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Rutin ameliorates glycemic index, lipid profile and enzymatic activities in serum, heart and liver tissues of rats fed with a combination of hypercaloric diet and chronic ethanol consumption

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Alcoholism and obesity are strongly associated with several disorders including heart and liver diseases. This study evaluated the effects of rutin treatment in serum, heart and liver tissues of rats subjected to a combination of hypercaloric diet (HD) and chronic ethanol consumption. Rats were divided into three groups: Control: rats fed a standard diet and drinking water *ad libitum;* G1: rats fed the HD and receiving a solution of 10% (v/v) ethanol; and G2: rats fed the HD and ethanol solution, followed by injections of 50 mg/kg^{-1} rutin as treatment. After 53 days of HD and ethanol exposure, the rutin was administered every three days for nine days. At the end of the experimental period (95 days), biochemical analyses were carried out on sera, cardiac and hepatic tissues. Body weight gain and food consumption were reduced in both the G1 and G2 groups compared to control animals. Rutin effectively reduced the total lipids (TL), triglycerides (TG), total cholesterol (TC), VLDL, LDL-cholesterol and glucose levels, while it increased the HDL-cholesterol in the serum of G2 rats, compared to G1. Although rutin had no effect on total protein, albumin, uric acid and cretinine levels, it was able to restore serum activities of alkaline phosphatase (ALP), lactate dehydrogenase (LDH), aspartate aminotransferase (AST), alanine aminotransferase (ALT) and creatine kinase (CK) in animals fed HD and receiving ethanol. Glycogen stores were replenished in both hepatic and cardiac tissues after rutin treatment. Moreover, rutin consistently reduced hepatic levels of TG and TC and cardiac AST, ALT and CK activities. Thus, rutin treatment was effective in reducing the risk factors for cardiac and hepatic disease caused by both HD and chronic ethanol consumption.

Keywords: Rutin, Hypercaloric diet, Ethanol, Heart, Liver, Rat.

Alcoholism and obesity represent two of the major public health problems of modern society[1-3]. Both chronic ethanol consumption and a hypercaloric diet (HD) intake are strongly associated with several diseases, including hypertension, cardiomyopathy, hepatic esteatosis and certain types of cancers[4-9]. It has been reported that chronic ethanol consumption and a HD can contribute to the development of cardiovascular and liver diseases by generating free radicals, which may disturb glucose metabolism and lipid profiles, leading to increased serum levels of triglyceride, low-density lipoprotein (LDL), very low-density lipoprotein (VLDL) and decreased high-density lipoprotein (HDL)[10-13].

Polyphenolic natural flavonoid has powerful antioxidant properties in neutralizing reactive oxygen and nitrogen species formation[14]. In this context, rutin, a natural flavonoid commonly distributed in various vegetables and fruits, such as buckwheat, passion flower, apples, onions, red wine and tea[15a & b] is known to have beneficial effects in decreasing TG, LDL and VLDL levels, besides restoring the normal concentration of cardiac and hepatic glycogen in diabetic rats[16]. Notably, rutin also has potential anti-tumor efficacy and anti-inflammatory effects[17,18].

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Abbreviations: ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CK, creatine kinase; HD, hypercaloric diet; HDL, high-density lipoprotein; HMG-CoA, 3-hydroxy-3-methyl-glutaryl-CoA reductase ; LDH, lactate dehydrogenase; LDL, low-density lipoprotein; NAD, nicotinamide adenine dinucleotide; NADH, nicotinamide adenine dinucleotide plus hydrogen; TC, total cholesterol; ROS, reactive oxygen species; TG, triglycerides; VLDL, very low-density lipoprotein; XO, xantine oxidase.

It also exerts hepatoprotective effect in rat, causing depletion of ALT, AST and ALP[19]. In general, the effects of polyphenols on lipid metabolism are linked to the antioxidant action against LDL oxidation, changes in hepatic cholesterol uptake and synthesis of triglycerides[20].

To date, no study has established the relationship between alcoholism and HD consumption and the role of rutin in restoring the nutritional status and biochemical alterations in the serum, hepatic and cardiac tissues. Therefore, this study has been aimed to evaluate whether the flavonoid rutin can improve the lipid and glucose profile, as well as the cardiac and hepatic enzymatic activities in serum, liver and heart tissue of rats subjected to a combination of hypercaloric diet and chronic ethanol consumption.

Materials and Methods

Animals and experimental protocol

Thirty male 56-day-old Wistar rats, weighing 200–250 g were used in this study. All rats were housed in polypropylene cages and maintained under conditions of lighting, temperature- and humidity-controlled room (12-h light/dark cycle; 25 ± 3ºC; 60 ± 5% humidity). The experimentation followed the principles and guidelines of the Canadian Council on Animal Care as outlined in the 'Guide to the Care and Use of Experimental Animals' and was approved by the Ethics Committee for Conduct of Animal Studies at the Institute of Biosciences, São Paulo State University (Permit number 71/01).

The animals were randomly divided into three experimental groups (n = 10/group): Control group: rats fed a standard diet and drinking water *ad libitum*; Group 1 (G1): rats fed a hypercaloric diet and receiving water *ad libitum* for 42 consecutive days, and after this period they received, instead of water, a solution of 10% (v/v) ethanol diluted in water for 53 days; Group 2 (G2): rats fed a hypercaloric diet and water *ad libitum* for 42 consecutive days and after this period they received, instead of water, a solution of 10% (v/v) ethanol diluted in water for 53 days plus administration of rutin as treatment. For both treated groups, the ethanol solution was offered *ad libitum* for seven consecutive weeks. Additionally, a hypercaloric diet was standardized according to a method previously described[21]. Briefly, the chow composition was standard rodent chow Purina® (3.78 kcal/g) added with skinless toasted peanuts (5.95 kcal/g), milk chocolate (6.11 Kcal/g) and corn starch wafer (3.55 Kcal/g), in the proportion of 3:2:2:1. The caloric density was 21.40 kJ/g (35% of calories as fat) for the palatable HD. Diets were given fresh as pellets and provided sufficient amounts of vitamins, minerals and essential lipids.

For the animals of G2, the treatment with rutin (Sigma, St. Louis, MO, USA) started after 42 days of combined HD and ethanol ingestion (Fig. 1). Rutin was dissolved in propyleneglycol as vehicle and injected intraperitoneally in a scheme of four doses of 50 mg kg^{-1} body weight[16,22] (Fig. 1). The animals received rutin every three days at the same time (9:00 h) for nine days (from 84 to 93 days of experiment; Fig. 1). Total food intake and ethanol consumption (g/kg/day) were measured daily in analytical balance and body weight (g) was monitored every week. Body weight gain was achieved by subtracting the final body weight from the initial body weight (FBW - IBW).

Sample preparation

At the end of experimental period (95 days), 48 h after the last dose of rutin, the rats were anesthetized (0.1 mL i.p. of 3% sodium pentobarbital) and euthanized by decapitation (Fig. 1A). Blood samples were collected and centrifuged at 6000 rpm for 15 min, and the serum was used for determination of several biochemical parameters. Tissue samples (± 200 mg) of liver and heart (left ventricle) were dissected, washed with ice-cold saline solution and homogenized with 0.01 M sodium phosphate buffer (pH 7.4), using a tissue homogenizer, motor-driven teflon glass Potter Elvehjem (1 min, 100 g). Thereafter, the homogenate was centrifuged at 10,000 *g* for 15 min and the supernatant was collected and processed for biochemical analysis.

Biochemical analyses

Biochemical parameters (urea, uric acid and creatinine levels) were measured in serum samples with a spectrophotometer Pharmacia Biotech (Ultrospec 2000, Cambridge, England). The analyses were performed with a CELM kit (Modern Laboratory Equipment Company, São Paulo, Brazil).

The glycemic index was determined by an enzymatic method utilizing glucose oxidase and peroxidase. The serum insulin concentration was determined using an enzyme immune assay kit (EIA kit, Cayman Chemical, USA), using an ELISA microplate reader (Biotech Instruments, Inc, USA).

Total cholesterol levels were enzymatically measured through the cholesterol ester/oxidase ratio. HDL concentrations were measured after precipitation of

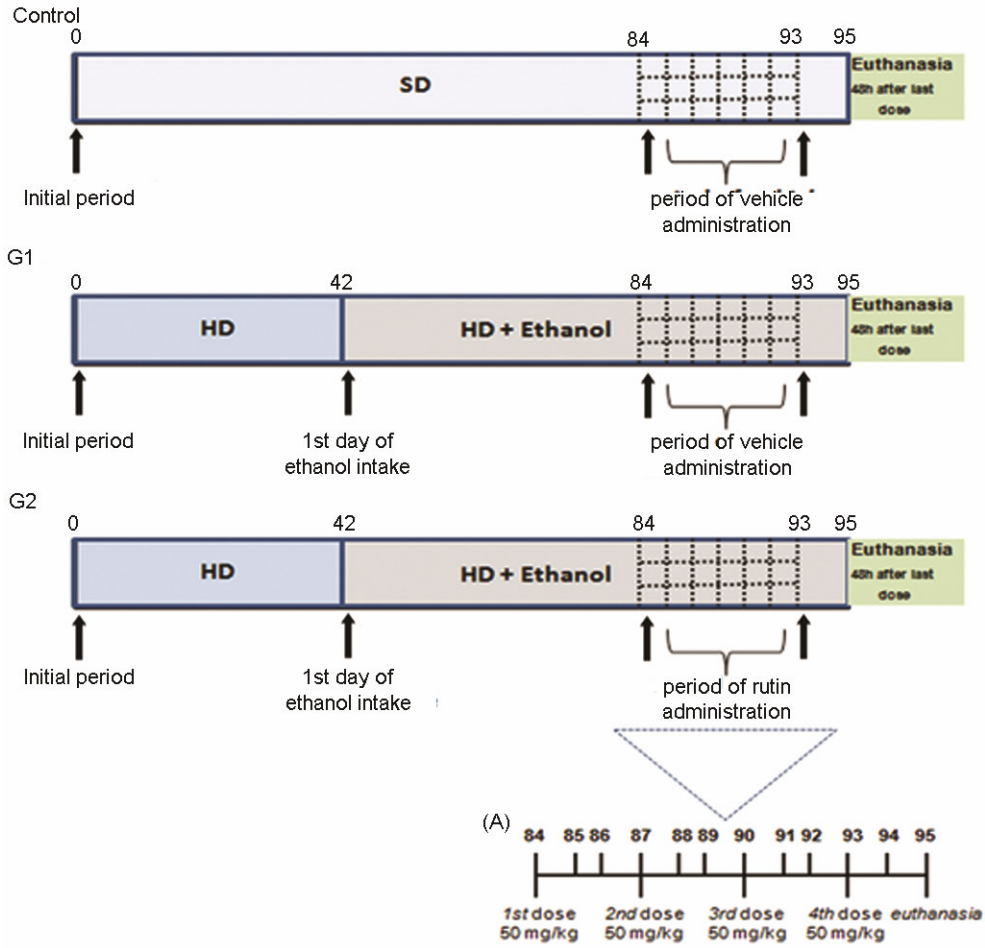

Fig. 1—Detailed schedule for overall experimental design (days) applied to individual group of animals. (A) Schematic protocol used for rutin administration based on four (4) doses of 50 mg kg^{-1}, followed by an interval of three (3) days between each dose. HD: hypercaloric diet; Control group: animals receiving standard diet (SD) and water *ad libitum*; G1: animals treated with ethanol and high-fat diet (HFD); G2: animals treated with ethanol and HFD receiving rutin as treatment

VLDL and LDL by the sodium phosphotungstate/Mg^{2+} method[23], using an enzymatic colorimetric method that incorporated polyethylene glycol-modified cholesterol ester oxidase. LDL concentrations were calculated by the Friedewald formula. Triacylglycerol concentrations were enzymatically assayed with glycerol kinase after lipoprotein lipase-catalyzed hydrolysis followed by oxidation to dihydroxyacetone phosphate and hydrogen peroxide[24].

The enzymatic activities of alkaline phosphatase (ALP–E.C. 3.1.3.1), creatine kinase (CK–E.C. 2.7.3.2), alanine aminotransferase (ALT–E.C. 2.6.1.2), aspartate aminotransferase (AST–E.C. 2.6.1.1) and lactate dehydrogenase (LDH–E.C. 1.1.1.27) were measured by enzymatic methods in serum, liver, and heart tissue[25].

Hepatic and cardiac (left ventricle) tissues were homogenized in 0.6 M perchloric acid, and the concentration of free glucose was determined by the glucose oxidase procedure. The stored glycogen was then hydrolyzed with amyloglucosidase (Sigma, St. Louis, MO, USA) and the total glucose released was measured[26]. The hepatic triacylglycerol was extracted using the procedure developed above[27]. Part of the sample (approximately 200 mg) was homogenized in chloroform-methanol 2:1 (v/v), with the chloroform layer containing all the lipids and the metabolic layer containing all the non-lipids. The hepatic triacylglycerol was measured as described for serum[24]. Homogenates were prepared on ice at the ratio of 200 mg hepatic and cardiac (left ventricle) tissues per 5 mL of 0.01 M phosphate buffer (pH 7.4) in a Potter-Elvehjem homogenizer. The homogenates were centrifuged at 12,000 *g* for 20 min at 48°C[28] and the supernatant was used for determination of total protein and activities of ALT and LDH.

Statistical analysis

Statistical differences were assessed by analysis of variance (ANOVA), followed by Tukey's test. The statistical significance was set at $p< 0.05$ (Systat Software, San Jose, CA). All results were expressed as the mean ± standard deviation (SD). The Sigma Plot program (version 11.0) was used for graphic design.

Results

Nutritional parameters

Animals of the control group had higher body weights and food consumption compared to the G1 and G2 groups, which consequently had higher body weight gains (Table 1). There were no differences in both food and ethanol consumption between G1 and G2; however, these groups presented the highest ingestion of food-derived calories. As expected, rutin treatment did not significantly affect the body weight gain during the experimental period (Table 1).

Lipid profile and serum biochemical parameters

Figure 2 shows that ethanol and HD consumption were able to induce a dyslipidemic profile by increased levels of total lipids (TL), total triacylglycerol (TG), total cholesterol (TC), VLDL, LDL and reduced HDL concentrations. Administration of rutin had a protective effect on serum lipid fractions. TL, TG, TC, VLDL and LDL concentrations were reduced after rutin treatment, while HDL increased (Fig. 2). Glucose and urea levels were respectively higher and lower in the G1, when compared with the control group. Conversely, rutin effectively reduced the glucose concentration and increased the urea levels in animals receiving HD and ethanol. Also, serum levels of nitrogen derivatives, such as total protein, albumin, uric acid and creatinine were unchanged after treatment (Table 2).

Table 1—Nutritional parameters
[Values expressed as mean ± SD]

Parameters	Groups		
	Control	G1	G2
Body weight (g)	379.1 ± 73.4	359.5 ± 56.6 [a]	362.8 ± 63.0 [a]
Body weight gain (g)	230 ± 3.6	168 ± 1.8 [a]	164 ± 1.8 [a]
Ethanol consumption (g/Kg/day)	—	0.41 ± 0.1	0.43 ± 0.7
Energy from ethanol (kcal/Kg/day)	—	2.9 ± 0.7	3.1 ± 0.5
Food consumption (g/Kg/day)	11.3 ± 2.8	6.7 ± 2.9 [a]	6.4 ± 3.0 [a]
Energy from food (kcal/Kg/day)	42.9 ± 10.7	129.2 ± 15.5 [a]	124.8 ± 15.6 [a]

[a] $P < 0.05$ *vs* Control group. One-way ANOVA complemented by Tukey test. Control group: animals receiving standard diet and water *ad libitum*; G1: animals treated with ethanol and high-fat diet (HFD); G2: animals treated with ethanol and HFD receiving rutin as treatment.

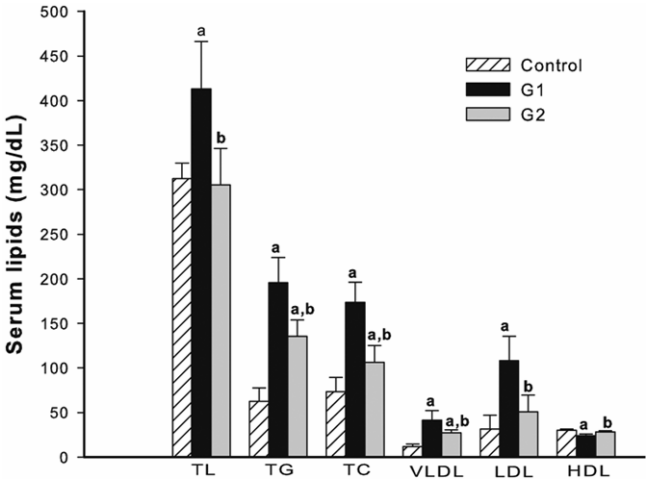

Fig. 2—Serum lipid profile (mg/dL) of control, G1 and G2 groups [TL: total lipid; TG: total triglyceride; TC: total cholesterol; VLDL: very low-density lipoprotein; LDL: low-density lipoprotein; HDL: high-density lipoprotein. [a] $P < 0.05$ different from control group; [b] $P < 0.05$ different from G1 group. All results are expressed as the mean ± SD]

Table 2—Serum glucose concentration, total protein, nitrogen metabolism-related parameters and serum enzymatic activities
[Values expressed as mean ± SD]

Parameters	Groups		
	Control	G1	G2
Glucose (mg/dL)	120.79 ± 10.24	181.71 ± 23.16 [a]	138.53 ± 13.02 [b]
Total protein (g/dL)	7.94 ± 1.03	9.83 ± 2.96	11.04 ± 1.65 [a]
Albumin (g/dL)	4.04 ± 0.45	4.07 ± 0.45	4.14 ± 0.27
Urea (mg/dL)	59.24 ± 6.51	41.66 ± 4.84 [a]	52.49 ± 4.99 [b]
Uric acid (mg/dL)	1.65 ± 0.73	1.63 ± 1.37	2.82 ± 0.88
Creatinine (mg/dL)	0.99 ± 0.12	0.95 ± 0.39	1.03 ± 0.15
ALP (U/L)	118.70 ± 7.67	182.28 ± 25.32 [a]	119.98 ± 8.87 [b]
Amylase (U/L)	156.82 ± 38.34	165.03 ± 6.76	160.38 ± 7.71
LDH (U/L)	130.94 ± 6.93	266.60 ± 8.77 [a]	137.54 ± 5.74 [b]
ALT (U/L)	52.01 ± 4.54	89.23 ± 8.95 [a]	67.55 ± 4.38 [b]
AST (U/L)	57.94 ± 2.55	75.14 ± 4.70 [a]	54.20 ± 3.23 [b]
CK (U/L)	157.82 ± 16.66	263.37 ± 10.50 [a]	144.82 ± 6.75 [b]

[a] $P<0.05$ different from *c*ontrol group; [b] $P<0.05$ different from G1 group. One-way ANOVA complemented by Tukey test. ALP: alkaline phosphatase; ALT: alanine aminotransferase; AST: aspartate aminotransferase; CK: creatine kinase; LDH: lactate dehydrogenase. Control group: animals receiving standard diet and water *ad libitum*; G1: animals treated with ethanol and high-fat diet (HFD); G2: animals treated with ethanol and HFD receiving rutin as treatment.

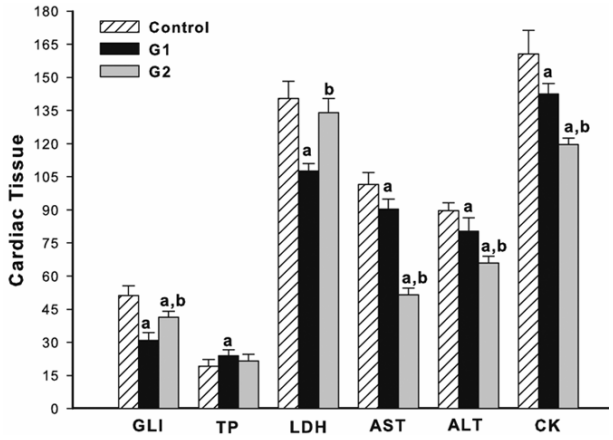

Fig. 3—Biochemical parameters in cardiac tissue of control, G1 and G2 groups [GLI: glycogen (mg/g); TP: total protein (g/100 g tissue); LDH: lactate dehydrogenase (U/g soluble protein); AST: aspartate aminotransferase (U/g soluble protein); ALT: alanine aminotransferase (U/g soluble protein); CK: creatine kinase (U/g soluble protein). [a] $P < 0.05$ different from control group; [b] $P < 0.05$ different from G1 group. All results are expressed as the mean ± SD]

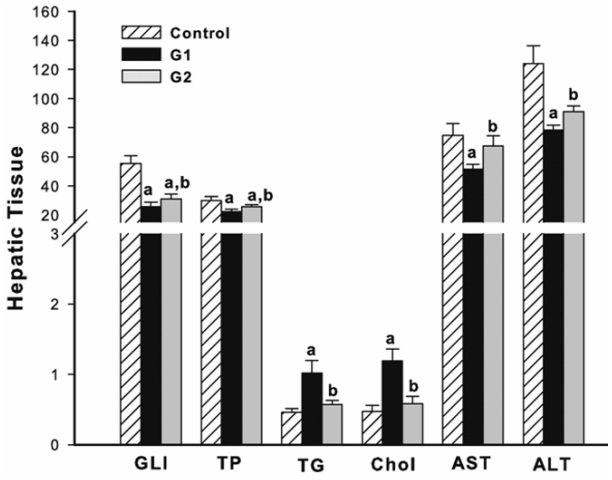

Fig. 4—Biochemical parameters in hepatic tissue of control, G1 and G2 groups [GLI: glycogen (mg/g); TP: total protein (g/100 g tissue); TG: total triglyceride (g/100 g tissue); Chol: cholesterol (g/100 g tissue); AST: aspartate aminotransferase (U/g soluble protein); ALT: alanine aminotransferase (U/g soluble protein). [a] $P < 0.05$ different from control group; [b] $P < 0.05$ different from G1 group. All results are expressed as the mean ± SD]

Serum ALT, AST, ALP, LDH and CK activities were increased in the G1 group compared with the control group. Administration of rutin significantly lowered these activities to normal levels (Table 2).

Biochemical parameters in cardiac and hepatic tissues

The glycogen concentration was enhanced after rutin treatment in cardiac tissue and total protein was unaltered by the treatment (Fig. 3). Ethanol and HD consumption caused a decrease in cardiac LDH, AST, ALT and CK activities, as well as reduced the cardiac glycogen stores. Considering the cardiac enzyme activities, administration of rutin increased LDH activity and reduced the activities of AST and ALT, as well as CK and augmented cardiac glycogen concentrations (Fig. 3).

Hepatic tissue was also injured by ethanol associated with HD ingestion. Glycogen, protein levels, AST and ALT activities were lower than those found in the control group. Notably, TG and cholesterol concentrations were higher in the G1 group (Fig. 4). Rutin augmented glycogen concentration, total protein, and the activities of AST and ALT. Additionally, administration of rutin diminished the hepatic levels of TG and total cholesterol (Fig. 4).

Discussion

It is expected that high caloric food consumption leads to increased body weight gain[29], while chronic ethanol consumption causes a severe reduction[6]. In addition, quercetin-like flavonoids may reduce body weight gain by either a direct effect on food and energy intake or by modulating liver and adipocyte lipid metabolism in obese animals[30]. Other flavonoids, such as resveratrol do not change these parameters[29]. This observation was similar to that found in our investigations of rutin treatment (Table 1), and it could be assumed that rutin did not alter the final body weight in rats receiving ethanol and HD under *ad libitum* feeding and drinking conditions. Interestingly, the effects of ethanol intake over body weight loss surpassed the effects caused by HD ingestion, since animals from G1 and G2 showed lower body weights. Indeed, ethanol intake is associated with reduced food consumption and consequent low body weight[8]. Furthermore, the empty calories of ethanol do not contribute to the body weight gain, and the lipid synthesis pathway is blocked by inhibition of glucose-6-phosphate dehydrogenase, a key enzyme for generating NADPH that is essential during lipogenesis.

The elevation of serum lipid profiles in the G1 group might be associated with increased hepatic TG and cholesterol. Most of the consequences linked to serum hyperlipidaemia and hyperlipoproteinaemia are related to the high risks for developing cardiovascular diseases such as atherosclerosis[11]. Additionally, the TG/HDL ratio has been proposed to be used as an indicator of heart failure[31], dyslipidaemia, and insulin resistance[32]. It is well-known that either HD or chronic ethanol consumption significantly alter cholesterol metabolism,

leading to serum dyslipidaemia[33,34]. In addition, ethanol *per se* can be responsible for an imbalance of the NAD^+/NADH redox state in hepatocytes[35], thus diminishing fatty acid β-oxidation and contributing to TG biosynthesis and further to the onset of fatty liver or hepatic steatosis[36,37].

Rutin treatment was able to reduce the concentration of TG and cholesterol in the liver of animals receiving both a hypercaloric diet and ethanol. These findings indicated that rutin minimized the harmful effects on lipid metabolism. A possible explanation is the fact that rutin-like flavonoids can inhibit HMG-CoA reductase production, an essential enzyme for cholesterol synthesis[38,39]. Conversely, the increase of HDL levels induced by rutin is beneficial, since it removes cholesterol from the organism and sends it to be metabolized by a mechanism known as reverse cholesterol transport[40]. This event explained the increase of HDL in association with reduced serum levels of TG, total cholesterol, VLDL and LDL in animals treated with rutin. It has recently been demonstrated that rutin has hypolipidemic functions, acting as a hepatoprotector and cardioprotector in diabetic animals[16]. There are several flavonoids implicated in preventing atherosclerosis by inhibiting lipid peroxidation of LDL particles[41] and lowering the serum concentration of cholesterol and TG[42,43].

Rutin also positively affected glucose and glycogen metabolism in both liver and cardiac tissues. It seems possible that glycogen content tends to be depleted after long-term exposure to ethanol[44], in response to inhibition of the gluconeogenesis pathway[45]. Furthermore, ethanol may also trigger its hypoglycemic effects through inhibition of glycogenolysis[46]. Moreover, the high serum glucose concentration in G1 animals indicated that glucose metabolism was only significantly affected by HD ingestion and not by ethanol consumption.

Experimental and epidemiological studies have shown that a hypercaloric diet causes disturbances in glucose metabolism related to glucose intolerance and decreased insulin sensitivity[10]. The effects of alcohol and the impact of hypercaloric diet consumption on glucose metabolism are not fully understood[10]. Rutin was capable of restoring glycogen and glucose to near normal levels in G2 animals. Notably, general flavonoids may suppress or inhibit the gluconeogenic enzymes or even the liver glucose-6-phosphatase, thereby reducing the release of glucose into the blood stream[47]. Flavonoids also exert their function on glucose metabolism by stimulating glycogenic enzymes, which increase glucose storage and also reduce glycogenolysis[48]. Thus, rutin possibly restored the homeostasis of gluconeogenesis and glycogenolysis. This partially explained the decrease in blood glucose associated with increased glycogen storage in both liver and cardiac tissues.

In this study, no differences were observed in total protein of serum and cardiac tissue. However, total hepatic protein levels were increased, demonstrating that rutin reestablished the liver protein metabolism. Rutin treatment increased serum urea in the G2 group (Table 2) and it might be considered as a consequence for enhanced activities of carbamoyl phosphate synthetase and arginine succinate synthetase[49], since our previous study has revealed that alterations in lipid metabolism are linked to reduced urea levels[30]. Creatinine levels were unchanged after treatment, suggesting that no animal had developed renal insufficiency. Similar to other studies, ethanol consumption did not cause pancreatic injuries[50] even when associated with HD, due to its hyporesponsiveness in altering the homeostasis of serum amylase.

Administration of rutin promoted elevation of uric acid levels in a model of alcoholic and obese rats. Hyperuricemia has been described as a result of increased uric acid production, impaired excretion of uric acid by the kidney, or a combination of these two changes[51]. However, creatinine levels, which can be useful indicators of renal function[52], remained unchanged among the experimental groups. Thus, renal functions were not affected by rutin treatment and the increased levels of uric acid might result from another pathway. On the other hand, flavonoids have been identified as inhibitors of uric acid synthesis by blocking the activity of xanthine oxidase (XO)[53,54], which catalyzes the oxidation of xanthine and hypoxanthine into uric acid (metabolism of purine). Furthermore, our results were supported by those described recently[51], showing the effects of rutin in normal and hyperuricemic mice.

Not surprisingly, either HD or ethanol intake could alter the activities of serum ALP, as well as AST, ALT, LDH, CK in liver and heart tissue[55,56]. In heart tissue (Fig. 2), the low activity of the enzymes was associated with the toxic effects caused by ethanol associated with HD ingestion. One possible explanation is the generation of ROS, which may lead to cell membrane lipid peroxidation, resulting in

increased membrane permeability and consequent release of AST, LDH and CK from tissue into the bloodstream[57]. In liver tissue, AST and ALT showed lower activities, indicating hepatic injuries arising from ethanol consumption and HD (Fig. 3). In both conditions, these findings were corroborated with an elevation of serum ALT, AST and CK activities, good predictors for disturbances in the integrity of cell membranes (Table 2). Conversely, treatment with rutin decreased the activity of serum enzymes, such as ALT, AST, ALP, CK and LDH, and increased the activity of AST, ALT, LDH and CK in the heart, as well as ALT and AST in the liver, showing cardioprotective and hepatoprotective effects. The flavonoid quercetin also exhibits hepatoprotective effect on the serum activities of ALT, AST, ALP, and LDH in animals with hypothalamic obesity[30]. Rutin also effectively acts as a potent antioxidant[58], protecting the integrity of cell membranes and preventing the escape of cytotoxic enzymes from liver and heart tissue.

In conclusion, the present study demonstrated that the flavonoid rutin was able to reduce the risk of cardiovascular and liver diseases by reducing the levels of lipid content, lipoproteins, serum glucose and enzymatic biomarkers for tissue toxicity in animals fed a HD and ethanol.

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