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EFFECTS OF AQUEOUS EXTRACT OF YERBA MATE (ILEX PARAGUARIENSIS) ON THE OXIDATIVE STRESS IN RATS FED A CAFETERIA DIET

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ABSTRACT

Ilex paraguariensis A. St.- Hil., Aquifoliaceae, is a native species of subtropical and temperate regions of South America, whose leaves and steams are used to make tea and traditional infusions, such as chimarrão and tereré. These beverages are considered beneficial to health because of their physiological effects on the treatment of rheumatism, obesity, fatigue, fluid retention, hypertension and liver disorders. This study examined the effects of aqueous extract of I. paraguariensis leaves on the oxidative stress of rats fed a cafeteria diet. A total of 40 male Wistar rats were equally divided into four groups and fed distinct diets for 15 days: group C (rat chow and water), group E (rat chow and aqueous extract of I. paraguariensis), group CD (cafeteria diet and water) and group ED (cafeteria diet and aqueous extract of I. paraguariensis). Lipid peroxidation (TBARS), reduced glutathione (GSH) and catalase activity (CAT) were subsequently assessed. Brain and liver histological analyses were also carried out. The results showed that aqueous extract of I. paraguariensis was able to reverse the oxidative stress caused by the cafeteria diet on serum, as well as significantly reduce lipid peroxidation in liver and brain tissues. A significant increase in GSH levels in the brain of animals fed the cafeteria diet was observed. However, this effect was only observed in the liver of animals fed with rat chow. Therefore, the aqueous extract of I. paraguariensis has important effects on the modulation of antioxidant defenses and oxidative stress, possibly being beneficial to health after eating the cafeteria diet.

Keywords: Ilex paraguariensis, Oxidative stress, Cafeteria diet, Antioxidant, Lipid peroxidation, Chimarrão.

1. INTRODUCTION

Obesity is a serious public health problem in many countries [1]. The intake of a high fat and calorie diet is directly related to the genesis of obesity [2, 3]. This type of diet, also known as "cafeteria diet", is characterized by containing a large proportion of carbohydrates or fats, or both [4], thus causing adverse health effects related to its potent ability to induce oxidative stress [5].

Oxidative stress is a state of imbalance between reactive oxygen species (ROS), generally known as free radicals, and antioxidant defense. It occurs when there is insufficiency of antioxidant defenses or overproduction of ROS Ferreira and Matsubara [6].

This imbalance between free radicals and pro-oxidant and antioxidant systems results in the production of harmful compounds to the tissues, which may be related to the etiology of various diseases, including cancer, diabetes, hypertension, cardiovascular, autoimmune, inflammatory and ocular problems, malnutrition, pancreatic changes, rheumatic and renal disorders, skin lesions and premature aging [7, 8].

In order to minimize or reverse the damage caused by oxidative processes from cafeteria diets, several plants have been studied to demonstrate the preventive role of antioxidant. Plants containing phenolic compounds have been the subject of several studies on the prevention and treatment of many health problems.

Yerba mate (*Ilex paraguariensis*) is among the plants containing significant phenolic compounds, both qualitatively and quantitatively. It is widely consumed as a leaf and steam infusion in Brazil, Argentina, Paraguay and Uruguay, called *chimarrão* in Brazil, *mate* in Argentina and Uruguay and *tereré* in Paraguay [9, 10].

Studies show that yerba mate based drinks are high in antioxidant, anti-inflammatory, antimutagenic, hypolipidemic [9], antifungal [11], anticancer [12], antiobesity [13] and anti-atherosclerotic substances [14].

Considering yerba mate as a potential source of phenolic compounds, whose biological properties have been reported in the literature, the aim of this study was to evaluate the effect of subchronic ingestion of aqueous extract of *I. paraguariensis* on oxidative stress in Wistar rats fed a cafeteria diet.

2. MATERIAL AND METHODS

2.1 Preparation of the Yerba Mate Extract

Tertúlia® (Jaborá/SC, Brazil) yerba mate was used in this study. A previous analysis of the aqueous extract of this product resulted in a significant amount of total polyphenols (65.5 ± 2.6 mg/100 ml) [15]. For the present study, an aqueous extract similar to the traditional *chimarrão* was prepared with 100 g of *I. paraguariensis* in a 1,000 ml beaker tilted at 45 ºC compacted with cool water on its surface and kept in contact with 500 ml of distilled water at 60 °C for 10 min. The extract was filtered and kept in the water bottle as the only fluid source. A freshly prepared extract was offered *ad libitum* to groups E (aqueous extract of yerba mate and rat chow) and ED (aqueous extract of yerba mate and cafeteria diet) every day. Mosimann, et al. [14] have described the technique of providing the extract as the only fluid source through water bottles.

2.2. Animals

Wistar albino male rats (14-15 weeks old) from the vivarium at Universidade do Oeste de Santa Catarina (UNOESC) were used in the study. The animals were housed in individual plastic cages lined with sawdust, which was changed three times a week. They received rat chow and

water *ad libitum* for one week, to acclimate to the new environment. The animals were kept under controlled temperature (22 ± 2 °C) and light-dark cycle (12 h light/12 h dark) throughout the experimental period. The experiment was conducted in accordance with the protocol 010/2011 approved by the Animal Research Ethics Committee of Universidade do Oeste de Santa Catarina.

2.3. Experimental Design

2.3.1. Treatment of Animals

The animals were divided into four groups and treated for 15 days, as follows: group C (control, n = 10): water *ad libitum* and rat chow (Nuvilab®); group E (n = 10): aqueous extract of yerba mate *ad libitum* and standard chow; group CD (control, cafeteria diet, n = 10): water *ad libitum* and cafeteria diet; and group ED (n = 10) aqueous extract of yerba mate *ad libitum* and cafeteria diet. A cafeteria diet consists of highly palatable processed human food, it is higher in calories and lipids, when compared to the standard rat chow: 138.6 kcal/30 g vs 87 Kcal/30 g and 19.4 % vs 4 %, respectively. Animals treated with cafeteria diet were fed daily with 10 g of each food item, totaling 30 g for each animal (table 1). The food and fluid intake was measured daily during the same period by weight difference from the previous day. The animals' body weight was determined every two days. The amount provided daily was determined by a pilot study that evaluated the average consumption of the same foods by Wistar rats at the same age. After 15 days, the animals were anesthetized and sacrificed by cervical dislocation. Blood was collected via intraocular puncture with heparinized capillary tubes. The serum was separated for determination of lipid peroxidation. Livers and brains were rapidly removed and fractionated for analysis of oxidative stress and histopathological tests.

2.4. Analysis of Oxidative Stress

2.4.1. Preparation of Tissue Homogenate

Livers and brains were rapidly removed and homogenized (1:10 w/v) in a 0.2 mM sodium phosphate buffer (pH 7.4) containing 0.1 % Triton X-100 and 0.12 M NaCl. The organs were homogenized in a tissue homogenizer (1 min, 100 g) and the homogenate was centrifuged at 10,000g for 10 min. The protein content was determined by the Lowry method [16]. Lipid peroxidation (TBARS), reduced glutathione (GSH) and catalase activity (CAT) were determined in the supernatant, in triplicate.

2.4.2 Lipid peroxidation analysis

The concentration of thiobarbituric acid reactive substances (TBARS) was determined according to method established by Bird and Draper [17], in which malondialdehyde and the final products of the peroxidation of fatty acids react with thiobarbituric acid to form a colored complex. The homogenates (liver and brain; 1 mg protein) were mixed sequentially in test tubes containing Tris-HCl 60 mM buffer (pH 7.4), 0.1 mM DTPA, 12% trichloroacetic acid and 0.73 % thiobarbituric acid. The tubes were incubated for 1 h at 100 °C, cooled and centrifuged (10,000 g

for 5 min). The absorbance of the supernatant was determined at 535 nm. The results are expressed in μmol MDA/mg protein.

2.4.3. Determination of Reduced Glutathione (GSH)

GSH was quantified according to Tietze [18]. This method is based on the reaction of GSH with 5,5'- dithiobis-(2-nitrobenzoic acid) (DTNB), generating the thiolate anion (TNB), which was determined spectrophotometrically at 412 nm. To determine GSH in the samples (liver and brain) 1 mg of protein was added to the reaction medium containing 20 μM DTNB in a 0.2 mM sodium phosphate buffer (pH 8). The results are expressed in mM/mg protein.

2.4.4. Determination of Catalase Activity (CAT)

Catalase activity was determined according to method described by Aebi [19], which quantifies the decomposition of hydrogen peroxide at 240 nm by the enzyme. An aliquot of sample (liver and brain) 0.1 mg protein was added to the reaction medium containing 10 μM hydrogen peroxide in a 50 mM sodium phosphate buffer (pH 7.0). The decrease in absorbance was monitored at 240 nm for 2 min, at 5 s intervals. The enzyme activity was calculated using the molar extinction coefficient of hydrogen peroxide (43.6 M^{-1} cm^{-1}) at 240 nm.

2.5 Histopathology

Liver and brain tissues were processed by standard histopathological techniques. The tissues were sectioned into 5-8 μm wide pieces with a rotary microtome at room temperature and fixed to a 10% formalin solution prepared in phosphate buffer at neutral pH. The samples were stained with hematoxylin and eosin.

2.6. Statistical Analysis

Results are expressed as mean ± standard error of the mean (SEM). The comparison between groups was carried out using the Student's t-test. Multiple comparisons were made by one-way analysis of variance (ANOVA) followed by the post-hoc Dunnett's test. A value of $p < 0.05$ was considered statistically significant.

3. RESULTS

3.1. Fluid and Food Consumption and Body Weight Variation

The results of this study demonstrated that treating animals for 15 days with rat chow + aqueous extract of *I. paraguariensis* (E) significantly decreased body weight gain (Fig. 1), compared to group C, which was fed rat chow + water. Additionally, *I. paraguariensis* was also able to reduce the body weight gain in animals treated with the high calorie diet (ED). Animals treated with aqueous extract of *I. paraguariensis* (E) consumed less food than those taking water (C). Fluid intake was lower in group E compared to group C (table 2).

Group CD ingested less food, but a higher amount of calories, when compared with group C. Among the animals treated with aqueous extract of *I. paraguariensis*, the total calorie intake was higher in group ED than in group E. Fluid intake was lower in ED when compared to CD and E.

3.2. Oxidative Stress in Animals Treated with Cafeteria Diet and Aqueous Extract of *I. Paraguariensis*

Animals treated with water + cafeteria diet (CD) showed a significant increase in lipid peroxidation when compared to the control group (C). The aqueous extract of *I. paraguariensis* reduced significantly lipid peroxidation in groups treated with aqueous extract of *I. paraguariensis* and treated with cafeteria diet (E and ED), as shown in Fig. 2.

Animals treated with aqueous extract of *I. paraguariensis* showed a significant decrease in TBARS levels in the liver (Fig. 3A) in both groups (ED and E), when compared to CD and C, respectively.

In the brain, the aqueous extract of *I. paraguariensis* significantly reduced lipid peroxidation in both groups E and ED. TBARS levels were not increased by the cafeteria diet, when compared to groups CD and C (1185.15 ± 267.91 vs 1110.00 ± 88.00 μmol MDA/mg protein), as shown in Fig. 4A.

3.3. Glutathione (GSH) Levels in Brain and Liver

The consumption of *I. paraguariensis* extract caused no significant change in GSH levels in the liver of rats treated with cafeteria diet, when groups ED and CD are compared (0.84 ± 0.11 vs 0.76 ± 0.08 mM/mg protein) (Fig. 3B). However, there was a significant increase in the group treated with extract of *I. paraguariensis* + rat chow (E) compared to the group treated with water + rat chow (C), (0.92 ± 0.10 vs 0.57 ± 0.05 mM/mg protein), respectively.

Glutathione levels were significantly increased in brain tissue from both groups ED (cafeteria diet) and E (rat chow) after ingestion of the extract of *I. paraguariensis* (Fig. 4B). Compared with the control group, the cafeteria diet caused no significant change in GSH levels in the brain.

3.4. Catalase (CAT) Antioxidant Activity in Brain and Liver

Catalase activity is shown in Fig. 3C and 4C (liver and brain, respectively), in order to evaluate enzyme antioxidant action. Catalase activity in liver tissues from different groups can be observed in Fig. 3C. There was no significant difference between the groups treated with the cafeteria diet (CD and ED). However, it is noteworthy that catalase activity in the liver increases after eating the cafeteria diet, as observed in groups CD and ED, compared to C and E.

Fig. 4C shows that catalase activity in the brain was significantly increased by the cafeteria diet in group CD compared to group C (37.43 ± 6.0 vs 11.08 ± 0.82 mM/min/mg protein). After ingesting extract of *I. paraguariensis*, a decreased in catalase activity in groups E and ED was observed, compared to C and CD.

3.5. Histopathology

Morphological investigations performed in hepatocytes animals from group C or E not shows alterations significant. Liver tissues from all animals in these groups were normal (Fig. 5A and B).

Animals receiving the cafeteria diet showed moderate to severe swelling of hepatocytes, as well as poorly defined vacuoles, disorganization of hepatocyte cords and reduced capillary lumen. These signs were not diminished in the liver tissue of animals treated with aqueous extract of *I. paraguariensis* and cafeteria diet (Fig. 5C and D; 6C and D). No changes in brain tissue were observed in both groups (results not shown).

4. DISCUSSION

There is evidence that plant-derived compounds may have beneficial effects on health. *I. paraguariensis* infusions contain numerous bioactive compounds, such as polyphenols [20]. Yerba mate has had various biological activities reported in the literature, including its potential antioxidant activity, which is attributed to a great additional source of natural polyphenols [21].

Cafeteria diets, such as that employed in this study, have been used in experimental research in animal models with the objective of analyzing food consumption in modern societies [22]. Increased levels of glucose and fatty acids in tissues and blood stream, caused by this type of diet, link the occurrence of obesity to a high ROS production, generating oxidative stress [23].

The fact that animals consume significantly less food when treated with aqueous extract of *I. paraguariensis* reinforces the hypothesis that weight loss (Fig. 1) can be considered, at least partially, due to increased satiety induced by *I. paraguariensis*. Although *I. paraguariensis* intake has not reduced significantly the energy consumption, a reduced body weight in animals ingesting the cafeteria diet suggests that other mechanisms, independent of increased satiety, may be involved in body weight loss, such as increasing metabolism and oxidation of fatty acids.

The bitter taste or the short adaptation to the aqueous extract of *I. paraguariensis*, as the only fluid source, may have been responsible for the lower intake verified by the average daily consumption of fluid. However, this has not affected the antiobesity effects seen on animals taking the aqueous extract.

Levels of malondialdehyde (MDA), used as a TBARS measure, were evaluated. MDA formation occurs when free radicals attack the fatty acids in biological membranes, leading to structural and permeability changes. This may cause loss of selectivity during ion exchange, release of organelle contents, formation of cytotoxic products (such as malondialdehyde), culminating in cell death [24, 25].

Milagro, et al. [26] showed that animals treated with cafeteria diet for 56 days had increased oxidative stress in the liver. Conversely, Moreira, et al. [27] found no increase in lipid peroxidation in rats treated with cafeteria diet for four weeks. Controversy about the induction of lipids oxidation may be related to the variation in the concentration of polyunsaturated fatty acids in this type of diet, because the increase in polyunsaturated fatty acids leads to a higher susceptibility to oxidation by ROS, thus representing a risk factor for lipid peroxidation in various cells [28].

This study demonstrated that the cafeteria diet did not induce an increase in oxidative stress by the formation of MDA in liver and brain tissues. However, only on the serum this increase was significant. Two weeks of treatment with the cafeteria diet may not be sufficient to cause the depletion of antioxidant defenses, which occurs at the initial phase (latency) of the lipid peroxidation process, when antioxidants are consumed with no significant oxidation of fatty acids [29]. On the other hand, the treatment with extract of *I. paraguariensis* was able to reverse the oxidative stress caused by the diet on the serum, whereas there has been a significant reduction in this parameter in the evaluated tissues. Thus, the aqueous extract of *I. paraguariensis* showed antioxidant action, being a potential protective mechanism against damage caused by oxidative stress.

The ingestion of aqueous extract of *I. paraguariensis* had distinct effects on the markers of antioxidant defenses in liver and brain tissues. In normal conditions, the antioxidant defense system is responsible for keeping the redox-active species under control. The primary defense system is composed of substances that prevent the generation or sequester ROS, thus blocking the initiation of the root chain. Antioxidant enzymes (CAT, SOD, GPx) and non-enzymatic substances, such as reduced glutathione (GSH), are found in this system. The secondary defense system is composed of aromatic amines or phenolic compounds, such as tocopherols and flavonoids [25].

The tripeptide GSH is one of the most abundant and effective antioxidants in the human body. The results observed in GSH levels showed variation among the analyzed tissues. The GSH increase in the brain of rats consuming the aqueous extract of *I. paraguariensis* is necessary due to its use in the detoxification of free radicals from lipid peroxidation, where GSH is oxidized to GSSG by the action of glutathione peroxidase (GPx) to remove H_2O_2 [25]. Glutathione reductase (GR), an enzyme not evaluated in this study, catalyzes the reduction of GSSG allowing the continuous regeneration of GSH produced by GPx action [30]. Thus, the decreased catalase activity in the brain may be associated with a lower need in the detoxification of free radicals, because the aqueous extract of *I. paraguariensis* could be acting as a modulator antioxidant of the endogenous defenses, which also explains the decrease of lipid peroxidation. The increased activity of this enzyme in the liver is directly related to the cafeteria diet consumption, suggesting that the defense system is responsible for the modulation of the enzymatic activity in an attempt to detoxify the liver after exposure to free radicals.

The results suggest that the significant GSH increase in the liver was caused by ingestion of aqueous extract of *I. paraguariensis*. However, this does not happen when the cafeteria diet is also consumed. GSH maintenance is important because demonstrates the integrity of the oxidation-reduction system, with high levels of glutathione being necessary in the liver, due to the organ's high exposure to xenobiotics [31]. In this way, free radical formation would be inhibited by the system involving GSH and related enzymes, without causing depletion.

Although the cafeteria diet induced an increase in the oxidative stress only on serum, treatment with aqueous extract of *I. paraguariensis* showed significant effects in the modulation of antioxidant defenses in both tissues studied, showing antioxidant potential in the presence or

absence of the cafeteria diet. Further studies should be developed to better characterize the effects of cafeteria diets on oxidative stress in different periods, and evaluate other models able to detect the antioxidant potential of the aqueous extract of *I. paraguariensis* to better understand its mechanisms of action in various tissues.

5. CONFLICT OF INTEREST

The authors declare that are no conflicts of interest.

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LEGENDS

Table 1 - Cafeteria diet used in this study (groups CD and ED, for15 days).

Table 2 – Food and fluid intake by groups: C (water and rat chow); E (aqueous extract of *I. paraguariensis* and rat chow); CD (water and cafeteria diet); ED (aqueous extract of *I. paraguariensis* and cafeteria diet).

Fig. 1. Variations in body weight in groups C (water and rat chow), E (aqueous extract of *I. paraguariensis* and rat chow), CD (water and cafeteria diet) and ED (aqueous extract of *I. paraguariensis* and cafeteria diet). Results are expressed as mean ± standard error of the mean (n = 10). * significantly different to C; @ significantly different to E; # significantly different to CD. $p < 0.05$ using analysis of variance (ANOVA) followed by the post hoc Dunnett 's test.

Fig. 2. Evaluation of TBARS levels in serum. Where C (water and standard chow). E (aqueous extract of *I. paraguariensis* and feed). CD (water and cafeteria diet). ED (aqueous extract of *I. paraguariensis* and cafeteria diet). Results are expressed as mean ± standard error of the mean (n = 10). * $p < 0.05$= significant difference between ED and E and between CD and C. # $p < 0.05$= significant difference in relation to CD, using analysis of variance (ANOVA) followed by the post-hoc Dunnett's test.

Fig. 3. Evaluation of oxidative stress in the liver of animals treated with rat chow and cafeteria diet, ingesting or not aqueous extract of *I. paraguariesis*. **(A)** TBARS levels. **(B)** GSH concentration. **(C)** Catalase activity. Where: C (water and rat chow), E (aqueous extract of *I. paraguariensis* and rat chow), CD (water and cafeteria diet), ED (aqueous extract of *I. paraguariensis* and cafeteria diet). Results are expressed as mean ± standard error of the mean (n = 10). **(A)** *different from C, * different from E, # different from CD. **(B)** *different from C, * **(C)** *different from E, *different from C. * $p < 0.05$. # $p < 0.05$ by analysis of variance ANOVA followed by the post-hoc Dunnett's test.

Fig. 4. Evaluation of oxidative stress in the brain of animals treated with rat chow and cafeteria diet, ingesting or not aqueous extract of *I. paraguariesis*. **(A)** TBARS levels. **(B)** GSH concentration. **(C)** Catalase activity. Where: C (water and rat chow), E (aqueous extract of *I. paraguariensis* and rat chow), CD (water and cafeteria diet), ED (aqueous extract of *I. paraguariensis* and cafeteria diet). Results are expressed as mean ± standard error of the mean (n = 10). * significantly different to C ; * significantly different to E; # significantly different to CD. * $p < 0.05$. # $p < 0.05$ by analysis of variance ANOVA followed by the post-hoc Dunnett's test.

Fig. 5. Histopathological analysis of the liver of animals treated with: A = rat chow and water (10x magnification), B = rat chow and water (40x magnification), C = water and cafeteria diet

(10x magnification) and D = water and cafeteria diet (40x magnification). The arrows indicate the histological changes observed in animals treated with cafeteria diet.

Fig. 6. Histopathological analysis of the liver of animals treated with: A = aqueous extract of *I. paraguariensis* and rat chow (10x magnification), B = aqueous extract of *I. paraguariensis* and rat chow (40x magnification). C = aqueous extract of *I. paraguariensis* and cafeteria diet (10x magnification) and D = aqueous extract of *I. paraguariensis* and cafeteria diet (40x magnification). The arrows indicate the histological changes observed in animals treated with cafeteria diet.

TABLES

Table-1.

Food item	Weight (g)	Energy (Kcal)	Fat (%)	Protein (%)	Carbohydrate (%)
Bacon-flavored chips (Baconzitos)®	10	50	30	5	55
Sweet cookie Isabela®	10	48.6	0	8.8	22.2
Peanut and molasses candy Yoki®	10	45.3	28.2	14.7	34.7
Total	30	138.6	19.4	9.5	37.3
Rat chow (Nuvilab®)					
Nuvilab® rat chow	**30**	**87**	**4**	**14**	**72**

Adapted from Rothwell, et al. [32].

Table-2.

Treatment	Total food intake (g)	Total food intake (Kcal)	Fluid intake (ml)
C	23.8 ± 0.4	69.1 ± 1.2	40.0 ± 1.0
E	20.5 ± 0.3 *	59.5 ± 0.9 *	34.4 ± 2.1 *
CD	19.1 ± 0.3*	91.8 ± 1.7 *	36.0 ± 0.6
ED	19.8 ± 0.5	95.1 ± 2.2 @	25.8 ± 1.0 @ #

* significantly different from group C;

@ significantly different from group E;

significantly different from group CD.

FIGURES

Figure 1

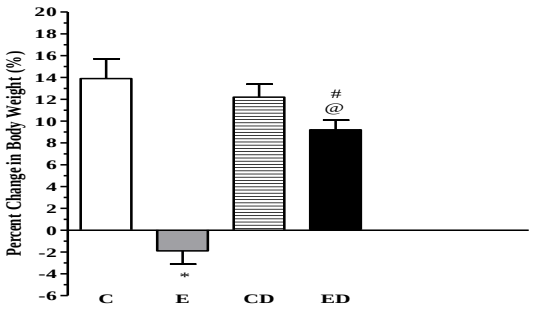

Figure 2

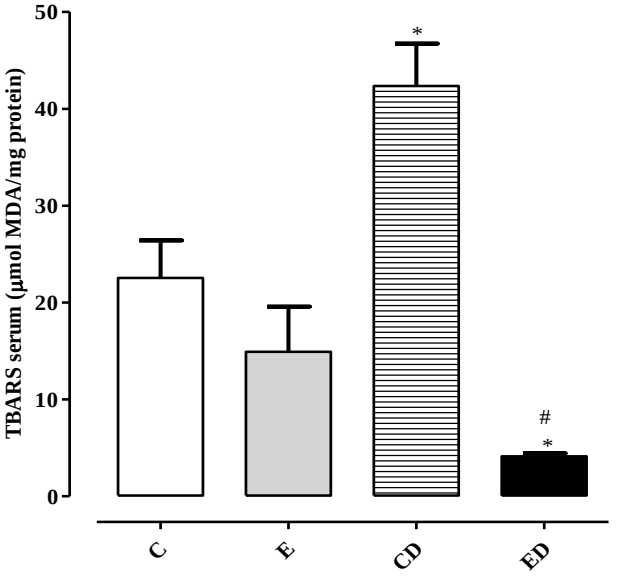

Figure 3

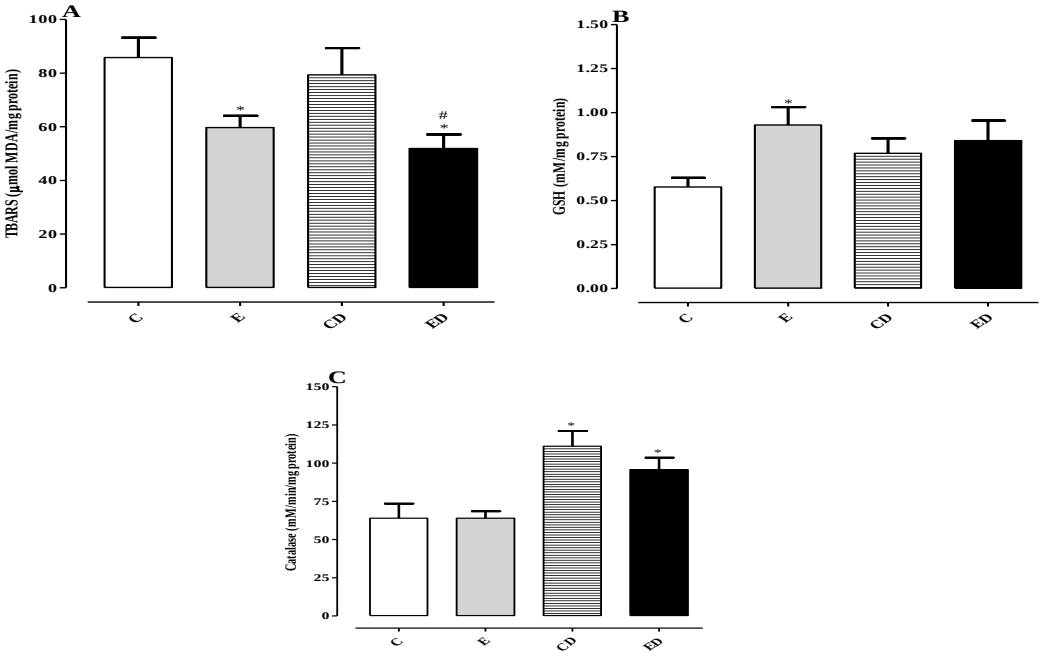

Figure 4

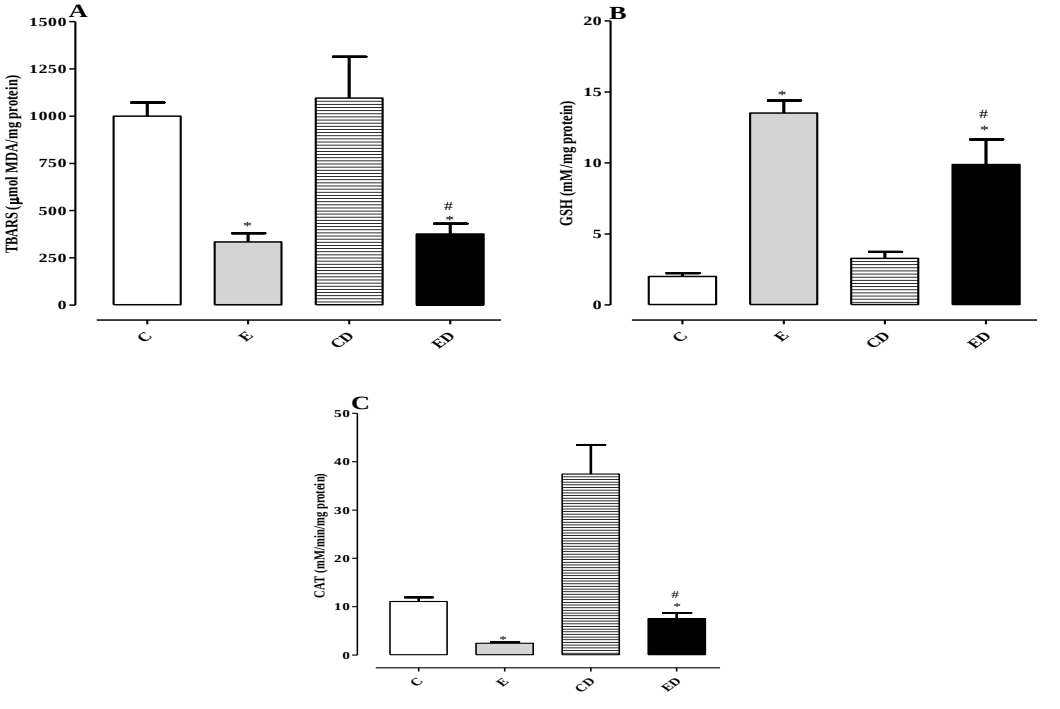

Figure-5.

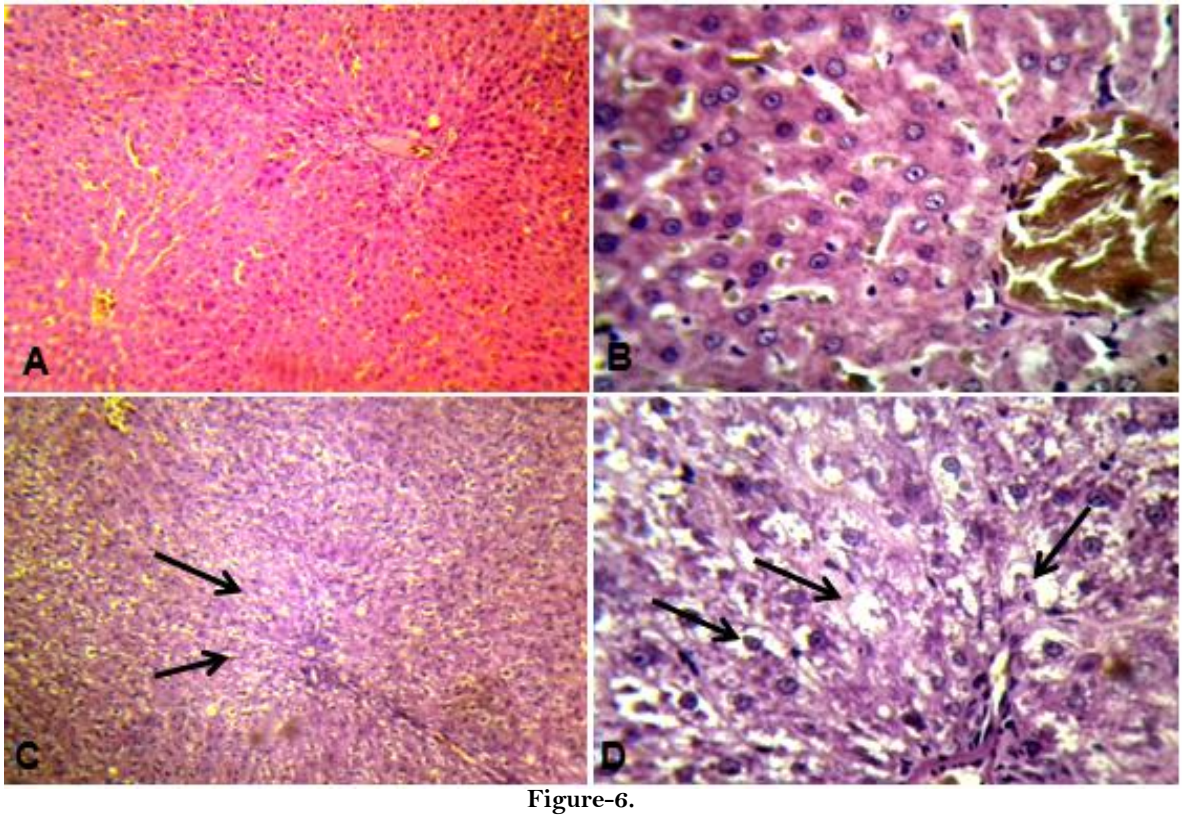

Figure-6.