Effect of Conjugated Linoleic Acid (CLA) in Rats Subjected to Damage Liver Induced by Carbon Tetrachloride

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Abstract: The conjugated linoleic acid (CLA) consists in a group of positional and geometrical isomers of octadecadienoic acid (18:2 ω-6) in which the two double bonds are conjugated and may have *cis* or *trans* configurations. CLAs have been the subject of extensive investigations for their various biological activities, including antiatherosclerotic, anticarcinogenic and antioxidative activities. The aim of this study was to evaluate the antioxidant activity and hepatoprotective properties of CLA in rats subjected to liver injury induced by carbon tetrachloride (CCl_4). Eighteen male Wistar rats were divided into three groups: control; CCl_4 and CLA. The CLA was administrated daily by gavage for 21 days, and then subjected to liver injury induced by CCl_4. The serum and liver were collected and analyzed. CLAs supplementation take to attenuation of the liver damage in treated animals, since the reduction in the levels of thiobarbituric acid reactive substances (TBARS) in the liver suggests hepatoprotective activity of these fatty acids. The levels of catalase (CAT) and glutathione reductase (GR) and reduced glutathione (GSH) levels in liver tissue as a result of reduced oxidative damage induced by CCl_4, and increased after treatment with CLA, suggesting antioxidant capacity of CLAs. Moreover the analysis of reverse transcription / polymerase chain reaction (RT / PCR) showed increased expression of the CAT gene, suggesting inducing effects on this enzyme. These results suggest that CLAs can be used as adjuncts to attenuate hepatic damage.

Keywords: Conjugated linoleic acid, hepatoprotective, carbon tetrachloride, antioxidant activity, rats.

INTRODUCTION

Liver injury often leads to apoptosis and necrosis of hepatocytes, regardless of its cause. They may be usually caused by viral infections, autoimmune diseases, ischemia, and xenobiotics, including: drugs, toxins or alcohol. The model of acute liver injury induced by carbon tetrachloride (CCl_4) is widely used to investigate the mechanisms of liver injury and regeneration [1]. Liver and lipid accumulation in the liver occur within 24 hours of oral administration only. The hepatocyte necrosis, damage to lysosomes and mitochondria of liver cells are induced by administration of CCl_4 [2]. Even during the acute phase of liver damage, fibrosis does not occur, there is the activation of hepatic stellate cells which are the key fibrogenic cells of the liver injury [3].

Epidemiological studies have consistently shown that nutrition is strongly associated with reduced risk of developing chronic diseases such as cancer and cardiovascular disease, and also in the pathogenesis of acute and chronic liver disease [3]. In this context fatty acids may have protective effects on the liver injury induced by endotoxin. Research reported protective effects of olive oil on liver injury caused by CCl_4 administration [4]. Olive oil prevents liver injury induced by CCl_4 and liver fibrosis, since the oleic acid inhibited the activation of hepatic stellate cells. Thus, the fatty acids may play an important role in liver fibrosis [3].

Polyunsaturated fatty acids (PUFAs) such as linoleic acid, α-linolenic acid and arachidonic acid are important for the maintenance of biological functions in mammals. In clinical studies with humans and animals is observed that other polyunsaturated fatty acids omega family, such as eicosapentaenoic acid and docosahexaenoic acid are correlated with the reduction in the risk of developing cancer and cardiovascular disease [5]. Some studies have shown that oils rich in polyunsaturated fatty acids have a hepatoprotective effect in rats and mice subjected to liver damage by CCl_4, by elevating the activity of endogenous antioxidant enzymes superoxide dismutase, catalase and glutathione peroxidase (SOD, CAT and GPx, respectively) and reduce lipid peroxidation [6, 7].

Recently, studies are focused on conjugated linoleic acid (CLA), which is the general term used to describe a group of positional and geometric isomers of linoleic fatty acid with conjugated double bonds [2, 8]. The interest in the possible health benefits is increasing, since they have been shown to have a antitumor activity, immunomodulatory activity and lipid lowering of

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serum [9]. The fatty acid conjugates are known to have pharmacological activities relevant for the prevention and treatment of atherosclerosis, obesity, hypertension and cancer [10]. *In vivo* study showed that a diet enriched with 1% CLA, significantly inhibited the increase of collagen fibers in the livers of animals, indicating hepatoprotective effects of CLAs on liver fibrosis induced by CCl_4 [11].

The aim of this study was to evaluate the protective effect of the isomers of conjugated linoleic acid (9cis, 11trans and 10trans, 12cis) in animals subjected to acute liver disease by the administration of CCl_4.

MATERIAL AND METHODS

Sample

Samples of CLA (Free Fat Acid - Conjugated Linoleic Acid) have been provided by the company Cognis, the product was Tonalin FFA 80, with validity of 12 months, consisting of free fatty acids of which approximately 80% conjugated linoleic acids. These samples of CLA are derived from safflower oil and the product comprises a pool (50/50) of the two active isomers: 18:2 *cis* 9 CLA, *trans*-11 and 18:2 *trans*-10, *cis*-12 CLA.

CLA samples were fractionated in amber bottles previously cleaned in volumes of 20mL and 50mL, sealed, identified and stored in a freezer at -20 °C. Daily bottles with sample amounts to be used were removed in advance to thaw at room temperature.

Identification of CLA Isomers

For the preparation of methyl esters of fatty acids present in the samples was conducted method of esterification Alkaline as described by Christie, Sébédio and Juanéda [12]. The lipid fraction (50 mg oil) was subjected to esterification, the sample was diluted in toluene, sodium methoxide methanolic 0.5 M ($NaOCH_3$) and heated at 50°C for 10 minutes. After cooling glacial acetic acid water and hexane was added. After phase separation the lower phase was washed with hexane, vortexed for 20 seconds and allowed to stand for phase separation. The upper phase was recovered and filtered. The solvent was evaporated under N_2, the methyl esters were resuspended with 2 ml of hexane and the solution was transferred to the vial and injected into the chromatograph.

Analysis of Fatty Acid Compositions

The fatty acid (FA) compositions of the oil and hepatic tissue, were determined by gas chromatography using a Shimadzu GC chromatograph model 2010 with flame ionization detector and a fused silica capillary column (100 m; 0.25 mm de internal diameter; 0,2 µm thick film / SP-2560) in column temperature was: isothermal at 162°C for 32 minutes warming of 1.4°C/minute up to 195°C, kept at this temperature for 15 minutes. Subsequently it was heated at 2°C/minute up to 235°C and this temperature was maintained for 5 minutes. The temperatures of the injector and detector were 250°C and 250°C, respectively [13]. Helium (1 ml / min) was used as the carrier gas and the split ratio of the sample injector equal to 1/50. The identification of fatty acids was based on comparison with the retention times for the mixture of methyl esters of standards C4-C24 (Sigma 18919).

Animals

This study was approved by the Ethics Committee on Animal Experiments of the Faculty of Pharmaceutical Sciences, University of São Paulo, Brazil. To evaluate the effects of FFA-CLA *in vivo* were used 18 rats, male lineage "Wistar", weighing between 50 and 70g, reared in Production and Experimentation of the Faculty of Pharmaceutical Sciences and the Institute Chemistry / USP. The animals had free access to water and using a balanced commercial diet (Nuvilab CR-1) decontaminated by irradiation at 12KGy offered ad libitum. Supplemental FFA-CLA were performed by gavage for 21 days.

The animals were housed in polypropylene cages (six rats each) in an environment with controlled lighting (12 h light / dark) at 25 ° C and 60% humidity throughout the experimental period. We adopted an adjustment period of seven days, and after this period, the animals were divided into three groups.

Table 1: Fatty Acid Composition of Oil

FAT	Fatty Acid Composition (%)		
	18:1	18:2 c9, t11	C18:2 t10,c12
CLA	7.47 ± 0.18	46.91 ± 1.52	45.62 ± 0.89

Scheme of distribution of animals:

Control: treated daily with water, orally (po).

CCl_4: daily treated with water (po) followed by treatment with CCl_4.

CLA: treated daily with 2% CLA (compared to the average daily consumption of diet, po), followed by treatment with CCl_4.

The hepatic damage was induced in the animals on day 21 of the experiment, by administration of a single dose of 3 mL of CCl_4 (1:1 dilution in olive oil) subcutaneously in groups CCl_4 and CLA. After 24 hours of induction, the animals were anesthetized with an intraperitoneal injection of a mixture of ketamine (90 mg / kg) and xylazine (10 mg / kg), the recommended dose Vivarium Production and Experimentation of the Faculty of Pharmaceutical Sciences and the Institute Chemistry / USP, and euthanized. Blood samples were collected by abdominal artery for determination of liver function enzymes - alanine (ALT) and aspartate (AST) aminotransferases and analysis of biochemical parameters - triglycerides, total cholesterol and HDL cholesterol using commercial kit Labtest® and to analyze the levels of thiobarbituric acid reactive substances (TBARS). The liver was perfused through the injection of 0.9% NaCl into the portal vein, collected and homogenized in potassium phosphate buffer 0.1M (pH 7.0) and the homogenate was centrifuged. The supernatant portion of the homogenate was intended for the testing of TBARS and antioxidant enzymes.

The feed intake and animal weight were monitored throughout the test, in order to check the weight gain and feed efficiency ratio (CEA).

Analysis of TBARS

TBARS were measured in serum and liver homogenates the method was described by Ohkawa *et al*. [14]. The TBARS concentrations were calculated using a standard curve for 1,1′,3,3′-(TEP) tetraethoxy-propane (10^{-4} mol/L) and were expressed as µmol of malondialdehyde (MDA) per milligram of protein.

Extraction and Esterification of Hepatic Lipids

Lipids from the hepatic tissue were obtained by the Folch method [15], using the original extration ratio of 20 parts 2:1 chloroform/metanol to 1 part tissue. A weak salt solution (0,88% KCL) is then added to achieve a final ration of 8:4:3 chloroform/metanol/water after including the water contained in the tissue. The fatty acid methyl ester content and analysis of fatty acid compositions was determined according to the methods already described previously.

Determining Superoxide Dismutase (SOD)

The cytoplasmic SOD activity was determined according method of Mccord and Fridovich [16] using a reaction containing cytochrome C (100 mM), xanthine (500 mM), ethylenediaminetetraacetic acid (1 mM) and KCN (200 mM) and potassium phosphate buffer (0.05 M - pH 7.8). The results were expressed as units per milligram of protein. One unit of SOD activity was defined as the amount of enzyme required to inhibit the reaction rate by 50% at 25 °C and pH 7.8.

Determination of Activity Catalase (CAT)

The catalase (CAT) activity in the liver homogenate was measured spectrophotometrically by calculating the rate of degradation of H_2O_2, the substrate of the enzyme at 37 °C and pH 8.0. The results were expressed as mmol of hydrogen peroxide decomposed per minute per milligram of protein, according to the method of Beutler [17].

Reduced Glutathione (GSH) Assay

The intracelular GSH contentes in the liver homogenate was quantifiied spectrophotometrically according to the method of Tietze [18]. The same is based on the reaction of reduced glutathione (GSH) present in homogenates with substance dithiobis 5.5 - 2.2 nitrobenzoic acid (DTNB) to produce a colored compound that absorbs at 412 nm spectrophoto-metrically. The concentration of GSH was quantified using a standard curve of GSH (50 to 800 mM).

Determination of Glutathione Peroxidase (GPX)

The activity GPx in the liver homogenate was determined as described by Sies *et al*. [19]. The results were expressed as units per milligram of protein. The unit of enzyme activity was defined as the amount of enzyme required to oxidize one mol of NADPH per minute at 30 ° C, pH 7.0.

Determination of Glutathione Reductase (GR)

The activity GR in the liver homogenate was determined spectrophotometrically according to the method of Sies *et al*. [19]. We added 50 µl of sample to a solution of 50 mM GSSG, 0.5 M EDTA, in 0.1 M

phosphate buffer (pH 7.0) and 4mM NADPH was added just before the enzymatic determination as the starting reagent. The assay was 6.22 nM run at 340 nm at 37 °C. GR activity was estimated using NADPH extinction coefficient of 6.2 $mM^{-1}.cm^{-1}$ and expressed as unit/mg of protein.

Analysis of Protein

The determination of the content of proteins in tissues was performed according to the colorimetric method [20]. The amount of protein in the sample was calculated from a standard curve of bovine serum albumin and results expressed in mg of protein per mL.

Reverse Transcription / Polymerase Chain Reaction (RT / PCR)

RNA Extraction

RNA extraction was carried out with a mixture of 100 mg of rat liver and 1000 μL of Trizol reagent (Invitrogen, New York, New York). Following by addition of 200μL of chloroform (Merck, Darmstadt, Hessen, Germany) by vortexing for 15 seconds, incubated at room temperature for 5 minutes and centrifugation at 12,000 x g and 4 °C for 15 minutes. The supernatant (400 μL) was collected, avoiding the interphase, and mixed with 500 μL of isopropanol by vortexing for 5 seconds. Then it was centrifuged at 12,000 x g at 4 °C for 5 minutes and the supernatant was discarded. The resulting pellet was washed with 1 ml of ethanol (75%), vortex gently shaken and centrifuged at 7,500 x g and 4 °C for 10 minutes. The supernatant was again discarded. The pellet was resuspended in 20 μl of RNase-free distilled water, incubated at 50 °C for 10 minutes, and stored at - 70 °C.

Reverse Transcription

Five μl of RNA was added to 1.0 μLof primer (Cu / Zn SOD or CAT) 1.0 μLdNTP (10 mM), and 4,0 μLof sterile distilled water. The reaction was initiated by a heating step at 65 °C for 5 minutes and then quickly cooled on ice. After addition of 4.0 μLof 5X buffer First-Strand (Invitrogen), 2.0 μLof DTT (0.1 M, Invitrogen), and 1.0 μLRNAseOUTribonuclease inhibitor (Invitrogen), the mixture was incubated at 37 °C for 2 minutes. Thereafter, 1.0 μLM-MLV reverse transcriptase (200 U / μl, Invitrogen) was added and the mixture was incubated at 37 °C for 50 minutes. The reaction was stopped by a heating step at 70 °C for 15 minutes. The PCR product (cDNA) was stored at -70 °C.

PCR Amplification

The amplification reaction was performed with five microliters of a mixture containing cDNA 5.0 μLTris (hydroxymethylaminomethan) at 20 mM, pH 8.4, 1.5 μl $MgCl_2$ (50 mM), 1.0 μL dNTP (10 mM), 1.0 μL of primer (SOD or CAT) and 0.4 μL to TaqDNApolimerase (5 U/uL). After initial denaturation at 94 °C for 3 minutes in a thermal cycler (Bio-Rad, Hercules, California, USA), 35 cycles (94 °C for 45 seconds, 55 °C for 30 seconds, 72 °C for 1.3 minutes and 72 °C for 10 minutes) were conducted. Finally, the mix was cooled to 4 °C. The PCR amplification products were analyzed by electrophoresis on an agarose gel 2,0% (Sigma, St. Louis, Missouri, USA) at 60 V. The gel was stained with 0.1 μL / mL Sybr safe (Invitrogen), visualized on a table fluorescence (Vilber-Lourmat, Marne-la-Vallée, France), and photographed with a digital camera. The primers CAT-262bp (C to T) and SOD-242 bp (C to T) were genotyped using the following sequences (Promega, Madison, AL, USA):

CAT 1 - 5'-GCG AAT GGA GTG GAG GCA TAC - 3 '

CAT 2 - 5'-GAG TGA CGT TGT CTT CAC CAT TAG TG - 3 '

Cu / Zn SOD 1 - 5'-TCT AAG CAT GGC AAA GGT CC - 3 '

Cu / Zn SOD 2 - 5 '- CAG GGC TTAGCA CAGCAG AT - 3'

Statistical Analysis

The experiments were performed in triplicate and results were expressed as mean and standard deviation, p value <0.05 was considered statistically significant. It was used analysis of variance (ANOVA) followed by Tukey's comparison test using Prism 5.0 software (GraphPad).

RESULTS DISCUSSION

The results of consumption, food intake, weight gain and CEA are shown in Table **2**. The CLA supplementation does not significantly alter food intake, weight gain and animal CEA. This pattern was observed [21] which investigated the effect of CLA supplementation and phytosterols alone or in combination, on the process of auto-oxidation of lipids and activities of antioxidant enzymes in healthy Sprague-Dawley rats for nine weeks. Concerning the feed intake and weight gain of the animals during the period of supplementation, it can be said that, although

Table 2: Food Intake, Weight Gain and Feed Efficiency Ratio (CEA) in Animals Supplemented with CLA for 21 Days and Undergoing Liver Injury by CCl_4

Groups	Sample (ml/d)	Food Intake (g/ d)	Weight Gain (g)	CEA
Control	0.5	26.40 ± 3.41	155.91 ± 31.81	0.20
CCl_4	0.5	25.88 ± 3.20	155.59 ± 17.54	0.20
CLA	0.5	23.29 ± 3.73	127.21 ± 28.08	0.18

*****Data are presented as mean and standard deviation (n = 6).

there were slight differences among the groups, the development of the animals were normal.

The levels of total cholesterol and HDL-cholesterol in the present study, it was observed that there was no statistical difference between groups (Figures **1a** and **1b**). As for the levels of triglycerides, there was an increase in levels for the group supplemented with CLA (Figure **1c**). Other study [22] also observed an increase in triglyceride levels after supplementation with CLAs, besides the increase in abdominal circumference of animals and induced cardiac oxidative stress. However, other research has shown that supplementation with CLAs significantly reduces body weight gain [23], plasma levels of free fatty acids and triglycerides [24], and total cholesterol [24, 25].

The results obtained in this study regarding serum levels of AST (Figure **2a**) and ALT (Figure **2b**), did not differ significantly ($p > 0.05$) of the results found in controls. In contrast, other study [2] demonstrated that the levels of AST and ALT were significantly higher after administration of CCl_4, and CLA supplementation was not effective in reducing these levels of AST and ALT induced by CCl_4. It is possible in this study were already in the liver regeneration process and the levels of markers of liver damage, such as ALT and AST detectable in the bloodstream were already normalized. Research demonstrated that the acute liver injury induced by CCl_4 reversible and is temporarily accompanied by significant inflammation and necrosis of hepatocytes, followed by complete restoration with hepatocyte regeneration in the delayed phase of injury [26]. These authors report that the genes responsible for representative liver function such as catalase, albumin, and PPAR-α were significantly dysregulated in 24 h and 48 h were restored after administration of CCl_4.

The proposed mechanism of hepatotoxicity of CCl_4 involves the bioactivation of CCl_4 to give reactive free radicals. Radicals originating cause peroxidation of fatty acids in membrane phospholipids, resulting in lipid peroxide radicals, lipid hydroperoxides and other products, acting as active oxidizing agents. When the liver is the toxic condition with excess trichloromethyl radical, the activity of the enzyme xanthine oxidase, which produces superoxide anion and hydrogen peroxide, increases with the progression of cell injury. Therefore, lipid peroxidation plays a key role in liver caused by CCl_4 [27, 28].

The biochemical mechanisms involved in the development of hepatotoxicity have been investigated

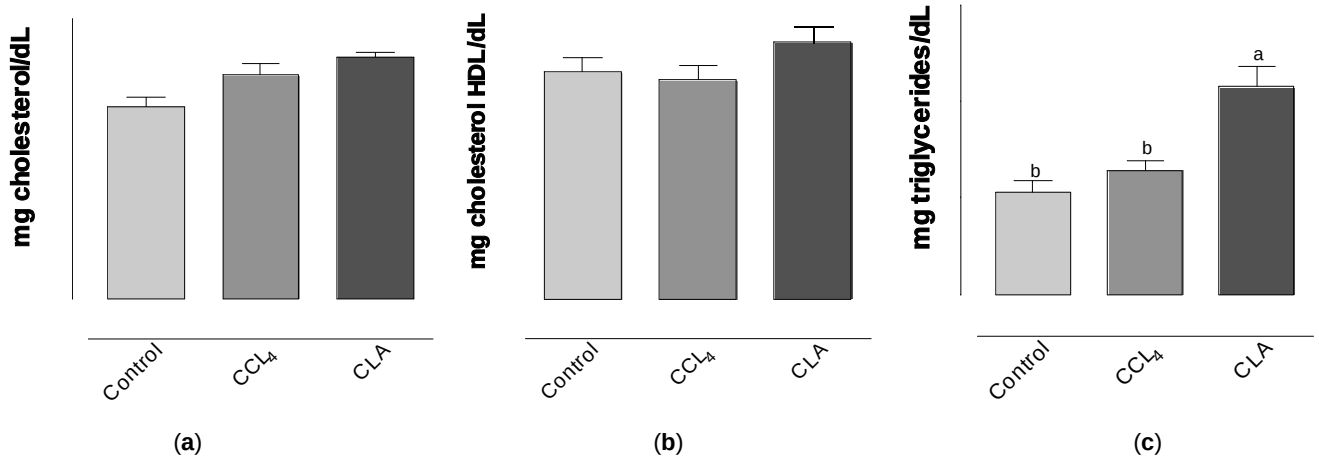
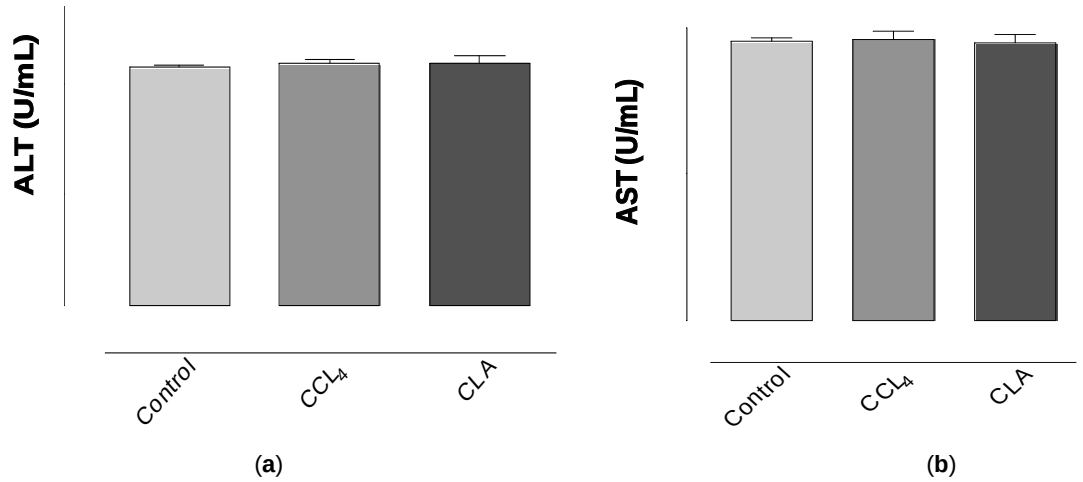


Figure 1: Levels of total cholesterol (**a**), HDL-cholesterol (**b**), and triglycerides (**c**) in the serum of rats. Groups (n = 6): control, CCl_4 and CLA. Different letters indicate significant differences ($p < 0,05$).



(**a**) (**b**)

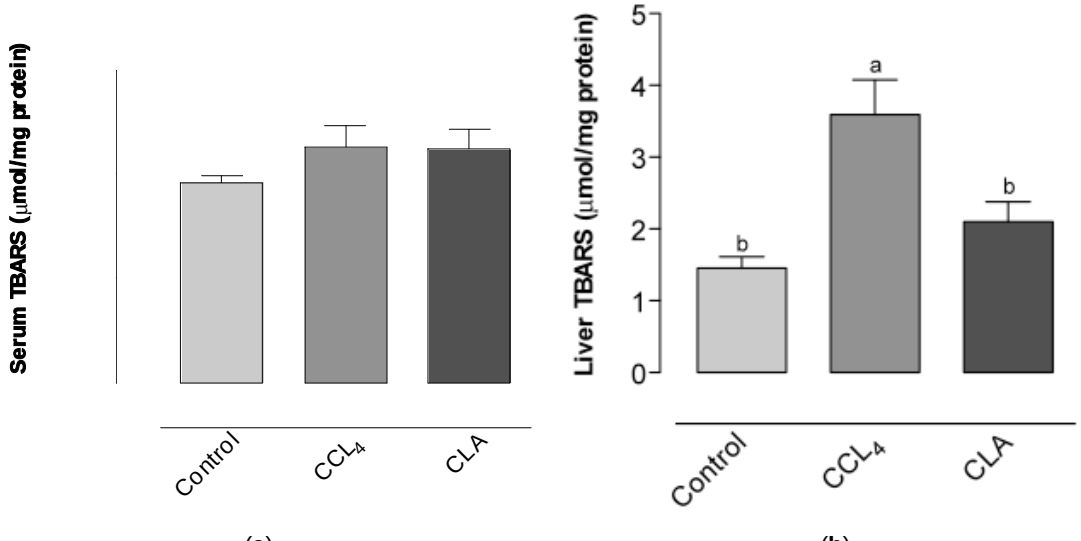
Figure 2: Plasma levels of alanine aminotransferase (**a**) and aspartate aminotransferase (**b**) in rats. Groups (n = 6) control, CCl_4 and CLA.

and malondialdehyde (MDA) is widely used as a marker of lipid peroxidation [6]. Liver injury induced by CCl_4, comes a significant increase of approximately 270% and 188% in the liver content of malondialdehyde and hydroxyproline, respectively, and supplementation with olive oil, significantly reduced hepatic levels of malondialdehyde and hydroxyproline to 48 and 74% respectively [7]. Another study also showed that almond oil significantly inhibited the formation of MDA in the liver during the acute damage caused by administration of CCl_4 [6].

The TBARS are produced as a result of lipid peroxidation. In this study we found that serum showed no statistical difference ($p > 0.05$) between groups. As for liver tissue, there was a reduction in the rate of lipid peroxidation in animals treated with 2% CLAs, when compared to CCl_4, illustrated in Figure **3b**. This result indicates that CLAs have the ability to protect hepatic tissue damage caused by altered redox state by the administration of CCl_4.

In this study the incorporation of CLA isomers on hepatic tissue was evaluated. The results are shown in Table **3**. The isomers 18:2 cis-9, *trans*-11 and 18:2 *trans*-10, *cis*-12 CLA, were incorporated in the liver of animals supplemented with CLA, the incorporation was 2.7%.

The reactive oxygen species (ROS), such as anionic superoxide and hydrogen peroxide (H_2O_2), are produced during normal cellular aerobic metabolism. The main components of the antioxidant system in mammalian cells is composed of three enzymes,



(**a**) (**b**)

Figure 3: TBARS levels in serum (**a**) and liver tissue (**b**) in rats Groups (n = 6). control, CCl_4 and CLA. Different letters indicate significant differences ($p < 0,05$).

Table 3: Fatty Acid Composition in Liver Tissue

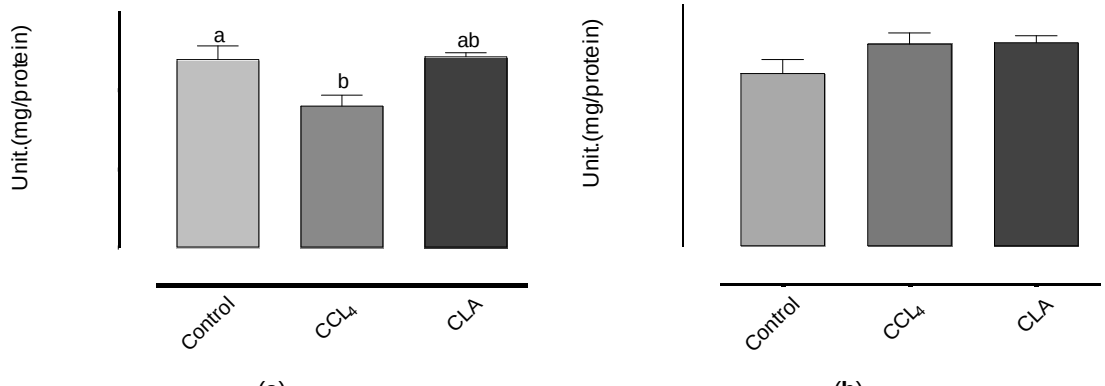
FA Composition (%)	Control	CCl_4	CLA
C 13:0	4.5 ± 0.9	5.3 ± 1.1	4.0 ± 0,1
C 16:0	17.8 ± 0.7	17.2 ± 0.9	16.8 ± 0,4
C 18:0	16.1 ± 0.8	17.2 ± 0.8	19.0 ± 0,9
C 18:1t	6.4 ± 0.8	7.0 ± 2.1	5.7 ± 1,4
C 18:1c	2.5 ± 0.2	2.4 ± 0.4	1.7 ± 0,1
C 18:2c	24.3 ± 1.2	23.1 ± 1.7	22.1 ± 1,0
CLA 9c11t			1.7 ± 0.3
CLA 10t12c			1.0 ± 0.2
C 20:4 n6	24.7 ± 1.1	23.8 ± 1.8	23.6 ± 2.0
C 22:6 n3	3.5 ± 0.4	4.0 ± 0.4	4.3 ± 0.4
Saturated	33.9 ± 0.8[a]	34.4 ± 0.9[a]	35.8 ± 0.6[b]
Monounsaturated	2.5 ± 0.2	2.4 ± 0.4	1.7 ± 0.1
Polyunsaturated	52.6 ± 0.9	51.0 ± 1.3	52.8 ± 0.8
Trans	6.4 ± 0.8	7.0 ± 2.1	5.7 ± 1.4

[ab]Different letters indicate significant differences (p <0,05).
FA – Fatty Acid.

namely superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx). These enzymes constitute a mutually supportive team of defense against ROS. The decrease in SOD activity in the liver in rats treated with CCl_4 may be due to increased lipid peroxidation or inactivation of antioxidant enzymes. The decrease in GPx activity caused by CCl_4 toxicity may be due to decreased availability of GSH, which results in increased lipid peroxidation. The reduction in the level of SOD, CAT and GPx are consequences of liver damage in rats following administration of CCl_4 [6].

The activities of enzymatic antioxidants, assessed in this study, are presented in Figures **4**, **5** and **6**. In liver tissue, there was a significant reduction in catalase activity ($p < 0.05$), CCl_4 group (Figure **4a**) compared to the control group. Supplementation with CLA increased the activity of CAT activity values close to the control group. There was no significant difference in SOD activity between the groups (Figure **4b**).

The evaluation by RT / PCR shows the changes in gene expression of catalase and superoxide dismutase between groups evaluated (Figure **5**). The levels of CAT increased in the liver tissues after repeated administration of CLA, this figure shows changes in the expression of catalase evaluated by RT / PCR gene. The bands representing the expression of the SOD gene show no change, by reproducing the results of



(**a**) (**b**)

Figure 4: Activity of catalase (CAT - **a**) and superoxide dismutase (SOD - **b**) in rat liver. Groups (n = 6) control, CCl_4 and CLA. Different letters indicate significant differences (p <0,05).

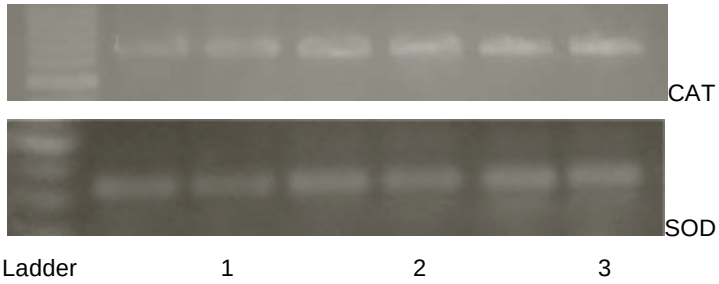


Figure 5: Gene expression (RT-PCR) of the activity of catalase (CAT) and superoxide dismutase (SOD) in liver tissue of rats. Groups (n = 6): control (1), CCl_4(2), CLA (3).

antioxidant activity for this enzyme. It is possible that for the CAT, the CCl4 increase the expression of this enzyme and that there is a physical barrier, or CLA may be being used as substrate for the tetrachloride or acting as an antioxidant and thus would remain available for more CAT enzyme activity as comproved in the Figure **4**.

Regarding the group of glutathione, one can observe a significant increase ($p < 0.05$) reduced glutathione (GSH), the CLA-supplemented group, reaching levels similar to the control group (Figure **6a**). It is clear, also a slight increase in the activities of GR to the group treated with CLA (Figure **6b**) confirming the effectiveness of the antioxidant capacity of the CLA. The administration of CCl_4 did not interfere in the activity of GPx in the control group, on the other hand, the activity of GPx in the group supplemented with CLA was lower than the control group, although not presenting difference from CCl_4 group, results shown in Figure **6c**.

Research shows that administration of oils with claims antioxidant properties reduce the levels of markers of lipid peroxidation, and provide increased antioxidant activity, but because they are olive oil [7], almond oil [6], and pumpkin seed oil [29], the effects can be attributed to the presence of unsaturated fatty acids, phenolic compounds and tocopherols.

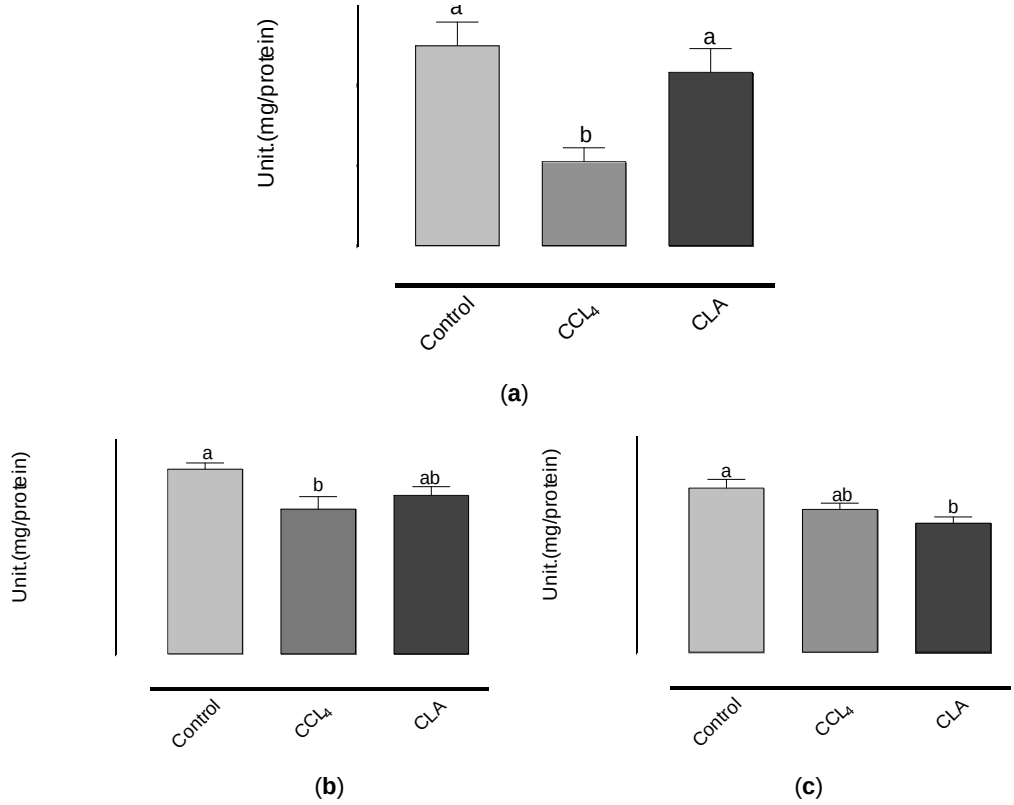


Figure 6: Values of reduced glutathione (GSH - **a**), and the activity of glutathione reductase (GR - **b**) and glutathione peroxidase (GPx - **c**) in rat liver. Groups (n = 6): control, CCl_4 and CLA. Different letters indicate significant differences ($p < 0,05$).

Oxidative stress plays a critical role in the pathophysiology of several liver diseases, and many complications of these diseases are mediated by oxidative stress, oxidative stress related mediators, and inflammation. Oxidative stress in the liver is induced by numerous systemic diseases such as hypertension, diabetes mellitus, and hypercholesterolemia; by agents such as antibiotics, chemotherapeutics, and radio contrasts; and environmental toxins, occupational chemicals, smoking, as well as alcohol consumption [29].

CLA has different isomers in respect to position and configuration of double bonds. Different isomers have different *in vitro* and *in vivo* anti-oxidative and anti-inflammatory effects that already have been studied extensively [26, 30-32]. Some researchers report the antioxidant effect of the AGC as a possible explanation of the beneficial health effects [30, 33], However few studies have evaluated the antioxidant activity of these components *in vitro*. The conjugated fatty acids are lipidic components, which make the application of very limited testing due to its lipossoluble. Faced with this feature, assessment is confined to test scan DPPH • radical (2,2-diphenyl - 1 - picrylhydrazyl) in determining the antioxidant capacity of conjugated linoleic acid (CLAs) isomers [31]. These authors [30-33], found that CLAs have high scanning activity of free radical by DPPH•, which may contribute to its biological activity. Another study also demonstrated the ability of CLAs isomers in reducing free radicals by the methods photoemission (using tert-butyl hidroxiperóxide to induce peroxidation of PUFAs and measuring the reduction of the chemiluminescence reaction by adding the CLAs isomers) and spectrophotometric evaluation by measuring the decrease the absorbance DPPH• solution with the addition of CLAs isomers [32].

The CLAs activity as antioxidants has been studied in recent years, but the results are contradictory and do not allow clear conclusions on the antioxidant activity of these compounds. Research evaluated the supplementation effect of a high fat diet plus 3% of CLAs (*cis*-9, *trans*-11 and *trans*-10, *cis*-12) for 30 days in a healthy animal model (CF1 mice). The results showed that the CLAs fed group showed an increase in the content of reduced glutathione (GSH) levels in liver tissue. The activity of glutathione peroxidase (GPx) and catalase (CAT) did not increase with supplementation of CLAs [34].

In another study [21], reported that supplementation with CLAs in healthy Sprague-Dawley provides reduction in MDA levels in plasma and liver tissue when compared to the control group supplemented with soybean oil. CLAs supplementation significantly increased by about 3.7 times, the CAT activity in plasma, since the activity of SOD, GPx, GR and GSH plasma levels did not differ significantly in the control group.

Studies on the effects of CLAs on models with altered redox state are scarce. Research examined the biological effects of a commercial mixture of isomers of CLAs in a model of fibrosis induced by carbon tetrachloride (CCl_4). The CLAs diet significantly inhibited the increase of collagen fibers in the liver of animals in the control group was observed an increase in the number of collagen fibers and fibrosis around the central vein. Although the control group was observed an increase in the number of cells α-SMA (α-smooth muscle actin) around the portal vein. However, in the group fed the cell number CLAs α-SMA was similar to that observed around the vein of a normal liver [11]. These results indicate hepatoprotective effects of CLAs on liver fibrosis induced by CCl_4. In the present study, the results also suggest that supplementation with CLAs has a hepatoprotective effect in model rats by CCl_4 oxidative stress by reducing lipid peroxidation and increasing the activity of CAT and GSH content.Some researchers attempted to explain the appearance of steatosis and steatohepatitis by the determination of the expression of α and γ peroxisome proliferator activated receptors (PPAR α and PPAR γ). There is a hypothesis that CLA acts as a ligand of PPAR, especially PPAR α and PPAR γ [23, 35, 36]. If this is indeed the case, CLA supplementation may be efficient in hepatic lipid disease prevention, and may act as an adjuvant in its treatment.

CONCLUSIONS

CLAs supplementation led to attenuation of the liver damage induced by CCl_4 in rats, as shown by reduction in the levels of thiobarbituric acid reactive substances and an increase in the content of reduced glutathione, as well as the activity of catalase and glutathione reductase enzymes in the liver. Although oils and fats are considered risk factors for liver damage due to susceptibility to free radical attack, the data presented here suggest a hepatoprotective activity of CLAs, due to its ability to improve the oxidative stress caused by CCl_4.

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