E</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAT</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>24 h</td>
<td>0.00 - 0.20</td>
<td>0.20 - 0.40</td>
<td>0.40 - 0.60</td>
<td>0.60 - 0.80</td>
</tr>
<tr>
<td>SOD</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>24 h</td>
<td>0.00 - 0.20</td>
<td>0.20 - 0.40</td>
<td>0.40 - 0.60</td>
<td>0.60 - 0.80</td>
</tr>
<tr>
<td>GPx</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>24 h</td>
<td>0.00 - 0.20</td>
<td>0.20 - 0.40</td>
<td>0.40 - 0.60</td>
<td>0.60 - 0.80</td>
</tr>
</tbody>
</table>

**FIGURE 2.** Histological analysis.
TABLE 2. Evaluation of antioxidant enzymes SOD, CAT, GPX and GST activities in the different studied groups and two studied times: 24h and 48h.

<table>
<thead>
<tr>
<th>Groups</th>
<th>SOD (USOD/min/mg Prot)</th>
<th>CAT (pmol/min/mg Prot)</th>
<th>GPX (nmol/min/mg Prot)</th>
<th>GST (nmol/min/mg Prot)</th>
</tr>
</thead>
<tbody>
<tr>
<td>24h</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CO</td>
<td>36.47 ± 7.49</td>
<td>3.43 ± 0.68</td>
<td>0.76 ± 0.09</td>
<td>246 ± 11.41</td>
</tr>
<tr>
<td>Vit. E</td>
<td>28.46 ± 5.13</td>
<td>4.60 ± 0.21</td>
<td>0.73 ± 0.08</td>
<td>262.57 ± 8.68</td>
</tr>
<tr>
<td>TAA</td>
<td>98.46 ± 15.48</td>
<td>1.65 ± 0.21</td>
<td>0.41 ± 0.04</td>
<td>561.57 ± 64.56</td>
</tr>
<tr>
<td>TAA + Vit. E</td>
<td>49.48 ± 9.47</td>
<td>3.40 ± 0.44</td>
<td>1.01 ± 0.16</td>
<td>350.57 ± 36.93</td>
</tr>
<tr>
<td>48h</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CO</td>
<td>31.54 ± 7.68</td>
<td>3.299 ± 0.23</td>
<td>0.68 ± 0.08</td>
<td>318.71 ± 18.94</td>
</tr>
<tr>
<td>Vit. E</td>
<td>12.96 ± 6.48</td>
<td>3.45 ± 0.24</td>
<td>0.71 ± 0.07</td>
<td>299.57 ± 11.81</td>
</tr>
<tr>
<td>TAA</td>
<td>154.13 ± 21.46</td>
<td>1.86 ± 0.42</td>
<td>1.76 ± 0.21</td>
<td>673.43 ± 38.13</td>
</tr>
<tr>
<td>TAA + Vit. E</td>
<td>62.45 ± 18.47</td>
<td>3.015 ± 0.35</td>
<td>1.19 ± 0.17</td>
<td>453.29 ± 13.84</td>
</tr>
</tbody>
</table>

CO: control group, Vit. E: Vitamin E group; TAA: Thiocetamide group; TAA+Vit.E: Thiocetamide with Vit. E group. Data are expressed as mean ± standard error of mean. n=7 per group. 

a = significant increase (P<0.001) TAA vs Controls; b = significant decrease (P<0.001) of TAA+Vit.E vs TAA; c = significant increase (P<0.05) of TAA vs Control groups, and d = significant decrease (P<0.01) TAA+Vit.E vs TAA.

Nitrites e Nitrates

Figure 2 shows that nitric oxide metabolites increased significantly (P<0.001) in the TAA groups at both times and were significantly reduced in the Vitamin E-supplemented groups (P<0.001) at both studied times.

Histology

Histological evaluation of liver tissue was performed by hematoxylin & eosin (HE) staining at 200x magnification. As can be seen in Figures 3A and B (24h) and 4-A and B (48h), the CO and Vit.E groups showed normal hepatic parenchyma, with clear-cut hepatocyte cordons with well-preserved cytoplasms and nuclei. In the histology of the TAA group, shown in Figures 3C (24h) and 4C (48h), there is evidence of hepatocyte cordon disorganization, inflammatory infiltrate, and necrosis. In the TAA+Vit.E group, seen in Figures 3D (24h) and 4D (48h), note the preservation of hepatocyte cordons and decreased incidence of necrosis and inflammatory infiltrate in response to Vitamin E.
DISCUSSION

Severe SALF is an acute hepatic disorder with varied, poorly known physiopathology and established physiopathology with variants concerning etiology. Knowledge of these mechanisms is important to determine the pathways of the lesion and any potential effects of external intervention. The utilization of experimental models and antioxidant drugs comes to contribute to its understanding. Drugs that can delay the progress of the disease or reorganize the hepatic parenchyma are potential therapeutic agents for the disease.

In the present study, vitamin E was found to attenuate oxidative stress and inflammation in TAA-induced SALF. Enzymes AST and ALT were increased by about 1200% in rats receiving TAA as compared to controls at 24 h and 48 h. Other authors also obtained similar results by inducing toxicity in rat livers through administration of AA doses (350 mg/kg). In Vitamin E-treated groups, there was a significant decrease of about 75% in AST and ALT as compared to TAA groups at 24 h and 48 h. In another study also observed a reduction of these parameters in an experimental model of toxicity, treating with flavonoid quercitin.

In LPO evaluation, TBARS presented an increase of 59.5% and 94.1% at 24 h and 48h, respectively, in the TAA group. In the Vit. E group, however, values were similar to those of the control group and significantly reduced as compared to TAA groups, with reductions of 51.3% and 42.4% at 24 h and 48 h, respectively. Such reduction demonstrates decreased LPO in the hepatic tissue due to the antioxidant action of vitamin E administered to these animals, which can be evidenced by histology, where one notices reorganization of the hepatic tissue and reduction of the inflammatory infiltrate and necrosis.

Administration of Vitamin E significantly reduced SOD activity by 49.7% at 24 h and 59.48% at 48 h, suggesting decreased production of O$_2^-$ and consequent decreased LPO, as demonstrated by histology and TBARS levels. In a study investigating the preventive effect of quercetin in animals with TAA-induced SALF, de Oliveira et al., showed significantly increased SOD activity from the administration of the flavonoid. Other works have reported decreased SOD activity from the administration of other antioxidants.

CAT activity was significantly reduced in TAA groups as compared to CO groups by 51.9% and 43.6% at 24 h and 48 h, respectively. TAA acts directly on the formation of O$_2^-$, which act on membrane lipids and form hydroperoxide lipids, thus accounting for reduced CAT activity. The use of Vit. E significantly restored CAT activity at 24 h and 48 h, with increases of 106% and 61.8%, respectively, making it similar to CO groups and reinforcing the antioxidant effect of Vitamin E. Another study found significant reduction of CAT activity in rats, evaluating the damage caused by oxidative stress induced by high-carbohydrate diet in a model of hepatic encephalopathy.

The behavior of GPx activity varied across the studied times. While in the TAA group there was a reduction of 46% in GPx activity in relation to the CO group at 24 h, there was an increase of it (158.8%) at 48 h, possibly explained by the reestablishment of the hepatic tissue. As compared to the TAA group, GPx activity in the TAA+Vit.E group presented an increase of 146.3% at 24 h and a decrease of 32.3% at 48 h, which can be explained by the decreased tissue aggression due to the protective effect of Vit. E, evidenced by lower TBARS levels and histology. The increase in GPx appears to accompany the accumulation of organic peroxides formed by LPO caused by TAA. Similar results were reported in a work using antioxidant quercetin and glutamine.

The high toxicity of TAA in hepatocytes triggered an increase in enzyme GST, through its detoxifying protective action, with increases of 128.2% and 111% at 24 h and 48 h, respectively. In the Vitamin E-treated group, however, the reduction was of 37.5% and 32.6% at 24 h e 48 h, respectively, owing to the preservation of hepatocytes by the exogenous antioxidant, as evidenced again by decreased TBARS levels and liver histology. Our findings corroborate those of other authors who evaluated GST activity in experimental model, using TAA as hepatotoxic inducer.

On histological evaluation of hepatic tissue using hematoxylin & eosin (HE) staining at a magnification of 200x, CO and Vit. E groups showed hepatic cells arranged in hepatocyte cords around capillaries with aspects of normal distribution, and visible nuclei without inflammatory infiltrates or necrosis. On the other hand, the hepatic tissue in the TAA group displayed disorganization of hepatocytes, inflammatory infiltrates, and necrosis, which accounts for increased TBARS levels at both times. Histological evaluation of the Vit.E-treated group showed a reorganization of hepatocyte cords and smaller incidence of inflammatory infiltrate or necrosis. These evidences coincide with significant reduction of TBARS and lower lipoperoxidation in the hepatic parenchyma at both studied times, promoted by Vitamin E antioxidant action. Another author also demonstrated Vitamin E antioxidant role when administered at 200 mg/Kg/day for 3 days.

The rise of metabolites NO$_2$ and NO$_3$ indicates an increase in NO production, which participates closely in the inflammatory and destructive process of the hepatic tissue. Animals in TAA groups presented increase of NO metabolites that associate with the superoxide anion to form peroxinitrite, which is extremely damaging to hepatocytes. This increase was of 476% (24 h) and 646% (48 h) in relation to control groups, while Vitamin E-supplemented groups showed a reduction of 36.2% (24 h) and 29.8% (48 h) as compared to groups receiving TAA, which in a way can be explained by the improvement of the hepatic parenchyma. Similar findings were reported by other authors who assessed nitric oxide levels.

From the findings it is possible to see that the Vitamin E group at 48 h, despite having received two additional 100 mg/Kg doses of Vitamin E, presents values that are close to those obtained at 24h, suggesting that the dose of 100 mg/Kg administered intraperitoneally thirty minutes after TAA administration was already sufficient to protect the liver against the oxidative stress triggered by the drug.

CONCLUSION

Oxidative stress plays a key role in the aggravation of liver injury and structural and/or functional disorders of the liver. The use of antioxidants, such as vitamin E, seems promising as an attempt to prevent the complications resulting from oxidative stress and in the continuation of the disease. In the present study, TAA administration induced liver damage in rats at both 24 h and 48 h and administration of Vitamin E was effective in protecting the tissue at both these times. We suggest that Vitamin E can be an effective tool in the treatment of SALF and its resulting complications. However, studies with new approaches taking in
consideration apoptosis markers, inflammatory route, and DNA damage caused by oxidative stress will be helpful in elucidating these mechanisms.

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Authors’ contributions

Miguel FM and Schemitt EG performed all of the research work; Colares JR and Hartmann RM were in charge of the experimental model; Morgan-Martins MI and Marroni NP designed the research work and the article review.

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