

Research Article

The Coffee Protective Effect on Catalase System in the Preneoplastic Induced Rat Liver

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This study aimed to evaluate the effect of organic/conventional coffee in liver tissues in the cancer process, taking into account the level and activities of catalase. The experiments were carried out with 8 groups of rats during 12 weeks. They received two injections of ethylenediaminetetraacetic acid solution 1.5% (v/v) prepared in 0.9% NaCl or 1,2-dimethylhydrazine (DMH) subcutaneous dose of 40 mg·kg⁻¹·bw⁻¹ for 2 weeks. The organic/conventional coffee infusions were at 5, 10, and 20% and were incorporated to feed (100 mL of infusion·kg⁻¹ of diet). The catalase activity showed a decrease for livers which received DMH and DMH plus organic coffee at 5% and 10%. However, an increase was observed for those receiving organic 20% and conventional 10% coffee, slowing down and favoring the reversibility of the carcinogenic process. By SDS-PAGE, we observed an intensity decrease of 59 kDa bands, as the percentage of coffee was increased. The iron concentration (by ET-AAS) confirmed the electrophoretic results, suggesting that the DMH influenced the catalase expression conditions, reducing the activity by the loss of iron ions. Thus, the coffee may restore the catalase system in the liver, exerting its chemopreventive effects.

1. Introduction

Liver cancer is considered one of the most aggressive ones and corresponds to the third leading cause of cancer deaths worldwide [1–3]. Among the new treatment alternatives and clinical procedures, the use of chemopreventive approaches has been researched and applied. Dietary components have shown great potential to suppress carcinogenesis in pre-clinical neoplastic models as well as prevent or delay the occurrence of this disease [4].

In this scenario, coffee emerges as one of the alternatives for these chemopreventive approaches [5]. Coffee is the third most popular beverage in the world after water and tea, with average daily consumption of 150 mL per person [6–8]. In addition, roasted coffee infusion has thousands of compounds such as phenolic polymers, amino acids, sugars and polysaccharides, niacin, chlorogenic acids, minerals, caffeine,

organic acids, lipids, and aromatic compounds [9]. They form a complex mixture of different concentrations which depend on environmental factors, cultivation, crop management, and grain processing [10]. The differences between conventional and organic coffee are mainly attributed to fertilization methods. According to Carvalho et al. [10], the concentrations of chlorogenic acids, trigonelline, and caffeine in organic arabica coffee (ORC) were higher than in the conventional coffee (CC) (4.6, 9.6, and 4.4% higher, resp.). The antioxidant compounds in coffee contribute to the reduction of free radicals that naturally occur or are chemically induced in the human body [11]. For example, caffeine is used in *in vitro* studies, as an inhibitor in N-demethylation reactions [12, 13]; it is also able to reduce liver fibrogenic processes, which regulate the growth of liver cells [6, 14, 15]. Polyphenols and melanoidins also act as protectors, reducing liver chronic diseases [16, 17].

Considering that researches related to the functional properties of the bioactive constituents of coffee, as well as the influence and interaction of these compounds to human health, we decided to compare the protein changes, especially catalase (CAT), in liver tissues of animals under colon neoplastic lesions, induced by DMH. In this context, proteomics has played an important role in the global monitoring of protein changes in tissues or individuals with cancer [18], once these modifications are catalyzed by critical enzymes in numerous cellular functions.

The CAT are tetrameric metalloproteins (heme or linked to Mn), which act as antioxidants [19] that catalyze the degradation reaction of hydrogen peroxide into oxygen and water. CAT has been found to significantly decrease chromosomal aberrations and also to delay or prevent the onset of spontaneous neoplastic transformation in mice fibroblasts and epidermal keratinocytes [20].

It is known that liver catalase activity becomes reduced in the presence of a growing tumor. However, after the tumor is removed, this activity returns to normal, showing that the tumor tends to suppress it as an antioxidant [21] but there is no evidence about the food interference in this mechanism yet.

The use of 1,2-dimethylhydrazine (DMH) and its metabolites are an excellent experimental model for studies of new cancer treatments [22]. It is an aliphatic methylating carcinogen, metabolized in the liver to form active intermediates like azomethane, azoxymethane, methylazoxymethanol, and ultimate carcinogenic metabolite, diazonium ion (by NAD⁺-dependent dehydrogenase), which are actively transported subsequently into the colon via bile and blood [23]. Once metabolized in the liver, the active methyl diazonium ions, capable of methylating DNA, RNA, or protein of colonic epithelial cells, induce more oxidative stress, resulting in overproduction of reactive oxygen species (ROS).

Therefore, the liver was chosen as the study tissue, and catalase activity can be used as excellent preventive chemical marker [24] to investigate cancer occurrence, as well as treatment efficiency. The objectives were to compare the differences of catalase activity in this tissue caused by the ingestion of coffee infusion, obtained from organic and conventional management systems. We also compared the electrophoretic liver protein band intensities and determined the iron concentrations in CAT SDS-PAGE bands, relating these differences with chemopreventive mechanisms.

2. Materials and Methods

To evaluate the coffee protective effect on catalase system, we performed an *in vivo* experiment aiming to obtain preneoplastic induced rat livers. From these livers all analyses were carried out such as protein extractions, determination of liver's total protein concentration, determination of catalase activity, liver's proteins separation by SDS electrophoresis, microwave-assisted acid digestion of catalase electrophoretic bands, and iron determination in these electrophoretic bands, as described below.

TABLE 1: Experimental design.

| Group | Reagent | Coffee |
|------------------|---------|--------------|
| G1/DMH + CD | DMH | — |
| G2/EDTA + CD | EDTA | — |
| G3/DMH + ORC 5% | DMH | Infusion 5% |
| G4/DMH + ORC 10% | DMH | Infusion 10% |
| G5/DMH + ORC 20% | DMH | Infusion 20% |
| G6/DMH + CC 5% | DMH | Infusion 5% |
| G7/DMH + CC 10% | DMH | Infusion 10% |
| G8/DMH + CC 20% | DMH | Infusion 20% |

Animals/group = 10, CD: commercial diet, ORC: organic coffee diet, CC: conventional coffee diet, DMH: 1,2-dimethylhydrazine 40 mg·kg⁻¹·bw⁻¹; and EDTA: 1.5% in NaCl 0.9% (DMH vehicle). Infusion 5%, 10%, and 20%: considering the incorporation of 100 mL of infusion·kg⁻¹ of ground feed.

2.1. In Vivo Experiment: Sample/Liver Collection. Samples of liver tissues were taken from Wistar rats, males, 21 days old, with acclimatization period of 2 weeks. Eighty animals were used, divided into 8 groups ($n = 10$); see Table 1. The procedures herein were approved by the Animal Ethics Committee in the Federal University of Alfenas, protocol: 235/09, and followed the Ethical Principles in Animal Experimentation adopted by the Brazilian College of Animal Experimentation (COBEA).

The experiment was conducted during 12 weeks. In the 1st and 2nd weeks, the animals were treated with either ethylenediaminetetraacetic acid (EDTA) solution 1.5% (v/v) prepared in 0.9% NaCl or 1,2-dimethylhydrazine (DMH) subcutaneous doses of 40 mg·kg⁻¹·bw⁻¹. These were supplied considering the proportion of 1.0 mL per 100 g of animal weight. The solutions were applied for two weeks, twice per week [25].

To analyze the coffee protective potential, 100 mL of each coffee infusion was incorporated to 1 kg of commercial diet (CD). Therefore, the organic coffee infusions (BCS-OKO Garantie Master Certificates numbers POCO-7569/07.08/14291-BR) at 5, 10, and 20% corresponded to groups G3, G4, and G5, respectively, whereas conventional coffee infusions at 5, 10, and 20% corresponded to groups G6, G7, and G8, respectively. G1 was the positive control group and G2 was the negative control group.

The animals were euthanized for liver collection in the end of the 12th week. The livers were subjected to perfusion with NaCl 0.9% (v/v) and stored in Ultrafreezer (U7386S60 Sanyo MDF, Japan) at -80°C. Samples of liver tissues were taken in triplicate (livers from three animals) from each group.

2.2. Chemical Analyses. All solutions were prepared employing deionized water from a purification system Milli-Q® water (Millipore®, Bedford, MA, USA), with 18.2 MΩ·cm resistivity. The sucrose and phosphoric acid were purchased from Isofar (Brazil). Acetone 90%, ethanol, acetic acid, nitric acid 10%, and bovine serum albumin were also used in the procedures and purchased from Sigma-Aldrich (Brazil). CBB G-250 colloidal, a solution of ammonium sulphate, 8% (w/v) Sigma-Aldrich (Brazil), phosphoric acid, 1% (v/v) Vetec (Brazil), CBB G-250, 0.08% (m/v) Serva Feinbiochemica GmbH & Co.

(Germany), and methanol, 20% (v/v) Sigma-Aldrich (Brazil), was used to reveal the proteins in the gels. Hydrogen peroxide 30% from Vetec (Brazil) and phosphate buffer and Triton X-100 from BioAgency (Brazil) were used for the sample preparation of activity measurements.

2.2.1. Protein Extractions. The thawed samples (2.0 g) were washed in 250 mmol·L⁻¹ sucrose solution [26] and ground in a mortar with the sucrose solution 1:4 (m/v) in an ice bath (at 0°C). Subsequently, the extracts were filtered in cheesecloth, centrifuged (5810R Eppendorf, Germany) for 30 min at 15,115 ×g, at 4°C. The precipitate was discarded, thereby obtaining the protein extract. The proteins of the supernatant were precipitated using a solution (3:1 v/v) containing acetone/ethanol (3:1 v/v) at -20°C, then maintained in contact for 2 h, and followed by centrifugation for 5 min at 804.96 ×g, at 4°C. The supernatant was discarded keeping the pellet. This cleaning procedure was repeated 3 times. Finally, after the complete evaporation of the solution acetone/ethanol, the pellet was resuspended in the appropriate buffer for analysis.

2.2.2. Enzyme Extraction to Catalase Activity Measurement. Fifty mg of liver tissues (in triplicate) was weighed and ground in a mortar with a solution (1:9 m/v) of Triton X-100, 1% (v/v) (Sigma-Aldrich, Brazil), at room temperature until lysing the organelles and enzyme exposure. Soon after, 50 mmol·L⁻¹ phosphate buffer (at pH = 7.0) was added at a ratio of 1:100 (v/v), and tissue extracts were immediately subjected to enzyme activity measurement.

2.2.3. Determination of Total Protein Concentrations. Bradford's method was applied [27], with bovine serum albumin solution, diluted in 100 mmol·L⁻¹ Tris HCl buffer (at pH = 7.4), as standard. Bradford's reagent solution was prepared daily, and the spectrophotometer Bel Photonics (SP 2000 UV, Brazil) at 595 nm was used. The total protein concentrations of the protein extracts, from three livers, randomly chosen from each group, were determined ($n = 3$) in relation to bovine serum albumin, with standard deviation. The total concentrations were between 246.42 ± 0.01 and 438.25 ± 0.02 mg·L⁻¹. These values were used for gel preparation, and also for catalase activity calculating, where the activity was calculated by the total protein amount in the tissue.

2.2.4. Determination of Catalase Activity. A spectrophotometer Biochrom Libra S22 (Biochrom, England) was used to determine the enzymatic activity, according to the method described by Aebi, 1984 [28]. The readings were at 240 nm in kinetic mode, from 0 to 15 s, and optical path of 10 mm, in quartz cuvette (1 mL). The activity of standard CAT (EC = 1.11.1.6) (Sigma-Aldrich, Brazil), 22.857 mg·L⁻¹ in 50 mmol·L⁻¹ phosphate buffer (at pH = 7.0), was measured in relation to 0.03 mol·L⁻¹ H₂O₂ (30%), with an initial absorbance of about 0.500. The analytical blank was 0.667 mL of enzyme solution plus 0.333 mL of 50 mmol·L⁻¹ phosphate buffer (at pH = 7.0). For sample absorbance measurements, 0.667 mL of enzyme solution and 0.333 mL of H₂O₂ (30%)

solution in 50 mmol·L⁻¹ phosphate buffer (at pH = 7.0) were used. As there is no international unit for the catalase activity, the rate constant of first-order reaction (k) was adopted. This rate constant is described by

$$k \cdot g^{-1} \text{ protein} = \left(\frac{2.3}{\Delta t}\right) \left(\frac{a}{b}\right) \left[\log\left(\frac{A1}{A2}\right)\right] \left(s^{-1}\right), \quad (1)$$

where Δt is time interval (15 s); a is the dilution factor, that is, the tissue protein concentration (mg protein·g⁻¹) divided by the protein concentration in the cuvette (mg protein·mL⁻¹); b is the mass of tissue divided by the protein amount in the extract (g·mg⁻¹), obtained by Bradford's method; $A1$ is the absorbance in t_{initial} and $A2$ is the absorbance in t_{final} . For this analysis, the chosen samples were 3 random livers of each of the following groups: the positive (G1/DMH) and the negative (G2/EDTA) control groups, the DMH + organic coffee at 5, 10, and 20% (G3 to G5 groups), and the DMH + conventional coffee at 10% (G7) group. This last group was chosen because this is the conventional percentage used as beverage.

2.2.5. SDS-PAGE Electrophoresis. The SDS-PAGE electrophoresis was carried out according to Laemmli's method [29] on discontinuous gel system, with the concentrating (3.5%) and the separating (12.5%) polyacrylamide gels (20 × 21 cm), using 6 μg of protein in each lane. An electrophoresis power supply EPS-601 (Amersham Pharmacia Biotech, Brazil) was used under the following electrophoretic conditions: 120 V, 30 mA, 5 W for 2 h (concentrating gel); and 200 V, 30 mA, 6 W for 12 h (separating gel). After electrophoresis, the proteins were stained with Coomassie brilliant blue (CBB G-250) colloidal. Densitometric quantification of CBB G-250-stained bands was performed by transmission acquisition, with an ImageScanner (GE Healthcare, ImageScanner™ II), and analyzed by Gel-Pro Analyzer® software (Media Cybernetics Inc., Silver Spring, USA). All estimated values (molecular mass, MM, and the protein mass of each band) were compared by 21 μg of standard proteins (# SM0431, Fermentas Life Sciences, EU): galactosidase (116 kDa), bovine serum albumin (66.2 kDa), ovalbumin (45.0 kDa), lactate dehydrogenase (35.0 kDa), restriction endonuclease Bsp98I (25.0 kDa), lactoglobulin (18.4 kDa), and lysozyme (14.4 kDa), calculating the average and standard deviation for 3 replicates. Each mass value from G1's protein bands was normalized (100%) and compared to the results of other samples from other groups. The normalization was chosen and applied to facilitate the better visualization and comparison of results.

2.2.6. Microwave-Assisted Acid Digestion of the Protein Bands. Protein bands were selected and carefully cut. These were weighed (wet weight) and put into a stove at 45.0°C up to constant mass. Thereupon, the bands were transferred to Teflon® vials, to which 5.00 mL of subdistilled nitric acid and 1.00 mL of hydrogen peroxide (30%) were added. After a predigestion time of 30 min, the vials were brought to a microwave oven (9 Milestone Ethos Plus, Sorisole, Italy). The optimized digestion program was as follows: step 1 (400 W at 100°C, for 3 min), step 2 (790 W at 150°C, for 6 min), and step 3 (0 W at 25°C, for 18 min). At the end of the digestion,

the samples were almost dried (max. 60°C) for about 1 h, and the volumes were adjusted with 0.20% subdistilled nitric acid solution (v/v) to 25 mL.

2.2.7. Determination of Iron by ET-AAS. The iron concentrations in the digested protein bands were determined using an atomic absorption spectrometer (AA-7000 Shimadzu Analytical and Measuring Instruments, USA) with the following conditions: pyrolysis temperature, 1,200°C; atomization temperature and time, 2,000°C and 4 s, respectively; sample volume, 20 μ L; wavelength, 248.3 nm; current, 6.0 mA; and slit, 0.2 nm. The digested ovalbumin band (45 kDa, standard proteins) was used as analytical blank, since this protein does not have the metal of interest in its structure. Measurements were made in duplicate. The iron concentration was calculated through the analytical curve equation, taking into account the dilutions and discounting the analytical blank.

2.3. Statistical Analysis. Principal component analysis (PCA) was performed followed by 95% confidence ellipses in order to infer about the (dis)similarity of groups. Such confidence regions were constructed through the bootstrap method, resampling the original replicates with reposition. Such procedure was performed using the software R 3.1.0 [30], through package FactoMineR [31].

3. Results and Discussion

In this work we aimed to assess the interference of organic and conventional coffee under DMH induced cancer rats, taking into account the oxidative stress generated and the endpoints, such as level and activities of proteins, highlighting catalase.

The DMH was chosen because it is a potent necrogenic hepatic carcinogen that alkylates hepatocellular DNA [32]. This carcinogen has been applied as a model to evaluate the hepatic protein profile since the initial phases of carcinogenesis. Moreover, its metabolization induces zonal necrosis that can promote hepatocarcinogenesis, corresponding to the first carcinogenesis steps, such as initiation and promotion, by enhancing growth of initiated hepatocytes resistant to toxicity [33, 34], followed by progression stage [35]. It was observed that the colons from the same animals used in this study had four crypts per focus, previously described by Carvalho et al. [10]. Therefore, it can be topographically considered that they are more likely to progress into tumors [36].

Recently, a general scheme has been proposed to describe the role of oxyradicals during the initiation and promotion stages of carcinogenesis [37]. An increase in the reactive oxygen metabolites (superoxide radical and hydrogen peroxide, precursors of a number of oxygen-derived radicals including hydroxyl radicals) in tumor cells is noted in the early stages, and the overproduction and/or the inability to destroy them may result in severe damage to cell molecules and structures [38]. Despite this aspect, we observed a decrease in the activity of catalase in all DMH-treated groups, as can be seen in Table 2.

This decrease of CAT activity can cause accumulation of superoxide anion, highly diffusible and potent oxidizing

TABLE 2: Catalase activity in the liver tissues ($n = 3$), with standard deviations. The rate constant of first-order reaction (k) was adopted ($s^{-1}\cdot g\text{ protein}^{-1}$), as a measurement of enzyme activity. Results are shown for liver samples from G1/DMH + CD, G2/EDTA + CD, G3/DMH + ORC 5%, G4/DMH + ORC 10%, G5/DMH + ORC 20%, and G7/DMH + CC 10%.

| Group | k ($s^{-1}\cdot g\text{ protein}^{-1}$) ^a |
|------------------|--|
| G1/DMH + CD | 47.23 \pm 0.11 |
| G2/EDTA + CD | 138.9 \pm 10.7 |
| G3/DMH + ORC 5% | 32.5 \pm 3.1 |
| G4/DMH + ORC 10% | 35.1 \pm 2.9 |
| G5/DMH + ORC 20% | 58.7 \pm 2.4 |
| G7/DMH + CC 10% | 58.5 \pm 0.5 |

^a k ($s^{-1}\cdot g\text{ protein}^{-1}$): the rate of first-order reaction.

radical capable of traversing membranes, causing serious injurious effects at sites far away from the tumor area [39]. Our results may be explained by Jrah-Harzallah et al. [40], which demonstrated that, after DMH treatment (during initiation, 10 weeks), the ROS-scavenging enzymes superoxide dismutase, CAT, and glutathione peroxidase were increased, instead of during the promotion (20 weeks), when they showed a reduced enzyme activity response. This latter aspect characterizes our samples as having passed the initiation phase (first 10 weeks), when an increased activity of these enzymes as an adaptive response to ROS stress to block the effects of the DMH metabolites occurs. Moreover, it confirms that the samples are in the beginning of progression phase due to the CAT activity decrease, suggesting an exhaustion resulting from prolonged oxidative stress, despite increased ROS generation. Therefore, increased levels of hepatic lipid peroxidation in DMH-treated rats could be regarded as a mechanism by which the colon tumor cells are better protected and allowed to grow further than their normal counterparts, thus, showing increased tumor incidence. Then, the reduced levels of CAT activities promote tumor growth, which is important for invasion and metastasis [24, 33, 41].

Based on Table 2, the liver samples from the positive control group had a decrease of approximately 66% in the enzymatic activity compared to the negative control group. We have observed that in groups treated with coffee, a CAT activity restoration of 24% for conventional 10% and organic 20% coffee occurred. The CAT activity in organic 5% and 10% groups was lower than in positive control group. However, for organic 20% and conventional 10% groups we observed an increase in its activity, higher than that in positive control group, slowing down and favoring the reversibility of the carcinogenic process. This protective effect can be a mechanism to prevent DNA damage by reactive oxygen metabolites implicated in the tumor development [39, 42]. Thus, the coffee may exert its chemopreventive effect by restoring the activities of CAT in the liver, resulting in scavenging of ROS, and turning the tissue less susceptible to oxidative damage [24].

Based on the above observations about the catalase activity, it was decided to investigate the liver tissue catalase

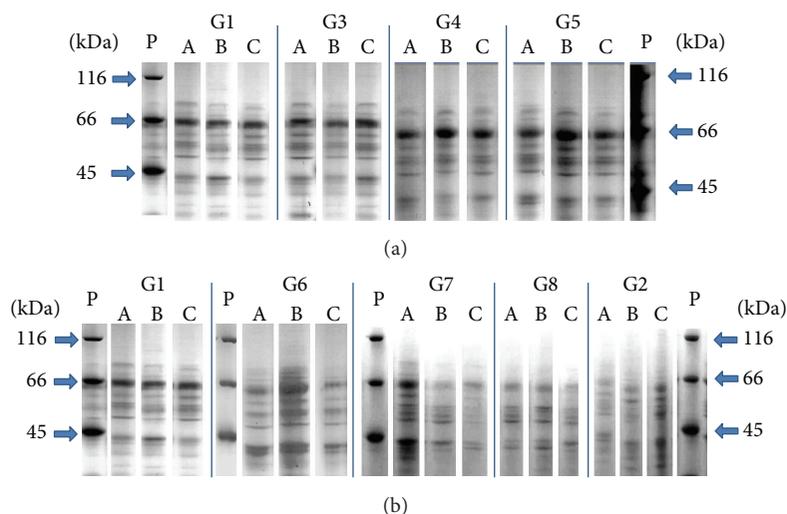


FIGURE 1: (a) Animal liver SDS-PAGE gels (from 116 to 45 kDa) of positive control group (G1), G3, G4, and G5 and protein standard (P). (b) shows SDS-PAGE gels of G1, G6, G7, and G8, negative control group (G2), and protein standard (P). Letters A, B, and C correspond to three random livers from each group.

expression. Interestingly, and in opposition of what we previously observed about the CAT activity decreasing under DMH action, we observed a CAT overexpression in this tissue. For this purpose, around 10 liver SDS-PAGE gel band intensities were compared among the treatments (see Figures 1(a) and 1(b)) in the region from 116 to 45 kDa. Distinct differences were observed in the liver protein bands after the treatment period, in which proteins were further enhanced in their intensities with some even being depleted.

The comparison of the percentage intensities (Table 3) among positive control (DMH), coffee (DMH + coffee), and negative groups (EDTA) highlighted that these treatments can interfere in the liver protein profiles. Some bands were completely suppressed from the coffee and the negative control groups, such as the following, with their possible respective identifications from rat liver [43]: 99 kDa, 76 kDa (bile acyl-CoA synthetase, EC = 6.2.1.7), and 70 and 69 kDa (solute carrier family 27, fatty acid transporter, member 2 or glutamate-cysteine ligase catalytic, EC = 6.3.2.2, and sodium- and chloride-dependent GABA transporter 2), which were only observed in the positive control group (DMH). However, others (e.g., 83 kDa) present in the positive control group suffered an intensity decrease of approximately 32% in organic 10% and 44% in organic 20% groups up to its complete depletion in the negative control group. Also, it can be noted that some bands had their intensities increased such as 82 kDa (sodium-dependent neutral amino acid transporter), 55 kDa (several cytochrome P450, EC = 1.14.14.1, such as 2B1, 2B2, 2C11, 2D1, or 3A2), and 43 kDa (erythrocyte membrane proteins such as LanC-like protein 1 or cystathionine gamma-lyase, EC = 4.4.1.1). For the conventional treatment, other bands were gradually reduced in their intensities, such as 73 kDa (solute carrier family 23 member 1), 62 kDa (gamma-glutamyltransferase 5, gamma-glutamyltransferase 1, or gamma-glutamyltranspeptidase, EC = 2.3.2.2), 59 kDa (solute carrier family member 1, member 5, or member 8),

and 49 kDa. It was also observed that some ones had their intensities increased (82, 64, and 43 kDa). The gel band proteins with 59 kDa, possibly catalase (EC = 1.11.1.6) [43], had their intensity increased by 35% in organic 5% group and for groups with higher coffee concentrations the intensity bands decreased, taking into account the negative control group, which had the lowest intensity (−31% in organic 10%, −36% in organic 20%, and −68% in negative control groups). This increase of intensity might be attributed to the induction condition triggered by genetic mutations as it occurs with genes like p53 and cyclin D1, which governs mitochondrial biogenesis. Mutations in these genes affect the rate of oxidative phosphorylation and decreases of ROS production, which, in turn, affects the expression of antioxidant enzymes [33, 44]. In addition, the CAT increasing intensity in both positive control and organic 5% groups may be due to an induction caused by their higher inactiveness, possibly as a compensating mechanism. Supplementation with the higher levels of organic and conventional coffee in these diets caused a decreasing in the band intensities, demonstrating a possible coffee protective effect in such enzyme expressions. These results match perfectly the higher activity and lower induction of CAT, and the enzyme production with coffee supplementation is closer to the spontaneous levels than when treated only with DMH.

Due to the above results and taking into account that CAT is a heme-protein, we decided to investigate the iron concentration changes in these protein bands. For this purpose, the negative and positive control, organic 10%, and conventional 10% groups were analyzed, since these infusion concentrations are usually ingested. The chosen analytical blank was the ovalbumin protein band from the standard proteins, since this protein has no iron in its structure.

The iron average concentration in the CAT protein bands (59 kDa), per gel band mass, was $950.88 \pm 0.12 \mu\text{g}\cdot\text{g}^{-1}$ for the negative control, and for the positive control group there was

TABLE 3: Optical density intensities (%) of the protein bands from gels G2 (negative control group); G3, G4, and G5 (DMH + ORC treatment); and G6, G7, and G8 (DMH + CC treatment) in relation to the normalized intensities of protein bands, observed in G1.

| MM (kDa) | G1 (DMH) | G2 (EDTA) | G3 (DMH + ORC 5%) | G4 (DMH + ORC 10%) | G5 (DMH + ORC 20%) | G6 (DMH + CC 5%) | G7 (DMH + CC 10%) | G8 (DMH + CC 20%) |
|------------|----------|-----------|-------------------|--------------------|--------------------|------------------|-------------------|-------------------|
| 83.0 ± 0.3 | 100 | — | — | 68.3 | 55.7 | — | n.d. | — |
| 81.8 ± 0.8 | 100 | 136 | 140.3 | 282.0 | 320 | — | 200 | — |
| 73.5 ± 0.5 | 100 | — | — | — | — | — | 68 | — |
| 63.6 ± 1.5 | 100 | 114 | — | — | — | — | 245 | — |
| 61.9 ± 0.1 | 100 | 79 | — | — | — | — | — | 82 |
| 59.2 ± 0.5 | 100 | 32 | 135.7 | 68.8 | 64.3 | — | 99 | — |
| 55.0 ± 0.1 | 100 | — | 177.9 | 157.9 | 142.1 | — | — | — |
| 53.4 ± 0.4 | 100 | 11 | — | — | — | 20 | — | 18 |
| 51.0 ± 0.6 | 100 | 45 | — | — | — | 18 | 48 | 35 |
| 49.6 ± 0.1 | 100 | 11 | — | — | — | 91 | 35 | 82 |
| 45.2 ± 0.5 | 100 | 100 | — | — | — | 75 | — | — |
| 43.5 ± 0.4 | 100 | 85 | 101.9 | 86.8 | 87.3 | 228 | 364 | — |

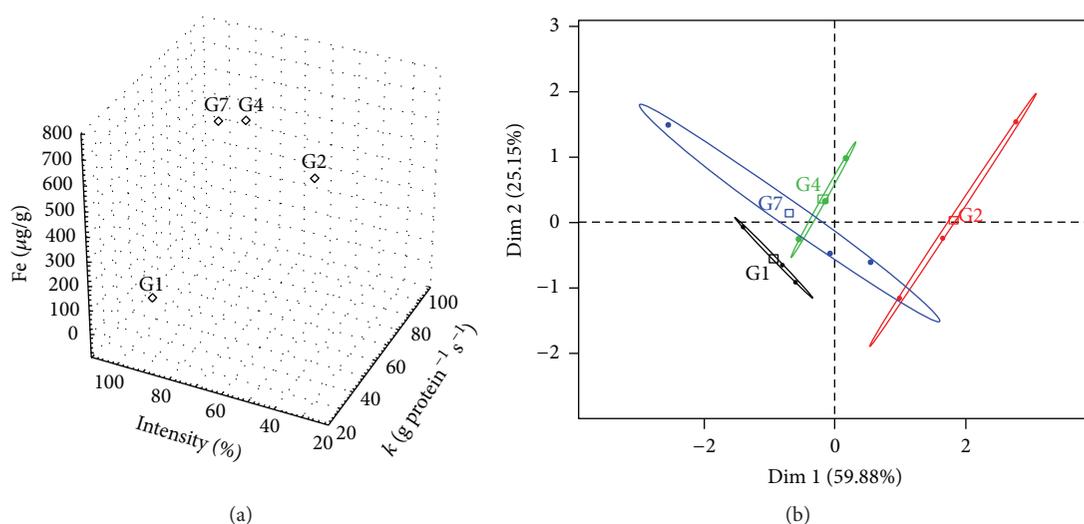


FIGURE 2: (a) Relation among the three studied variables: iron concentration ($\mu\text{g}\cdot\text{g}^{-1}$), catalase activity (k , $\text{s}^{-1}\cdot\text{g protein}^{-1}$), and band intensity percentage (%). (b) Principal component analysis (PCA). The first (x -axis) was able to explain about 60% of the total variability and the second (y axis) was able to explain about 25% with the ellipses of 95% confidence limits, constructed by bootstrap resampling, using replicates of the original data.

almost no iron ($6.45 \pm 0.16 \mu\text{g}\cdot\text{g}^{-1}$). It suggests that the DMH influenced the catalase expression conditions reducing the enzymatic activity by the loss of iron ions. The presence of iron in proteins agrees with the increased activity observed in Table 2. Interestingly, Andreoletti et al. [45], working with overexpression of recombinant catalase from *E. coli* by changing induction conditions, obtained low amount of heme content (29%) in catalase production. That result had repercussions on decreased activity by approximately 50%. On the other hand, organic or conventional coffee increased iron ion concentrations ($746.30 \pm 0.11 \mu\text{g}\cdot\text{g}^{-1}$ and $453.97 \pm 0.10 \mu\text{g}\cdot\text{g}^{-1}$, resp.), which are closer to the values determined for the protein band of the negative control group.

Principal component analysis (PCA) was performed to verify the (dis)similarity among the three studied variables in this paper. The three original dimensions (iron concentration, CAT activity, and intensity percentage, as shown in Figure 2(a)) were reduced to two principal components (Figure 2(b)), each of them being linear combinations of the original variables. The first (x -axis) was able to explain about 60% of the total variability and the second (y axis) was able to explain about 25%. Therefore, the plan explained 75% of the total variability, observing the proximity of the coffee treatment results. The 95% confidence limit ellipses showed overlaps between the ellipses from organic and conventional coffee groups, and between the negative control and conventional groups, highlighting the link among the three studied variables. Thus, the chemopreventive coffee compounds intake produced a mechanism for the metal ion preservation in the active sites of catalase, against the DMH action. To conclude, this protective effect is mainly emphasized for coffee infusion concentrations above 10% that restores CAT activity and production, nearing them to the healthy conditions.

4. Conclusions

This comparative study of the electrophoretic protein profiles provided an overview of how the coffee feedings might influence the liver metabolic mechanisms. Many different proteins varied in their intensities according to the treatments, opening a wide range of possibilities to study the coffee feed influence as a chemopreventive substance. The feeding of pre-neoplastic induced rats with the commercial diet containing coffee (organic or conventional) significantly increased CAT activities in the liver. Thus, we showed that the differences in the CAT activities in the intensities of 59 kDa protein band (attributed to catalase) and in the iron concentrations of protein were statistically related. Therefore, it confers a liver protective effect to the coffee, as a functional food.

Abbreviations

| | |
|------------|---|
| a : | Dilution factor |
| A1: | The absorbance in t_{initial} |
| A2: | The absorbance in t_{final} |
| b : | Mass of tissue divided by the protein amount in the extract |
| bw: | Body weight |
| CAT: | Catalase |
| CBB G-250: | Coomassie brilliant blue G-250 |
| CC: | Conventional arabica coffee |
| CD: | Commercial diet |
| COBEA: | Brazilian College of Animal Experimentation |
| DMH: | 1,2-Dimethylhydrazine |
| EC: | Enzyme commission number |
| EDTA: | Ethylenediaminetetraacetic acid |

| | |
|--------------|--|
| ET-AAS: | Electrothermal atomic absorbance spectrometry |
| G1: | Positive control group |
| G2: | Negative control group |
| G3: | The DMH + organic coffee at 5% group |
| G4: | The DMH + organic coffee at 10% group |
| G5: | The DMH + organic coffee at 20% group |
| G6: | The DMH + conventional coffee at 5% group |
| G7: | The DMH + conventional coffee at 10% group |
| G8: | The DMH + conventional coffee at 20% group |
| k : | Rate constant of first-order reaction |
| kDa: | Kilodalton |
| MM: | Molecular mass |
| m/v: | Mass per volume |
| ORC: | Organic arabica coffee |
| PCA: | Principal component analysis |
| ROS: | Reactive oxygen species |
| SDS-PAGE: | Sodium dodecyl sulphate-polyacrylamide gel electrophoresis |
| v/v: | Volume per volume |
| Δt : | Time interval. |

Competing Interests

The authors declare that there are no competing interests regarding the publication of this paper.

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