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Zingiber officinale formulation reduces hepatic injury and weight gain in rats fed an unhealthy diet

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Abstract: This study investigated the ability of formulation containing *Zingiber officinale* (ginger) to reverse health changes promoted by unhealthy diet in Wistar rats. Five compounds from the gingerol family and three from the shogaol family were identified in the chromatographic analyzes of the extract. The animals were fed a combination of unhealthy foods, the cafeteria diet, which promoted increases in body weight, hepatocyte nucleus area, total hepatocyte area and liver fat accumulation, as well as reduced hepatic glutathione S-transferase concentration, compared to the control group, which received commercial chow. The treatment with ginger improved all these results, highlighting the reduction of 10% of body weight and 66% of the total area of lipid droplets deposited, compared to the group that received the cafeteria diet. Ginger treatments also attenuated lipid peroxidation, with a mean reduction of 41% in malondialdehyde levels and a mean increase of 222% in glutathione S-transferase activity in the liver. The cafeteria diet and ginger extract did not promote significant changes in glycemic and lipid profile, liver weight and liver enzymes compared to the control group. We suggest that ginger can have beneficial effects on health complications associated with unhealthy diet, such as excessive adiposity, oxidative stress and hepatic injury.

Key words: cafeteria diet, gingerol, nonalcoholic fatty liver disease, oxidative stress, shogaol, *Zingiber officinale*.

INTRODUCTION

Reducing consumption of *in natura* foods and the inclusion of industrialized products in food habits contribute to the development of chronic noncommunicable diseases such as cardiovascular problems and type 2 diabetes. Non-alcoholic fatty liver disease (NAFLD) is a highly prevalent metabolic complication, which is directly associated with imbalance in food intake and obesity (Vernon et al. 2011). Its reversibility is possible from changes in dietary behavior and by specific nutritional therapies.

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Ginger (*Zingiber officinale*) belongs to the family Zingiberaceae is widely used as a medicinal plant in Brazil and in the world for cancer treatment, as anti-inflammatory and anti-oxidant (Isa et al. 2008). Its rhizome is also widely consumed food in the world adding an exotic flavor to food. The main ginger-derived components are gingerol and shogaol that have been shown reduce lipid peroxidation (Afshari et al. 2007) and metabolic changes (Isa et al. 2008, Sahebkar 2011, Tzeng and Liu 2013). In obese women, it promoted weight reduction and decreases in insulin, leptin, resistin and glucose levels (Attari et al. 2016). In animals fed a high-fat diet (Tzeng et al. 2015), as well as in those with ethanol-induced steatosis (Nwozo et al. 2014), administration of ginger attenuated the accumulation of fat in the liver and inflammation. These findings demonstrate the effect of ginger on different metabolic risk factors in adverse food style conditions. The objective of this study was to evaluate the action of ginger in reversing or attenuating the deleterious metabolic effects of an unhealthy diet.

MATERIALS AND METHODS

HARVESTING, DRYING AND EXTRACTING OF PLANT MATERIAL

Ginger rhizomes were collected at an experimental farm in the state of Espírito Santo, Brazil. The fresh vegetable material (4 kg) was washed in running water, sectioned and oven dried at 40°C. The material was transformed into powder (400 g) and then subjected to cold extraction in 1L 96% ethyl alcohol by maceration. After 72 hours, the alcohol extract was filtered, lyophilized (41 g yield, 10.2%) and manipulated as an oral formulation at 15% concentration of the extract.

PHYTOTHERAPEUTIC FORMULATION CONTAINING *Zingiber officinale*

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CHEMICAL COMPOSITION OF THE EXTRACT

The alcohol extract of ginger (5 mL) was centrifuged at 4000 rpm for 30 minutes. The supernatant was removed and filtered in a membrane with a porosity of 0.45 micrometers. The extract was injected into the gas chromatography coupled to mass spectrometry (Shimadzu GC-17A and Shimadzu GCMS-QP5050A) for the qualitative determination of the chemical composition and fragmentation of the compounds of the extract. The chromatographic conditions used were: entrainment gas He under flow of 0.8 mL x min^{-1}; Injector temperature 240°C, detector temperature 260°C; Initial column temperature 40°C, isothermal for 10 minutes, followed by heating at 8°C x min^{-1} to 300°C, remaining isothermal for 18 minutes; sample injection volume: 1.0 µL split ratio 1:5; column pressure 33 kPa. The ionization process occurred by electron impact (70 eV) and the sweep amplitude was of 40 to 600 Da. The obtained mass spectra were compared to the data available in the 7th edition of the Wiley equipment.

ANIMALS

The study was approved by the Ethics Committee of Animal Research of the Universidade Federal de Viçosa (protocol 80/2014). All procedures performed involving animals were in accordance with the ethical standards of the institution at which the study was conducted. Thirty male albino rats (*Rattus norvegicus*), Wistar line, with 52 days of age and approximately 200 g were used. The animals, obtained from the Central Husbandry of the Universidade Federal de Viçosa, were placed in individual polyethylene cages, closed with a stainless-steel lid, in an environment with temperature control (22°C ± 2°C), 12 h/12 h light/dark cycle and air exhaust system.

EXPERIMENTAL DESIGN

The rats received commercial chow and water *ad libitum* in the pre-experimental period. At 52 days

of age, the animals were divided into: (1) control group fed a commercial chow (CTR) (n= 6) and (2) group fed a cafeteria (CAF) diet (n= 24). The CAF diet (Table I), produced and offered in pellets, was used to induce metabolic effects associated with low quality and high caloric diet. After 45 days, the treatment of 20 days with the formulation containing *Zingiber officinale* (ZO) was started. The animals were divided into five experimental groups of six animals: (1) CTR without treatment; (2) CAF without treatment (CAF); (3) CAF + 75 mg/kg ZO (CAF+ZO1); (4) CAF + 150 mg/kg ZO (CAF+ZO2); (5) CAF + 300 mg/kg ZO (CAF+ZO3). The doses used in this study were based on previous studies that administered ginger concomitantly to hypercaloric or hyperlipidic diets (Nammi et al. 2009, Bhandari et al. 2005, Li et al. 2014).

The formulation containing ZO was administered daily at the same time, by gavage. Groups 1 and 2 received distilled water by gavage. The animals were weighed daily to calculate the dosage to be administered and to evaluate the weight gain. Food consumption was also measured daily.

At the end of the experiment, after 12 hours of fasting, the animals received inhaled anesthesia with isoflurane (Isoforine®, Cristália, Itapira, Brazil) and were euthanized by cardiac puncture. Samples of blood and liver were collected.

BIOCHEMICAL ANALYSIS

The blood was centrifuged at 3500 rpm for 10 minutes at 4°C. The plasma was used to determine the concentrations of total cholesterol (TC), high-density lipoprotein (HDL), triglycerides (TG), glucose, total bilirubin (TB), aspartate aminotransferase (AST) and alanine aminotransferase (ALT). Analysis were performed using colorimetric methods and specific commercial kits (Bioclin®, Belo Horizonte, Brazil).

TABLE I
Composition of the cafeteria diet.

Ingredients	grams/1000g	Kcal/100g
Pâté Ham	222	520
French fries	111	282
Bacon	111	625
Mortadella	111	270
Sweet cookie	111	440
Chocolate powder	111	385
Whole milk powder	111	538
Commercial chow	112	360
Total	1000	427

HISTOLOGICAL ANALYSIS

A fragment of the liver was fixed for 24 hours in 10% v/v buffered formaldehyde. The fragments were dehydrated, diaphanized in xylol and embedded in paraffin, using routine methods. 5 μm thick sections were made on a microtome and stained with hematoxylin and eosin. Ten random fields in each histological section were photographed using photomicroscope (Olympus x-41, Olympus Optical do Brasil Ltda, São Paulo, Brazil). The area of the hepatocytes (μm^2), area of the nucleus (μm^2), volume density of the hepatic sinusoids (%) and the lipid droplets (%) were analyzed by counting in a points system. For this analysis, the software used was Image Pro-Plus 4.5 (Media Cybernetics, Silver Spring, MD, USA).

OXIDATIVE STRESS

Samples of 100 mg of liver were homogenized in phosphate-buffer (pH 7.0) and centrifuged at 3500 g for 10 minutes at 5°C. The supernatant was used for the catalase (CAT), superoxide dismutase (SOD), glutathione S-transferase (GST) and malondialdehyde (MDA) analysis.

CAT activity was assessed by measuring the rate of decomposition of hydrogen peroxide (Aebi 1984). SOD activity was determined by the xanthine oxidase method based on the production of hydrogen peroxide and the reduction of nitroblue

tetrazolium (Sarban et al. 2005). Quantification of GST activity was done by spectrophotometry by measuring the product obtained from the complexation of reduced glutathione with 1-chloro-2,4-dinitrobenzene (Keen et al. 1976). Lipid peroxidation was evaluated by the quantification of MDA (Buege and Aust 1978). The Bradford method was used for the determination of total protein (Bradford 1976).

STATISTICAL ANALYSIS

Data distribution around the mean was verified by the D'Agostino-Pearson test. Asymmetric data were analyzed by the Kruskal-Wallis test and the symmetric data were analyzed using one-way ANOVA, followed by the Tukey test using GraphPad Prism 5.01 statistical software (GraphPad Software, Inc, CA, USA). The level of significance considered was of 5%.

RESULTS AND DISCUSSION

The main findings of this study were the control of body weight and hepatic injury, as well as the improvement of the antioxidant profile in rats treated with ginger.

CHARACTERIZATION OF THE EXTRACT

The chromatographic profile of the ethanolic extract of ginger indicated the presence of eight important bioactive compounds of the gingerol and shogaol families (Fig. 1a). The most prominent component in the extract was 6-shogaol. Concentrations of the other components, in relation to 6-shogaol, were around 85, 16, 27, 10, 19, 45 and 32%, corresponding, respectively, to 8-gingerol, 6-gingerol, 8-shogaol, 14-gingerol, 10-gingerol, 10-shogaol and 12-gingerol (Fig. 1b).

Ginger bioactivity has been attributed mainly to components of the gingerol and shogaol family, due to their antioxidant (Pournaderi et al. 2017), anti-inflammatory (Funk et al. 2016), hepatoprotective (Cheong et al. 2016), hypoglycemic (Sampath et al. 2017), insulin sensitivity (De Las Heras et al. 2017) and anti-obesogenic (Saravanan et al. 2014), among other functions, already described in the literature. In this study, eight components of these families were identified, among them 6-gingerol, 6-shogaol and 10-gingerol, which showed the highest antioxidant activity among the other bioactive compounds of these families (Lu et al. 2014). The use of gingerol promoted results similar to those of this study, such as reduction of weight gain and hepatic steatosis, but also improved plasmatic glycolic and lipid markers (Tzeng et al. 2015, Naidu et al. 2016).

FOOD INTAKE, BODY WEIGHT AND LIVER WEIGHT

In the period prior to treatment with ginger, there was no difference in food consumption and weight gain among the animals. At the beginning of the treatment with ginger, there was no difference in body weight among the animals, however, at the end, the animals that received only a CAF diet, but not those fed a CAF + ginger extract at any concentration, presented higher body weight compared to CTR group. Animals fed with CAF, with or without ZO (except CAF+ZO2), presented increased daily caloric consumption compared to the CTR group (Table II). These data demonstrate the effect of ginger on body weight control, even in similar conditions of caloric intake and nutritional density.

Mahmoud and Hegazy (2016) observed that ginger was able to reduce hepatic damage associated with the aging process in elderly rats, such as increased nucleus volume and sinusoidal congestion and collapse, similar to that observed in the present study. Reduction of body weight and fat vacuoles and droplets in the liver was also observed in rats with obesity induced by high fat diet and treated with ginger essential oil (Lai et al. 2016). However, in the aforementioned study, which

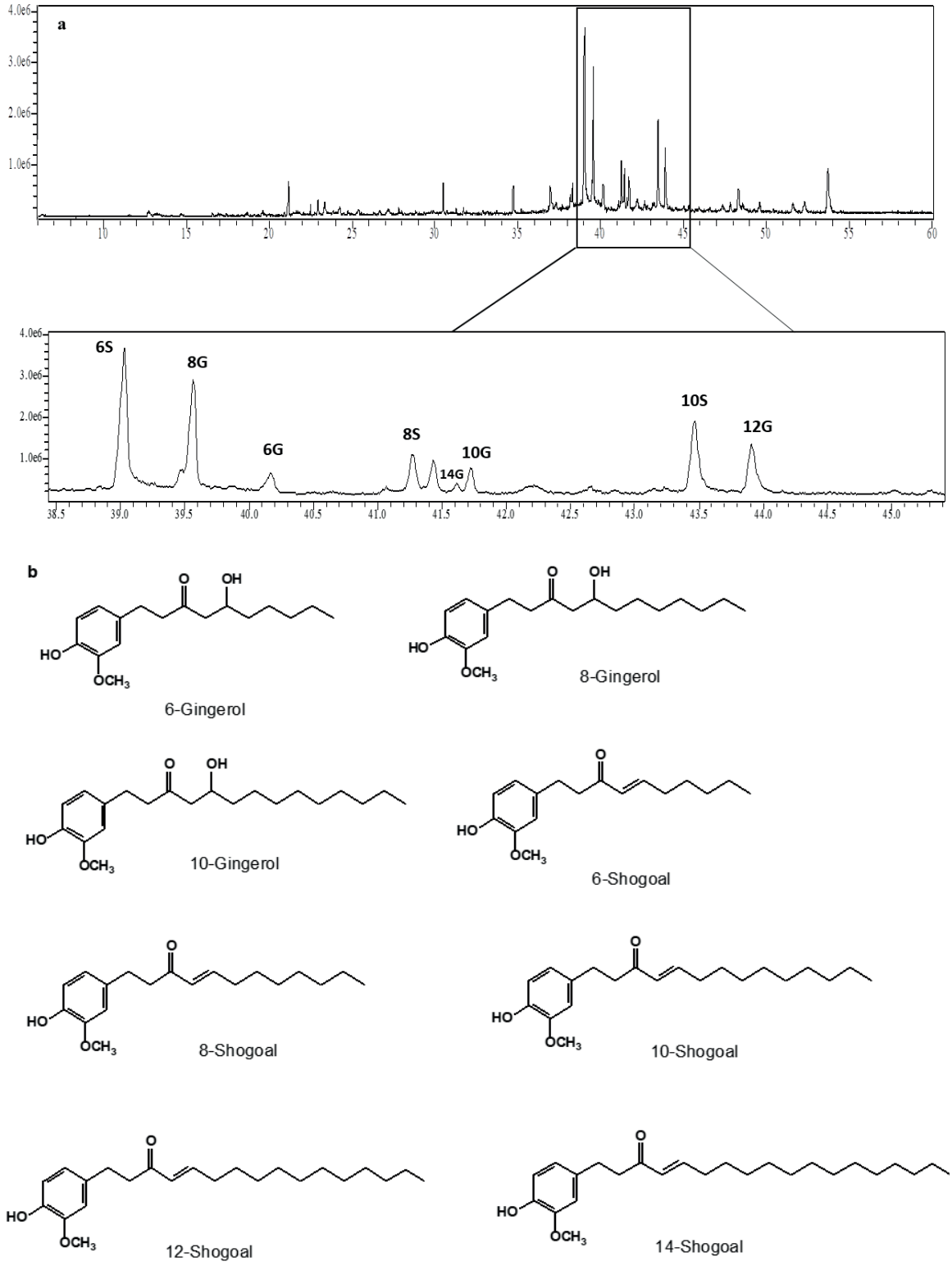

Figure 1 - **a** Chromatogram of the *Zingiber officinale* extract used in the study. 6S: 6-shogaol; 8G: 8-gingerol; 6G: 6-gingerol; 8S: 8-shogaol; 14G: 14-gingerol; 10G: 10-gingerol; 10S: 10-shogaol and 12G: 12-gingerol. **b** Chemical structure of the active compounds found in the formulation.

TABLE II
Caloric intake and body and liver weights in the different experimental groups.

	CTR	CAF diet	CAF+ 75 mg ZO/kg (ZO1)	CAF+150 mg ZO/ kg (ZO2)	CAF+300 mg ZO/ kg (ZO3)
Daily calories (kcal)	73 ± 8[b,2]	83 ± 5[a,1]	83 ± 4[a,1]	76 ± 5[b,2]	85 ± 5[a,1]
Body weight, day 45 (g)	326 ± 52	392 ± 27	357 ± 43	370 ± 21	371 ± 36
Body weight, day 65 (g)	333 ± 45[b]	444 ± 28[a]	396 ± 44[b]	400 ± 26[b]	409 ± 42[b]
Liver weight (g)	12 ± 2	14 ± 1	12 ± 1	13 ± 2	13 ± 1

Values expressed as mean ± standard deviation. Abbreviations: CTR: control fed a comercial chow; CAF: cafeteria diet; ZO: formulation containing ginger.
[a] and [b] indicate difference by the Tukey test;
[1] and [2] indicate difference by the Kruskal-Wallis test.

had a longer duration (12 weeks), attenuation of the increase in liver weight was observed due to ginger, which was not verified in the present study (Lai et al. 2016).

GLYCEMIC, LIPID AND HEPATIC MARKERS LEVELS

There was no statistical difference in glycemia among animals fed a CAF diet, with or without ZO, however, the CAF+ZO3 group presented 163 ± 13 mg/dL of glucose with a 14% reduction compared to CAF. The CAF group presented a 190 ± 53 mg/dL of glucose. This reduction presented as benefit of ZO3 formulation. The lipid profile, represented by TC, HDL and TG levels, also did not change among the animals. The ZO treatments did not promote hepatic toxicity, as observed by the absence of statistical difference of ASL and ALT between the groups. Only the CAF+ZO1 group presented higher values of bilirubin compared to the animals of the CTR group (Table III).

After confirming the in vitro activity of 6-gingerol in the suppression of TG formation induced by oleic acid in HepG2 cells, Tzeng et al. (2015) tested different concentrations of this compound in hamsters fed with high fat diet. Oral administration of 6-gingerol at a dose of 25, 50, or 100 mg/kg/day reduced plasma TC levels in a dose-dependent manner. Only the highest dose (100 mg/kg/day) reduced plasma TG and low-density lipoprotein (LDL) colesterol levels. Rats fed a similar dietary style presented, after thirty days of treatment with 75 mg/kg body weight of gingerol, reduced plasma glucose, plasma insulin and insulin resistance (Naidu et al. 2016). In the present study, the extract of ginger did not promote reduction of total cholesterol, however, ginger has been shown to reduce intestinal cholesterol absorption and increase fecal cholesterol excretion in animal models (Han et al. 2005). In rats with diet-induced hypercholesterolemia, 14-day treatment with two varieties of the ginger family (*Zingiber officinale* and *Curcuma longa*) promoted reductions in LDL and TG and increased HDL when compared to untreated controls (Akinyemi et al. 2016).

HISTOLOGICAL ANALYSIS

Compared to the CTR group, CAF diet, with or without treatment, promoted the increase of fat deposition in the liver of the animals ($p < 0.01$) (Fig. 2). However, the treatments with the formulation containing ZO, at all concentrations, promoted the mean reduction of 66% in the hepatic fat accumulation compared to untreated CAF group ($p<0.001$). There was no statistical difference in the accumulation of fat in the liver among the groups treated with ZO at the three different concentrations.

The groups CAF, CAF+ZO1 and CAF+ZO2 presented increased area of hepatocytes, and these groups, except for CAF+ZO2, also showed a larger

TABLE III
Circulating concentrations of glucose and lipidic and hepatic markers (mg/dL) in the different experimental groups.

Variables	CTR	CAF diet	CAF+ 75 mg ZO/kg (ZO1)	CAF+150 mg ZO/kg (ZO2)	CAF+300 mg ZO/kg (ZO3)
Glucose	152 ± 14	190 ± 53	195 ± 30	195 ± 32	163 ± 13
Δ%	- 20	0	3	2	- 14
TC	72 ± 15	54 ± 6	63 ± 16	60 ± 11	55 ± 6
Δ%	34	0	17	11	3
TG	108 ± 36	94 ± 31	88 ± 26	95 ± 29	92 ± 32
Δ%	14	0	- 6	0	- 3
HDL	24 ± 3	18 ± 2	20 ± 3	18 ± 3	18 ± 1
Δ%	29	0	10	- 1	- 1
AST	95 ± 15	87 ± 13	76 ± 20	77 ± 14	70 ± 7
Δ%	9	0	-12	-11	-20
ALT	40 ± 6*	58 ± 8*	45 ± 17	40 ± 8*	37 ± 10*
Δ%	-31	0	-22	-31	-36
TB	0.08 ± 0.01	0.09 ± 0.05	0.17 ±0.08*	0.11 ± 0.02	0.12 ± 0.03
Δ%	-11	0	88	22	33

Values expressed as mean±standard deviation (mg/dL). CTR: control fed a comercial chow; CAF: cafeteria diet; ZO: formulation containing ginger. Abbreviations: TC: total cholesterol; TG: triglycerides; HDL: high density lipoproteins; TB: total bilirubin; AST: aspartate aminotransferase; ALT: alanine aminotransferase.
Δ%: Variation (%) in relation to CAF group.
*$p<0.05$ in relation to the CTR group, by the Kruskal-Wallis test.

area of nuclei, compared to the CTR group ($p<0.01$). For both measurements, animals treated with ZO2 or ZO3 showed reductions compared to the CAF group ($p<0.01$). Animals fed a CAF diet, with or without ZO, presented higher volume density of hepatic sinusoids compared to the animals of the CTR group ($p<0.001$) (data not shown).

The ultraprocessed and sugar rich foods that make up the CAF diet are considered efficient to induce the metabolic syndrome in humans (Sampey et al. 2009). In the present study, CAF diet was able and induce excessive weight gain and hepatic injury, but no changes in the biochemical markers glucose, TC, TG and HDL. It is believed that some metabolic changes can be controlled by homeostatic mechanisms, but that prolonged exposure to inadequate diet could promote them. Insulin resistance, described as a precursor of biochemical changes, was not evaluated in the present study. The elevation of this hormone, related to unbalanced and excessive energy consumption, may increase the TG synthesis in the liver (Swenson 1992). Lipid accumulation and oxidative stress are central interdependent events in the pathogenesis of NAFLD (Tiniakos et al. 2010). Human studies involving the use of ginger and NAFLD are scarce, however, it was found that supplementation with 2g/day of ginger in patients with NAFLD for twelve weeks promoted the reduction of the degree of hepatic steatosis, insulin resistance and TNF-α, a pro-inflammatory cytokine (Rahimlou et al. 2016).

OXIDATIVE STRESS

There was no difference between the animals regarding the levels of SOD in the liver (Fig. 3a). CAT levels were similar between treatments with

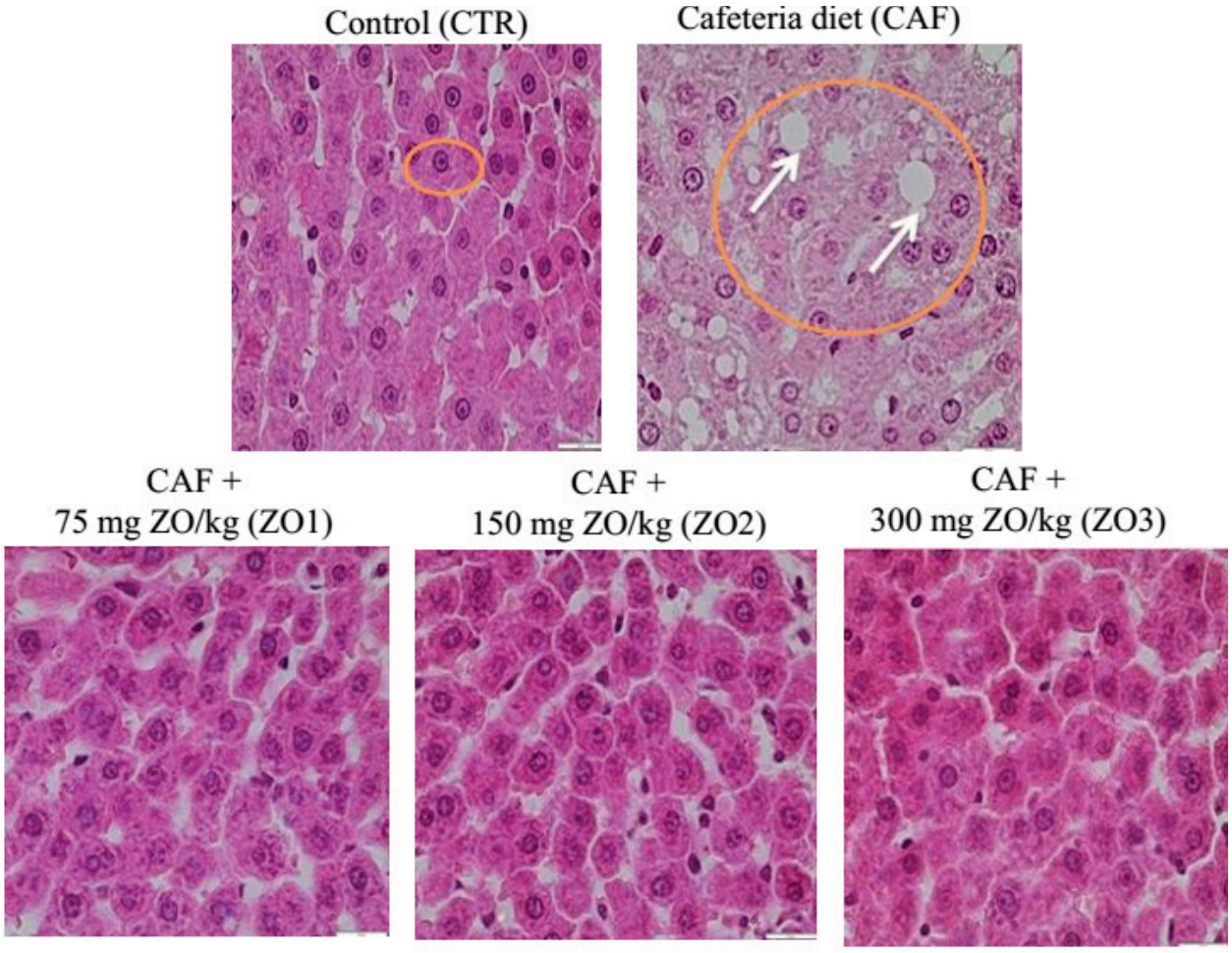

Figure 2 - Histological images of the liver of animals from different experimental groups. White arrows show fat deposition in the liver. CTR: control fed a commercial chow; CAF: cafeteria diet; ZO: formulation containing ginger. Scale bar, 20 μm.

ginger and between the CTR and CAF groups. CAF+ZO1 and CAF+ZO2 presented reduction in the hepatic CAT compared to the CTR and CAF groups (Fig. 3b). All ginger treatments had higher GST levels compared to the CTR and CAF groups (Fig. 3c). Lower lipid peroxidation, represented by lower levels of MDA, was observed in all treatments with ginger compared to the CTR group. Only the CAF+ZO1 group presented lower levels of MDA compared to the CAF group. There was no difference in the concentration of MDA between the CTR and CAF groups (Fig. 3d).

In the present study, in general, lower lipid peroxidation, verified by the lower hepatic concentration of MDA, was observed in animals treated with ginger. In addition, animals receiving ZO at all concentrations had higher levels of GST, a key enzyme in intracellular detoxification of endo and xenobiotic compounds (Chelvanayagam et al. 2001) compared to animals of CAF and CTR groups. Lai et al. (2016) observed increased MDA levels in animals fed for 12 weeks with high-fat diet compared with the control group, whereas animals treated with ginger essential oil showed a significant decrease of this enzyme concentration. Similar to the present study, the authors observed increased hepatic GST levels in animals treated with ginger essential oil, as well as no increase of CAT in animals treated with the lowest dosages.

As limitations of the present study, the duration may have been insufficient, and the animal

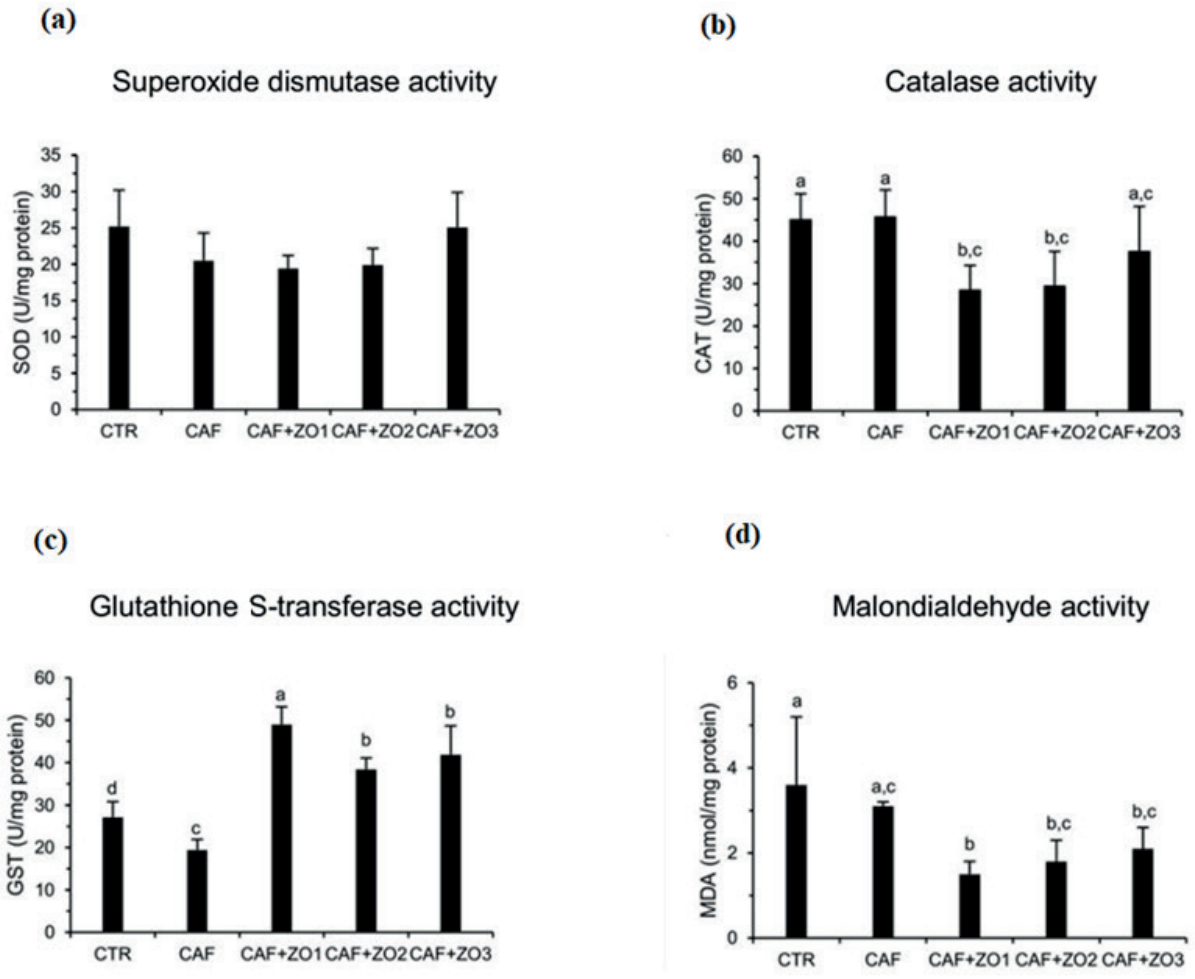

Figure 3 - Oxidative stress variables in the different experimental groups. Values expressed as mean ± standard deviation. **CTR**: control fed a comercial chow; **CAF**: cafeteria diet; **ZO**: formulation containing ginger; **CAF + ZO1**: 75 mg/kg **ZO**; **CAF + ZO2**: 150 mg/kg **ZO**; **CAF + ZO3**: 300 mg/kg **ZO**; (**a**) SOD: superoxide dismutase; (**b**) CAT: catalase; (**c**) GST: glutathione-S-transferase; (**d**) MDA: malondialdehyde.
[a,b,c,d]Different letters above the columns indicate a statistical difference ($p < 0.05$) between the groups, by ANOVA.

model may not have been ideal for induction of biochemical changes with CAF diet.

CONCLUSION

We conclude that, in general, the CAF diet did not alter biochemical parameters and treatment with ginger kept the balance in such markers. However, CAF diet promoted weight gain and hepatic injury in animals and the ginger-containing formulation improved these parameters by reducing fat deposition, nuclei area and hepatocyte size in an animal model. In addition, ginger treatment attenuated lipid peroxidation, improved the antioxidant profile in the liver of the animals and has an interesting effect as it helps prevent the progression of simple steatosis to a possible inflammatory steatosis.

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AUTHOR CONTRIBUTIONS

Dalila T. Leal, Gleide G. Fontes and Julia K.D. Villa contributed in collecting plant sample and identification, confection of herbarium, running the laboratory work, analysis of the data and drafted the paper. Rodrigo B. Freitas and Mateus G. Campos contributed to biological studies. Camilo A. Carvalho and Virginia R. Pizziolo contributed to chromatographic analysis. Marisa A.N. Diaz designed the study, supervised the laboratory work and contributed to critical reading of the manuscript. All the authors have read the final manuscript and approved the submission.

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