Chronic coffee and caffeine ingestion effects on the cognitive function and antioxidant system of rat brains

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A B S T R A C T

Coffee is a popular beverage consumed worldwide and its effect on health protection has been well studied throughout literature. This study investigates the effect of chronic coffee and caffeine ingestion on cognitive behavior and the antioxidant system of rat brains. The paradigms of open field and object recognition were used to assess locomotor and exploratory activities, as well as learning and memory. The antioxidant system was evaluated by determining the activities of glutathione reductase (GR), glutathione peroxidase (GPx) and superoxide dismutase (SOD), as well as the lipid peroxidation and reduced glutathione content. Five groups of male rats were fed for approximately 80 days with different diets: control diet (CD), fed a control diet; 3% coffee diet (3%Co) and 6% coffee diet (6%Co), both fed a diet containing brewed coffee; 0.04% caffeine diet (0.04%Ca) and 0.08% caffeine diet (0.08%Ca), both fed a diet diet supplemented with caffeine. The estimated caffeine intake was approximately 20 and 40 mg/kg per day, for the 3%Co-0.04%Ca and 6%Co-0.08%Ca treatments, respectively. At 90 days of life, the animals were subjected to the behavioral tasks and then sacrificed. The results indicated that the intake of coffee, similar to caffeine, improved long-term memory when tested with object recognition; however, this was not accompanied by an increase in locomotor and exploratory activities. In addition, chronic coffee and caffeine ingestion reduced the lipid peroxidation of brain membranes and increased the concentration of reduced-glutathione. The activities of the GR and SOD were similarly increased, but no change in GPx activity could be observed. Thus, besides improving cognitive function, our data show that chronic coffee consumption modulates the endogenous antioxidant system in the brain. Therefore, chronic coffee ingestion, through the protection of the antioxidant system, may play an important role in preventing age-associated decline in the cognitive function.

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1. Introduction

There is a general agreement on the existence of a normal cognitive decline from early to late adulthood and that disorders, such as Alzheimer’s disease (AD), are associated with an overall impairment of higher functions and cognitive faculties, one of which is a symptom of loss of memory (Bertain-Aanglade et al., 2006). Evidence suggests that a progressive accumulation of oxidative damage to the brain may well lead directly to such age-associated cognitive losses (Head, 2009; Martin and Grotewiel, 2006). Taking these arguments into consideration, the oxidative damage theory was proposed in an attempt to explain the underlying biological mechanisms of age-associated cognitive impairment. This theory postulates that the age-dependent accumulation of oxidative damage to macromolecules in the brain causes a progressive functional deterioration of cells, tissues, and organ systems, which commonly appear as functional senescence (Weinert and Timinas, 2003). Oxidative damage to lipids, proteins, and DNA occurs primarily through reactive oxygen species (ROS) actions. The brain, in particular, is highly vulnerable to ROS, as it consumes approximately 20% of the body’s total oxygen and contains a high content of polyunsaturated fatty acids and lower levels of endogenous antioxidant activity as compared to other tissues (Halliwell and Gutteridge, 1985). A wide array of enzymatic and non-enzymatic antioxidant defenses exist, including superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase (CAT), glutathione (GSH), beta-carotene, ascorbic acid (vitamin C), and alpha-tocopherol (vitamin E). A balance can be identified between both the activities and the intracellular...
levels of these antioxidants which are essential for the survival of organisms and their health (Matés and Sánchez-Jiménez, 1999).

In recent years, based on this possible link between oxidative damage and cognitive decline, there has been a growing interest in studying diet bioactive compounds that delay or prevent cell damage, provide symptomatic relief, and improve people’s quality of life. Coffee, a very popular beverage consumed worldwide, has been extensively studied as a protective food, able to produce physiological or metabolic effects on the human organism, adapting it to withstand environment adversities. Research has shown that caffeine, a major constituent of coffee, induces a broad spectrum of cellular and pharmacological responses, such as the central nervous system and motor activity stimulation (Friedholm et al., 1999), cognitive performance improvement (Cunha and Agostinho, 2010), anxiety and sleep disturbance (Nardi et al., 2009; Paterson et al., 2009), antioxidant activity (Noschang et al., 2009; Shi and Dalal, 1991), among others. Besides caffeine, coffee contains a number of other substances—phenolic polymers, chlorogenic acids, lipids, and terpenes—that have different biological effects, such as antioxidant (Cho et al., 2009; Natella et al., 2002), anticarcinogenic (Cavin et al., 2002), antimicrobial (Almeida et al., 2006), and neuroprotective activities (Herraz and Chaparro, 2006; Hwang and Jeong, 2008). Despite the known effects of coffee isolated components, little is known about the effect of brewed coffee, where functional interactions among components may well increase or decrease individual action, rendering the beverage a potential protective food against events that can lead to a decline in brain functions. Evidence suggests that in humans there is a link between chronic coffee/caffeine consumption and cognitive performance (Ritchie et al., 2007; Santos et al., 2010). However, very little is known about how coffee ingestion really affects cognitive function.

Therefore, the present study was carried out to assess the effect of chronic coffee and caffeine ingestion on cognitive performance in rats by modulating the endogenous antioxidant system of the brain.

2. Material and methods

2.1. Diets

The control diet was prepared using a mixture of 80% of a powdered rat lab chow (Labina, Purina, Paulínia, SP, Brazil) and 20% of powdered dog food (Bono, Purina, Paulínia, SP, Brazil). For every 90 g of the mixture, 100 ml of a solution containing 4% commercial gelatin, 1% cornstarch, and 5% sugar in hot water was added. The formed dough was pelleted and dried at 60 °C in a forced air oven (Nova Ética 420-D, Vargem Grande Paulista, SP, Brazil) for a time necessary to return the diet weight to 100 g.

The present study used the Coffea arabica species, cultivated in the southern regions of the state of Minas Gerais (Brazil), an exportation type, grain with no defects, with an average granulation and processed by toasting at a temperature of 160 °C for nearly 13 min, at classification 45. The classification of the toasting point was determined by using the Roast Color Classification System (AGTRON/SCAA, 1995), according to the standards applied by the Brazilian Coffee Industry Association (ABIC). The content of caffeine in coffee powder was 12.5 mg caffeine/g of coffee (1.25%), determined by HPLC.

Boiled, distilled water was added to milled toasted coffee at a concentration of 3% w/v and 6% w/v. The suspension was centrifuged (27 xg) for 10 min and the supernatant was collected (Centrifuge Hitachi CR21, Hitachinaka, Japan). The diet supplemented with coffee used the control diet as a basis. However, to this diet, 100 mL of 3% or 6% brewed coffee, containing the gelatin/starch/sugar solution described above, was added to prepare the dough. Similarly, the diet supplemented with caffeine was based on the control diet. However, in this case, the solution formulated to prepare the dough contained caffeine at the concentration of 0.04% or 0.08% (values corresponding to caffeine content in the diet with coffee at 3% and 6% respectively). The remaining procedure for preparing the control diet was then carried out. Control and experimental coffee and caffeine diets were prepared weekly in the laboratory and stored at 4 °C.

2.2. Animals

Female Wistar rats were mated. During the gestation and lactation period, they were fed a diet composed of rat lab chow (Labina, Purina, Paulínia, SP, Brazil), enriched with 20% dog food (Bono, Purina, Paulínia, SP, Brazil). Shortly after birth, when the pups were cleaned and fed by the mother, eight per dam were maintained, with as many male rats as possible. At weaning (21 days of life), the dams and female pups were discarded. The male rats were randomly divided into five groups (n = 16), with one animal per cage: (1) control diet (CD)—fed the control diet; (2) 3% coffee diet (3%Ca)—fed a diet containing 3% of brewed coffee; (3) 6% coffee diet (6%Ca)—fed a diet containing 6% of brewed coffee; (4) 0.04% caffeine diet (0.04%Ca)—fed a control diet supplemented with 0.04% caffeine; and (5) 0.08% caffeine diet (0.08%Ca)—fed a control diet supplemented with 0.08% caffeine.

Water was offered ad libitum. Every other day, the dietary consumption was determined by subtracting the weight of the amount of food offered (in the two days before) from that not ingested. The animal’s body weight was monitored weekly. At 90 days of life, the animals were subjected to two behavioral tasks: open field and object recognition memory (novel object preference). At approximately 100 days of life, the rats were sacrificed by decapitation. The brain was processed for further biochemical analysis of oxidative stress markers. The experimental protocol was in accordance with the Ethical Principles for Animal Experimentation as established by the Ethics Committee on Animal Experiments from the Federal University of Minas Gerais, Brazil (protocol no. 126/2007).

2.3. Behavioral test

Apparatus—Open field and novel preference tasks occurred in an open-topped arena (50 x 50 x 40 cm) made of acrylic. The walls inside the arena were surrounded with a black paper so that no external stimuli could be seen during the experiment, and the floor of the arena was divided into sixteen quadrants of equal size, using thin indentation marks, into a 4 x 4 matrix of squares (12.5 x 12.5 cm). The stimuli presented in the object recognition test were copies of objects composed of plastic pieces (Gulliver, São Caetano do Sul, SP, Brazil) that varied in shape, color, and size and were fixed to the floor of the arena to avoid moving during tasks. After each trial, the objects were thoroughly cleaned with a 75% ethanolic solution to remove odor cues.

Prior to each behavioral test, the animals were placed in the testing room 20 min before the task to allow habituation to the environment. All tasks were performed between noon and 4 p.m. The observer (unaware of the experimental group being tested) made direct visual observation of each animal. The observer counted the number of crossings that varied in shape, color, and size and were fixed to the floor of the arena to avoid moving during tasks. After each trial, the objects were thoroughly cleaned with a 75% ethanolic solution to remove odor cues.

The animal’s body weight was monitored weekly. At 90 days of life, the animals were subjected to two behavioral tasks: open field and object recognition memory (novel object preference). At approximately 100 days of life, the rats were sacrificed by decapitation. The brain was processed for further biochemical analysis of oxidative stress markers. The experimental protocol was in accordance with the Ethical Principles for Animal Experimentation as established by the Ethics Committee on Animal Experiments from the Federal University of Minas Gerais, Brazil (protocol no. 126/2007).

The observer also counted the number of crossings that varied in shape, color, and size and were fixed to the floor of the arena to avoid moving during tasks. After each trial, the objects were thoroughly cleaned with a 75% ethanolic solution to remove odor cues. The observer took note every time the animal crossed from one square to another (number of crossings). The observer also counted the number of rearings (“erect” posture sustaining weight on hind limbs, with fore limbs elevated and head usually in profile). The number of line crossings and the number of rearings were measured in 5 min periods on 2 consecutive days.

Novel preference task—The animals were habituated to the arena without stimuli for 20 min daily for 3 days before beginning the behavioral test. The task consisted of an acquisition or sample phase, followed by two preference tests, one after a delay of 90 min (short-term memory) and another 24 h after the sample phase (long-term memory).
In the sample phase, duplicate copies (A1 and A2) of an object were placed near the two corners at either end of one side of the arena (15 cm from each adjacent wall). The animal was placed in the arena facing the center of the opposite wall and allowed 5 min for exploration. After 90 min, the animal was replaced in the arena, presented with two objects in the same positions: one object (A3) was a third copy of the set of the objects used in the sample phase, while the other object was a novel object (B). Using two distinct chronometers, the observer quantified the total time spent exploring each object placed in the arena, within a 5-minute time window. The second test phase was performed after 24 h, with a copy of the object used in the sample phase (A4) and a new object (C) (Ennaceur and Delacour, 1988).

The positions of the objects in the test and the objects used as novel or familiar were counterbalanced between the animals.

Exploratory behavior of a particular object was defined as the animal directing its nose toward the object at a distance of <2 cm. Any other behavior, such as looking around while sitting on or resting against the object, was not considered as exploration.

The discrimination index was calculated as the time spent by each animal exploring the novel object divided by the total time spent exploring both objects.

2.4. Biochemical analysis

Brain homogenate—After removing the cerebellum, the brain was weighed, homogenized in 10 volumes (1:10 w/v) of sodium phosphate buffer at 50 mM plus KCl 140 mM, pH 7.4, and centrifuged at 750×g for 10 min (Centrifuge Hitachi CR21, Hitachinaka, Japan). The supernatant was collected. Aliquots of 1.0 ml of all samples were weighed, homogenized in 10 volumes (1:10 w/v) of sodium phosphate buffer at 50 mM plus KCl 140 mM, pH 7.4, and centrifuged at 750×g for 10 min (Centrifuge Hitachi CR21, Hitachinaka, Japan). The supernatant was collected. Aliquots of 1.0 ml of all samples were frozen in liquid nitrogen and subsequently stored in a freezer at −70 °C until further processing.

Biochemical analysis—The lipid peroxidation index was determined according to Yagi (1984), whereas the glutathione (GSH) content was determined according to Tietze (1969). The activity of glutathione reductase was determined according to Paglia and Valentine (1967), whereas the glutathione reductase was determined according to Carlbeg and Mannervik (1975). The superoxide dismutase activity was determined according to Marklund and Marklund (1974) and was based on the inhibition of pyrogallol auto-oxidation.

Measurements in all assays described were carried out in triplicate. The protein amount in all samples was determined according to Lowry et al. (1951) and modified by Hartree (1972).

2.5. Statistical analysis

Open field crossing, as well as memory data of rearing values and object recognition, underwent ANOVA analysis. The significant differences in the experimental values were determined using the Duncan test and Bonferroni test, respectively.

ANOVA, followed by the Duncan test, was applied to biochemical data. Values were considered statistically different at P < 0.05.

3. Results

Diet supplemented with of 3% or 6% coffee extract and 0.04% or 0.08% caffeine, as compared to the control group, had no effect on the rats’ locomotor and exploratory activities was evaluated by an open field test and is shown in Table 2. The intake of coffee or caffeine in the diet, as compared to the control group, did not modify the number of crossings and rearings in the open field test, indicating that the presence of coffee or caffeine in the diet did not increase the exploratory activity of these animals. All experimental groups showed a lower number of locomotor and exploratory activities on the second day and day 2 in crossings and rearings, respectively. Crossing is defined as the number of lines crossed by the animal in the arena. Rearing means the number of times the animal stood in erect posture. CD represents the control group; 3%Co, the group supplemented with 3% brewed coffee; 6%Co, the group supplemented with 6% brewed coffee; 0.04%Ca, the group supplemented with 0.04% caffeine; and 0.08%Ca, the group supplemented with 0.08% caffeine.

All chemicals used were of analytical grade and were purchased from Sigma Chemical Co. (St. Louis, MO, USA).

### Table 2

<table>
<thead>
<tr>
<th>Groups</th>
<th>Day 1</th>
<th>Day 2</th>
<th>Rearing Day 1</th>
<th>Rearing Day 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD</td>
<td>62.3 ± 4.0</td>
<td>47.5 ± 4.2</td>
<td>13.9 ± 1.2</td>
<td>8.1 ± 1.1**</td>
</tr>
<tr>
<td>3%Co</td>
<td>61.7 ± 4.1</td>
<td>38.0 ± 4.9*</td>
<td>9.3 ± 1.3</td>
<td>6.3 ± 0.6**</td>
</tr>
<tr>
<td>6%Co</td>
<td>65.4 ± 4.5</td>
<td>42.8 ± 2.4**</td>
<td>12.9 ± 1.4</td>
<td>5.4 ± 0.8**</td>
</tr>
<tr>
<td>0.04%Ca</td>
<td>66.6 ± 5.0</td>
<td>37.6 ± 4.8</td>
<td>14.3 ± 2.5</td>
<td>5.6 ± 0.9**</td>
</tr>
<tr>
<td>0.08%Ca</td>
<td>62.1 ± 3.7</td>
<td>38.1 ± 4.3*</td>
<td>12.2 ± 1.5</td>
<td>6.3 ± 1.1**</td>
</tr>
</tbody>
</table>

All values are mean ± SEM (n = 16). No statistical difference could be observed among the groups on the same day (column) according to ANOVA and Duncan test (P > 0.05). * and ** indicate statistical difference (P < 0.05—Duncan test) in the same group from day 1 to day 2 in crossings and rearings, respectively. Crossing is defined as the number of lines crossed by the animal in the arena. Rearing means the number of times the animal stood in erect posture. CD represents the control group; 3%Co, the group supplemented with 3% brewed coffee; 6%Co, the group supplemented with 6% brewed coffee; 0.04%Ca, the group supplemented with 0.04% caffeine; and 0.08%Ca, the group supplemented with 0.08% caffeine.

Table 1

| Diet ingestion and body weight gain in the rats. |
|---|---|---|---|---|---|
| Days | 21 | 35 | 50 | 65 | 80 | 95 |
| Food consumption (g) | | | | | | |
| CD | 3.12 ± 0.41 | 14.45 ± 0.35 | 22.05 ± 0.52 | 24.63 ± 0.65 | 24.07 ± 0.51 | 23.74 ± 0.52 |
| 3%Co | 3.77 ± 0.46 | 14.23 ± 0.58 | 23.08 ± 0.53 | 25.48 ± 0.79 | 25.79 ± 0.56 | 23.47 ± 0.65 |
| 6%Co | 3.79 ± 0.61 | 14.21 ± 0.45 | 22.79 ± 0.69 | 24.86 ± 0.89 | 25.32 ± 0.85 | 24.18 ± 0.69 |
| 0.04%Ca | 3.69 ± 0.45 | 13.79 ± 0.40 | 22.11 ± 0.57 | 23.93 ± 0.55 | 24.51 ± 0.42 | 23.63 ± 0.53 |
| 0.08%Ca | 3.89 ± 0.46 | 13.79 ± 0.37 | 22.50 ± 0.53 | 24.32 ± 0.67 | 24.67 ± 0.89 | 24.95 ± 0.57 |
| Body weight (g) | | | | | | |
| CD | 48.66 ± 1.12 | 125.16 ± 2.39 | 230.48 ± 4.55 | 316.24 ± 6.55 | 370.41 ± 7.11 | 395.21 ± 7.95 |
| 3%Co | 49.44 ± 1.14 | 123.83 ± 2.70 | 231.97 ± 4.02 | 322.38 ± 6.71 | 383.91 ± 7.79 | 408.87 ± 8.22 |
| 6%Co | 48.01 ± 1.21 | 120.71 ± 2.23 | 225.94 ± 3.67 | 312.82 ± 7.13 | 374.85 ± 7.90 | 398.11 ± 7.90 |
| 0.04%Ca | 47.75 ± 1.19 | 120.60 ± 2.17 | 225.01 ± 4.21 | 312.44 ± 5.22 | 378.54 ± 6.15 | 394.61 ± 7.13 |
| 0.08%Ca | 47.88 ± 0.73 | 123.22 ± 2.50 | 225.37 ± 4.86 | 319.50 ± 5.34 | 374.94 ± 6.77 | 403.09 ± 7.26 |

All values are mean ± SEM (n = 16). Although food consumption was assessed every couple of days and body weight was assessed weekly, this table depicts six chosen periods (days 21, 35, 50, 65, 80 and 95) in order to show the time evolution of coffee/caffeine consumption per body weight along the experiment. No significant difference was observed among the groups on the same day (column) according to ANOVA and Duncan test (P > 0.05). CD fed lab chow; 3%Co fed 3% brewed coffee supplemented diet; 6%Co fed 6% brewed coffee supplemented diet; 0.04%Ca fed 0.04% caffeine supplemented diet, and 0.08%Ca fed 0.08% caffeine supplemented diet. The estimated caffeine intake in these animals was about 20 and 40 mg/kg per day, for the 3%Co–0.04%Ca and 6%Co–0.08%Ca treatments, respectively.
The results are summarized in Table 3. A decrease in the content of TBARS (38%) in rats that ingested coffee or caffeine in the diet was observed. On the other hand, coffee 6% and caffeine 0.08% increased the GSH content by 60%. Diet supplementation with coffee or caffeine, as compared to the control group, induced a significant increase (56%) in GR activity but did not affect the GPx activity. Furthermore, an increase of 54% in superoxide dismutase (SOD) activity in the groups fed a diet supplemented with 6% coffee extract or caffeine could be observed.

4. Discussion

To evaluate the effect of chronic coffee ingestion on the cognitive function and antioxidant system modulation, the animals received a diet supplemented with brewed coffee (3% and 6%), and the corresponding doses of 0.04% and 0.08% caffeine after weaning. These diets were administered daily over the experimental life of the animals. According to prior studies conducted by our research group (Silva-Oliveira et al., 2010), and confirmed in this study, the addition of coffee or caffeine to the rat’s diet did not alter food intake nor did it have any observable impact on the body weight of all groups studied.

The concentrations of 3% and 6% of coffee in the diet were based on the literature (Huber et al., 2003; Turesky et al., 2003) and on data from our research group that showed that the intake of coffee/caffeine in this concentration range is capable to produce physiological and metabolic effects in different biological systems of rats, without toxic effects. However, these doses of coffee and caffeine used in this experimental animal model study were raised according to daily human consumption. The estimated caffeine intake in these animals was approximately 20 and 40 mg/kg/day, for the 3% and 6% coffee groups, respectively. By contrast, the estimated intake of caffeine in humans drinking 2 cups of coffee daily is approximately 4 mg caffeine/kg body weight, whereas for heavy coffee drinkers the amount may reach 15 mg/kg (Turesky et al., 2003). But it is important to consider, in addition to body weight, the rate of caffeine metabolism, which is faster in rats than in humans, see pharmacokinetics studies from Fredholm et al. (1999) for details. The caffeine dose chosen (4 mg/kg/day) is also based on the report from van Gelder et al. (2007) who found best protection in elderly cognition decline by drinking 3 cups of coffee per day (375 ml/day), which is around 255 mg/day of caffeine for an approximate body weight of 60–70 kg.

The effects of coffee’s individual components on learning and memory have been reported in different animal models; however, the results of these studies are contradictory (Costa et al., 2008a, 2008b; Kwon et al., 2010; Myoung-Eun et al., 2007; Soellner et al., 2009). Most examined the acute effects of these components. Thus, the long-term effects of coffee consumption on the central nervous system of mice have been poorly characterized. Functional interactions among components can increase or decrease individual actions. Therefore, this study focused on the effects of coffee consumption over the experimental life of the animals, which can better reflect an overall human consumption.

Our results showed that coffee (3% or 6%) or caffeine (0.04% or 0.08%) groups presented a better consolidation of long-term memory than did the control group. Costa et al. (2008a, 2008b) also showed that caffeine treatment, when compared to the control group, increases the object discrimination index, reinforcing its positive effects on short-term and long-term memories. In addition, caffeine administration during the adult life of mice prevented the age-associated decline in recognition memory of objects. Moreover, aged animals treated with caffeine showed a similar performance to adult animals in the novel preference task (Costa et al., 2008b).

The results obtained in the open field test showed that diet supplementation with coffee or caffeine had no effect on the locomotor and exploratory activities of animals, i.e., improvement in the cognitive performance of the animals by the ingestion of coffee or caffeine was not observed.

Fig. 1. Effect of daily coffee or caffeine ingestion in (A) sample phase, (B) test phase after 90 min and (C) test phase after 24 h of object recognition memory task. The discrimination index was calculated as the time spent by each animal exploring the novel object divided by the total time spent exploring both objects. Values are presented as the mean ± SEM (n = 16). CD represents the control diet; 3%Co, the group supplemented with 3% brewed coffee; 6%Co, the group supplemented with 6% brewed coffee; 0.04%C, the group supplemented with 0.04% caffeine; and 0.08%C, the group supplemented with 0.08% caffeine. *p < 0.05 (ANOVA and Bonferroni test).

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The chronic consumption of coffee in this study decreased the lipid peroxidation of the membranes; increased the concentration of glutathione, a potent endogenous antioxidant; and increased the activity of two antioxidant enzymes, glutathione reductase and superoxide dismutase (SOD). Thus, daily coffee intake, because of its protective effect on the endogenous antioxidant system, can also result in a beneficial effect on learning and memory.

### Table 3

<table>
<thead>
<tr>
<th></th>
<th>TBARS (nmol TBA/mg prot)</th>
<th>GSH (nmol GSH/mg prot)</th>
<th>GPx (μmol NADPH/min/mg prot)</th>
<th>CR (μmol NADPH/min/mg prot)</th>
<th>SOD (U/mg prot)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (CD)</td>
<td>2.37 ± 0.22</td>
<td>7.55 ± 0.67</td>
<td>10.28 ± 0.69</td>
<td>31.23 ± 3.20</td>
<td>1.56 ± 0.09</td>
</tr>
<tr>
<td>3% Co (3xC)</td>
<td>1.33 ± 0.07 *</td>
<td>8.44 ± 0.08</td>
<td>9.96 ± 0.77</td>
<td>47.20 ± 3.76 *</td>
<td>1.73 ± 0.13</td>
</tr>
<tr>
<td>6% Co (6xC)</td>
<td>1.31 ± 0.08 *</td>
<td>12.66 ± 0.52 *</td>
<td>12.01 ± 1.01</td>
<td>47.55 ± 4.56 *</td>
<td>2.29 ± 0.18 *</td>
</tr>
<tr>
<td>0.04% Ca (0.04Ca)</td>
<td>1.48 ± 0.09 *</td>
<td>9.88 ± 0.053</td>
<td>10.55 ± 0.72</td>
<td>49.76 ± 1.66 *</td>
<td>2.52 ± 0.25 *</td>
</tr>
<tr>
<td>0.08% Ca (0.08Ca)</td>
<td>1.79 ± 0.12 *</td>
<td>11.99 ± 1.13 *</td>
<td>12.30 ± 1.01</td>
<td>50.58 ± 4.97</td>
<td>2.42 ± 0.20 *</td>
</tr>
</tbody>
</table>

All values are mean ± SEM (n = 8). The antioxidant system was evaluated by determining the activities (normalized by mg of total protein) of glutathione (GSH), glutathione peroxidase (GPx), glutathione reductase (GR) and superoxide dismutase (SOD), as well as the lipid peroxidation and reduced glutathione content (TBARS). CD represents the control group; 3xC, the group supplemented with 3% brewed coffee; 6xC, the group supplemented with 6% brewed coffee; 0.04%Ca, the group supplemented with 0.04% caffeine; and 0.08%Ca, the group supplemented with 0.08% caffeine. *indicates statistical difference (p < 0.05—ANOVA and Duncan test) between groups for each biochemical analysis.


