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Article

Tyramine exerts hypolipidemic and anti-obesity effects *in vivo*

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Obesity and dyslipidemia are conditions often associated with cardiovascular risk, inflammation, oxidative stress, and death. Thus, a new approach has been highlighted to promote research and development of pharmacological tools derived from natural sources. Among the most widely studied groups of substances, polyphenols such as tyramine stand out. This study investigated hypolipidemic and anti-obesity properties of tyramine. Oral toxicity evaluation, models of dyslipidemia and obesity were used. To induce dyslipidemia, Poloxamer-407 (P-407) was administered intraperitoneally. In the hypercholesterolemic and obesity model, specific diet and oral tyramine were provided. After 24h of P-407 administration, tyramine 2 mg/kg (T2) decreased triglycerides (TG) (2057.0 ± 158.5 mg/dL vs. 2838 ± 168.3 mg/dL). After 48h, TG were decreased by T2 (453.0 ± 35.47 vs. 760.2 ± 41.86 mg/dL) and 4 mg/kg (T4) (605.8 ± 26.61 760.2 ± 41.86 mg/dL). T2 reduced total cholesterol (TC) after 24h (309.0 ± 11.17 mg/dL vs. 399.7 ± 15.7 mg/dL); After 48h, 1 mg/kg (T1) (220.5 ± 12.78 mg/dL), T2 (205.8 ± 7.1 mg/dL) and T4 (216.8 ± 12.79 mg/dL), compared to P-407 (275.5 ± 12.1 mg/dL). The treatment decreased thiobarbituric acid reactive substances and nitrite in liver, increased superoxide dismutase, reduced the diet-induced dyslipidemia, decreasing TC around 15%. Tyramine reduced body mass, glucose, and TC after hypercaloric feed. Treatment with 5 mg/L (0.46 ± 0.04 ng/dL) and 10 mg/L (0.44 ± 0.02 ng/dL) reduced plasma insulin (1.18 ± 0.23 ng/dL). Tyramine increased adiponectin at 5 mg/L (1.02 ± 0.02 vs. 0.83 ± 0.02 ng/mL) and 10mg/L (0.96 ± 0.04 ng/mL). In conclusion, tyramine has low toxicity in rodents, has antioxidant effect, reduces plasma triglycerides and cholesterol levels. However, further studies should be conducted in rodents and non-rodents to better understand the pharmacodynamic and pharmacokinetic properties of tyramine.

Keywords: Polyphenol. Oral toxicity. Hyperlipidemia. Cholesterol oxidation. Obesity.

INTRODUCTION

Dyslipidemias are a set of disorders interfering with blood lipid metabolism, such as cholesterol, triglycerides, phospholipids and free fatty acids. It has been ranked as one of the highest risk factors contributing to the prevalence and severity of cardiovascular diseases, such as atherosclerosis (Ye, Zhang, 2017). The systematic decrease of plasma lipids accompanied by the decline in cholesterol, emphasizing low density lipoproteins cholesterol (LDL-c), is a valuable approach to reduce the oxidative and inflammatory stress caused by dyslipidemia. (Bohula *et al*., 2018).

The prevalence of dyslipidemia varies demographically and is strongly influenced by cultural

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and behavioral factors, such as diet, physical activity, and alcohol use, in addition to genetics. Abnormalities in the lipid profile, specifically hypertriglyceridemia and low levels of high-density lipoprotein (HDL), have been shown to be a strong predisposing issue to many diseases, including obesity, diabetes, and cardiovascular disorders. The prevalence of hypercholesterolemia, hypertriglyceridemia, high levels of LDL-c and low levels of HDL-c are reported in about 40% of the population. As a result, coronary heart disease is the leading cause of death in developing countries (Hedayatnia *et al.*, 2020; Shabana, Shahid, Sarwar, 2020).

The accumulation of cholesterol and associated lipids within the vascular wall represent the most prominent feature of atherogenesis. LDL particles are particularly atherogenic because of its longer persistence in the bloodstream, preferential penetration through the endothelial barrier, and greater oxidative susceptibility compared to other lipoprotein particles, such HDL and VLDL. Enhanced oxidative stress, which results from either overproduction of reactive oxygen species (ROS) or decreased efficiency of the scavenger antioxidant defense system, plays a significant role in many diseases. Oxidative modification of cholesterol is considered an initial step in the conversion of LDL into more atherogenic particles (Ference *et al.*, 2017; Masuda, Yamashita, 2017).

Obesity and dyslipidemia, as well as metabolic syndrome, are conditions often associated with oxidative stress. The redox imbalance stimulates systemic inflammation, which correlates with tissue dysfunctions. Lipid lowering drugs are commonly used in such conditions, because of their antioxidant potential (Rosenson 2004). HDL is a lipoprotein that helps remove excess cholesterol from the bloodstream through reverse cholesterol transport. It also has antioxidant action due to its constitutive enzymes, such as paraoxonase, which prevent lipid oxidation and, consequently, atherosclerotic damage (de Sousa *et al.*, 2020).

A new approach has been highlighted to promote research and development of pharmacological tools derived from natural sources. Among the most widely studied groups of substances, polyphenols stand out. Polyphenols-rich plants are studied widely, for instance olive (Sánchez Macarro *et al.*, 2020), green tea (Liao *et al.*, 2019) and grape (Bedê *et al.*, 2020).

Tyramine is a monoamine in nature, largely found in vegetables and present in animal metabolism. In humans, it is considered a trace amine due to its low plasma levels (Pessione, Cirrincione, 2016; Gainetdinov, Hoener, Berry, 2018). Tyramine appears to affect lipids and carbohydrates metabolism. In a study performed in experimentally alloxan-induced diabetic rats, tyramine was able to reduce plasma glucose, as well as cholesterol and triglyceride levels in these animals (Lino *et al.*, 2007). In this context, this study aims to investigate the hypolipidemic, antiobesity and antioxidative properties of tyramine in mice.

MATERIAL AND METHODS

Animals

Female Wistar rats (180-200 g) and Male Swiss mice (25-30 g) were obtained from the Bioterium of the Federal University of Ceará, Brazil. Mice were chosen based on previous studies that revealed, through plasma lipid analysis, strong similarities between the fingerprints of lipids in humans and mice compared to other animal species (Kaabia *et al.*, 2018). The experiments were performed in accordance with the ethical guidelines. The animals were maintained under standard conditions, with access to food and water *ad libitum*. Experimental protocols were approved by the Animal Research Ethics Committee (Protocol 24/2010). The female rats were used only in the toxicity protocol. In the dyslipidemia and obesity tests, the male mice were used.

Extraction of tyramine and oral toxicity

Tyramine was extracted, purified and characterized according to Lino *et al.*, (2007). Fresh leaves of *Cissus verticillata* were collected at the Medicinal Plants Garden Prof. Francisco José Abreu Matos from Federal University of Ceará (UFC). The plant *exsiccate* was registered (n° 32240) at Prisco Bezerra Herbarium of UFC. Tyramine was isolated from the methanol soluble fraction using Sephadex LH-20 columns. The extract obtained was analyzed by nuclear magnetic resonance spectroscopy.

The oral toxicity study was performed according to the Organization for Economic Cooperation and Development (OECD) guidelines. The up-and-down procedure (OECD 423 and 425) was used to evaluate acute toxicity through the administration of tyramine at dose of 175, 550 and 2000 mg/kg orally to Swiss albino mice (25-30 g) (Oecd, 2001; Ehile *et al.*, 2018).

OECD 407 guidelines were followed for repeated-dose 28-day oral toxicity study in rodents (Oecd, 2008). Tyramine was administrated to Wistar rats by gavage daily for 28 days. Four groups (n = 10) were evaluated: negative control (NC) group, treated with the vehicle; T10 group, treated with tyramine 10 mg/kg; T20, treated with tyramine 20 mg/kg; and T40, treated with tyramine 40 mg/kg. The doses chosen were 10 times higher than the effective dose used in previous studies evaluating the hypoglycemic activity of tyramine (Amaro *et al.*, 2014). Afterwards, blood samples were collected for determination of red blood count (RBC), hemoglobin (HGB), hematocrit (HT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), glucose (GLU), total cholesterol (TC), triglycerides (TG), aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyl transferase (GGT), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), amylase (AMYL), total protein (TP), albumin (ALB), creatinine (Cr), urea (Ur), uric acid (UA), sodium (Na^+), potassium (K^+) and chloride (Cl^-). Likewise, the animals were euthanized, and the organs were collected and weighted. Liver samples were preserved for further evaluation.

Dyslipidemia induction models

In the present work, two experimental models of dyslipidemia were used. The first was the hyperlipidemic state induced by Poloxamer 407, while the second was induced by the hypercholesterolemic diet. In order to induce hyperlipidemia, a poloxamer 407 (P-407) solution was prepared in saline solution and administered at 400 mg/kg by intraperitoneal (i.p.) injection (Kim *et al.*, 2008). At 2, 24 and 48 hours after the administration of P-407, water (control group), tyramine (1, 2 or 4 mg/Kg) or fenofibrate (200 mg/Kg, reference drug) were administered by oral gavage to Swiss mice. Blood samples were collected 24 and 48 h after the treatment for TC and TG measurement. The liver was removed, washed with sterile saline solution and stored (-80ºC) for analysis of TBARS (Thiobarbituric acid reactive substances), nitrite and SOD (superoxide dismutase).

The hypercholesterolemic diet consisted of a conventional diet added with 10% coconut oil (*Cocus nucifera*), 1% powdered cholesterol and 0.1% powdered cholic acid, as previously standardized (Wilson *et al.*, 2007). Diet's formulation and composition were provided as Supplementary Material. Male Swiss mice (20-25 g) were fed a hypercholesterolemic diet for two weeks, while the negative control group received conventional feed. At the end of this period, the animals (n = 6) received water, tyramine (1, 2 or 4 mg/kg) or simvastatin (20 mg/kg) daily, for 8 weeks, orally. In dyslipidemia models, tyramine doses were based on studies by Morin *et al.*, (2002). Blood samples were collected after 4 and 8 weeks for TC and TG measurement and were analyzed to determine biochemical parameters.

Obesity induction model

Aiming at inducing obesity, a high-caloric diet was produced with conventional feed, peanuts, milk chocolate and sweet biscuits in a 3:2:2:1 ratio, as previously standardized (Estadella *et al.*, 2004). The energy content of the high caloric diet corresponds to 21.40 kJ/g, while the normocaloric diet is 17.03 kJ/g. Diet's formulation and composition were provided as Supplementary Material.

In the obesity induction model, animals were fed with a hypercaloric diet for 15 weeks; the negative control received conventional feed. Simultaneously during inducing obesity, Male Swiss mice also received filtered water or tyramine (5 and 10 mg/L) and sibutramine (50 mg/L) diluted in drinking water. Tyramine dose (0.05 and 0.1%) were established according to the work of Carpéné *et al.*, (2018). Weekly monitoring of body mass gain, as well as water and feed consumption was performed. At the end of this period, the animals were weighed, and their blood samples were collected for biochemical analysis of GLU, TC, TG, insulin, ghrelin and adiponectin. The animals were then euthanized, and abdominal fat was surgically dissected and weighed.

Biochemical and hematological analysis

Blood samples from our experimental groups were collected in heparinized and EDTA-microtubes. Heparinized plasma was obtained by centrifugation (4,000×g, 10 min, 4°C) to determine biochemical parameters using a biochemical analyzer (Roche Diagnostics Limited, Rotkreuz Switzerland). Similarly, hematological parameters were determined using a blood cell counter (Diagnocel, São Paulo, Brazil).

The samples were used to determine plasma concentration of insulin, adiponectin and ghrelin in animals submitted to obesity induced model. The quantification of these parameters was performed using immuno-enzymatic assays - ELISA (Millipore, Massachusetts, USA).

Evaluation of antioxidant potential in liver tissue

Aiming to evaluate the antioxidant potential of tyramine in liver tissue, livers were used to produce a 10 % homogenate in the 1.15% KCl buffer (pH 7.0). After centrifugation (4,000×g, 10 min, 4°C), the supernatant was used for protein content analysis by the Bradford method (Bradford, 1976) and oxidative stress determinations.

Lipid peroxidation was evaluated through the previously described TBARS method (Mihara, Uchiyama, Fukuzawa, 1980). Analyzes were performed by spectrophotometry at 532 nm using a standard malondialdehyde (MDA) curve. The results were expressed as ng/mg of protein. The nitrite concentration was determined using the Griess reagent (0.1% N-(1-naphthyl) ethylenediamine dihydrochloride) and then analyzed at 560 nm. The nitrite concentration was determined from a standard $NaNO_2$ curve. Nitrite concentrations were expressed as µmol/g of protein (Green, Tannenbaum, Goldman, 1981). The superoxide dismutase (SOD) activity was measured according to (Sun, Oberley, Li, 1988) using the wavelength of 560 nm. Results are expressed in "units of enzyme per gram of protein" (U/g).

Statistics analysis

The Shapiro-Wilk normality test was applied to the variables. All results are presented as mean ± S.E.M. The data were evaluated by one-way ANOVA and the differences between means were assessed by Tukey's test using the GraphPad Prism program. Results were considered statistically significant at $p < 0.05$.

RESULTS

Acute oral toxicity study

The initial oral dose of tyramine used was 175 mg/kg (animal 1), followed by 550 mg/kg (animal 2) and 2000 mg/kg (animals 3, 4 and 5); doses were administered progressively according to the acute oral toxicity-up-and-down procedure. No death was recorded. There were no significant change in behavior or any signs of toxicity throughout the experimental period. Mice body weight was not affected by tyramine administration. Therefore, the oral toxicity of this substance was classified as category 5, which contains substances with relatively low toxicity (lethal acute toxicity greater than 2000 mg/kg) according to OECD Globally Harmonized Classification System. Data from toxicological analysis were provided as Supplementary Material.

Repeated-dose toxicity

The consecutive treatment of animals with tyramine at oral doses of 10 mg/kg, 20 mg/kg and 40 mg/kg during 28 days of experiment did not cause any deaths. There were no macroscopic or microscopic changes in brain, heart, lungs, liver, spleen, stomach, intestine or kidneys. Absolute and relative weights were not affected by tyramine treatment. Treatment did not cause changes in pattern of water and food consumption when compared with C- group. Regarding general health issues, the animals treated with tyramine appeared healthy and showed no signs of toxicity such as weight loss, mucous membranes and breathing, tremor, inflammation, seizures, salivation, diarrhea, lethargy or coma. A similar natural body weight gain was observed in all experimental groups.

The hematological analyses showed that tyramine did not promote any significant changes (Table I). However, some biochemical parameters were affected by tyramine treatment. Plasma glucose was increased

in T20 (119.7 ± 6.07 mg/dL) and T40 (122.3 ± 3.11 mg/dL) when compared to NC (98.8 ± 3.34 mg/dL), corresponding to an increase of 21.15% and 23.78%, respectively. GGT levels were also altered, in two highest concentrations: T20 (49.42 ± 3.75 U/L) and T40 (49.84 ± 6.41 U/L) when compared to C- group (31.39 ± 4.40 U/L), corresponding to a percentage increase of 57.44% and 58.77%, respectively. An increased plasma urea was observed only in the T40 group (46.50 ± 1.33 vs. 37.30 ± 1,06 mg/dL), indicating a 24.66% increase.

TABLE I - Initial and final weights of the five mice in which tyramine was administered at concentrations of 175 mg/Kg, 550 mg/Kg, 2000 mg/Kg, 2000 mg/Kg, and 2000 mg/Kg according to OECD 425, as well as observation (30 minutes, 1 hour and 4 hours after administration) as to the appearance of signs of toxicity and mortality; NST = no signs of toxicity

	C-	T10	T20	T40
Glucose (mg/dL)	98.8 ± 3.34	100.8 ± 4.43	**119.7 ± 6.07***	**122.3 ± 3.11***
Triglycerides (mg/dL)	94.60 ± 5.71	129.00 ± 12.50	132.7 ± 9.86	116.6 ± 11.80
Total cholesterol (mg/dL)	77.10 ± 2.30	77.70 ± 2.43	71.20 ± 3.48	72.50 ± 2.31
AST (U/L)	69.30 ± 4.27	62.60 ± 4.01	60.50 ± 2.02	57.60 ± 1.61
ALT (U/L)	34.30 ± 1.44	33.10 ± 0.86	37.10 ± 1.97	36.80 ± 1.26
GGT (U/L)	31.39 ± 4.40	41.74 ± 2.88	**49.42 ± 3.75***	**49.84 ± 6.41***
ALP (U/L)	500.00 ± 21.69	459.4 ± 13.99	442.5 ± 10.97	467.10 ± 17.12
LDH (U/L)	120.40 ± 13.48	116.00 ± 10.96	83.56 ± 5.21	92.25 ± 7.93
Amylase (U/L)	771.30 ± 6.22	775.30 ± 1.24	774.60 ± 2.26	769.00 ± 1.73
Total proteins (g/dL)	5.53 ± 0.09	5.45 ± 0.05	5.39 ± 0.05	5.53 ± 0.04
Albumin (g/dL)	3.75 ± 0.04	3.74 ± 0.04	3.72 ± 0.04	3.80 ± 0.05
Creatinine (mg/dL)	0.59 ± 0.01	0.58 ± 0.01	0.61 ± 0.02	0.58 ± 0.01
Uric acid (mg/dL)	0.51 ± 0.05	0.52 ± 0.04	0.46 ± 0.05	0.38 ± 0.02
Urea (mg/dL)	37.30 ± 1.06	39.80 ± 1.66	35.40 ± 1.37	**46.50 ± 1.33***
Sodium (mEq/L)	141.30 ± 0.80	141.70 ± 0.62	143.50 ± 0.81	141.60 ± 0.38
Potassium (mEq/L)	2.95 ± 0.08	2.98 ± 0.07	2.86 ± 0.05	3.13 ± 0.05
Chloride (mEq/L)	110.70 ± 0.90	110.10 ± 0.50	111.60 ± 0.61	111.50 ± 0.37
Erythrocytes (10^6/µL)	7.12 ± 0.08	7.46 ± 0.10	7.38 ± 0.08	7.36 ± 0.10
Hemoglobin (g/dL)	14.01 ± 0.12	14.38 ± 0.18	14.12 ± 0.11	14.26 ± 0.16
Hematocrit (%)	43.84 ± 0.32	45.44 ± 0.59	45.09 ± 0.39	45.59 ± 0.50
MVC (fL)	61.71 ± 0.33	60.34 ± 0.50	61.15 ± 0.29	61.97 ± 0.30
MVH (pg)	19.71 ± 0.11	19.10 ± 0.30	19.18 ± 0.14	19.37 ± 0.09
MHCM (g/dL)	31.94 ± 0.10	31.66 ± 0.24	31.37 ± 0.12	31.41 ± 0.14

Subtitle: C- – Negative control treated with water; T10 – Animals treated with tyramine 10 mg/Kg; T20 – Tyramine 20 mg/Kg; T40 – Tyramine 40 mg/Kg. ALP – Alkaline phosphatase; ALT – Alanine aminotransferase; AST – Aspartate aminotransferase; CHCM – Mean corpuscular hemoglobin concentration; GGT – γ-glutamyltransferase; HCH – Mean corpuscular hemoglobin; LDH – Lactate dehydrogenase; VCM – Mean corpuscular volume. The results of the experimental groups (n = 10) were expressed as mean ± standard error of the mean (SEM). ANOVA with Tukey post-test; *p <0.05 vs. C-.

Effects of tyramine on P-407-induced hyperlipidemic mice

Our data showed that P-407 efficiently induces increased triglyceridemia in C+ group when compared to C- group after 24h (2838 ± 168.3 vs. 129.5 ± 4.8 mg/dL) and 48h (760.2 ± 41.86 vs. 152.3 ± 8.10 mg/dL), as shown in Figure 1 (A and B). These increases correspond to 2091.50% and 399.15%, respectively. It was observed that after 24 h, only the T2 group was able to decrease TG plasma concentration (2057.0 ± 158.5 mg/dL). However, after 48 hours, there was a reduction in T2 (453.0 ± 35.47 mg/dL) and T4 (605.8 ± 26.61 760.2 ± 41.86 mg/dL), corresponding to a decrease of 40.41% and 20.37%, respectively. It is important to compare this effect with that of fenofibrate, the reference drug, which induced a decrease of 45.32% in triglyceridemia, finding mean values of 416.0 ± 21.75 mg/dL.

Additionally, it was also noted that P-407 was able to cause an increase in plasma TC. The C-group presented mean of 110.0 ± 7.2 mg/dL; in comparison, the induction with P-407 increased these values to 399.7 ± 15.7 mg/dL after 24h and 275.5 ± 12.1 mg/dL after 48h, corresponding to an increase of 263.36% and 153.45%, respectively. The Figure 1 (C and D) shows the effect of tyramine on this parameter. After 24h, only T2 and FENO groups presented significant reduction to 309.0 ± 11.17 mg/dL and 303.8 ± 17.45 mg/dL, respectively. After 48h, tyramine reduced the plasma cholesterol in all studied concentrations, showing the following values: T1 (220.5 ± 12.78 mg/dL), T2 (205.8 ± 7.1 mg/dL) and T4 (216.8 ± 12.79 mg/dL). Fenofibrate also decreased cholesterol, presenting a value of (190.0 ± 9.3 mg/dL). In present study, there were no alterations on blood glucose after P-407 induction. Besides, tyramine was unable to revert changes in transaminases levels after induction with P-407 (Table II).

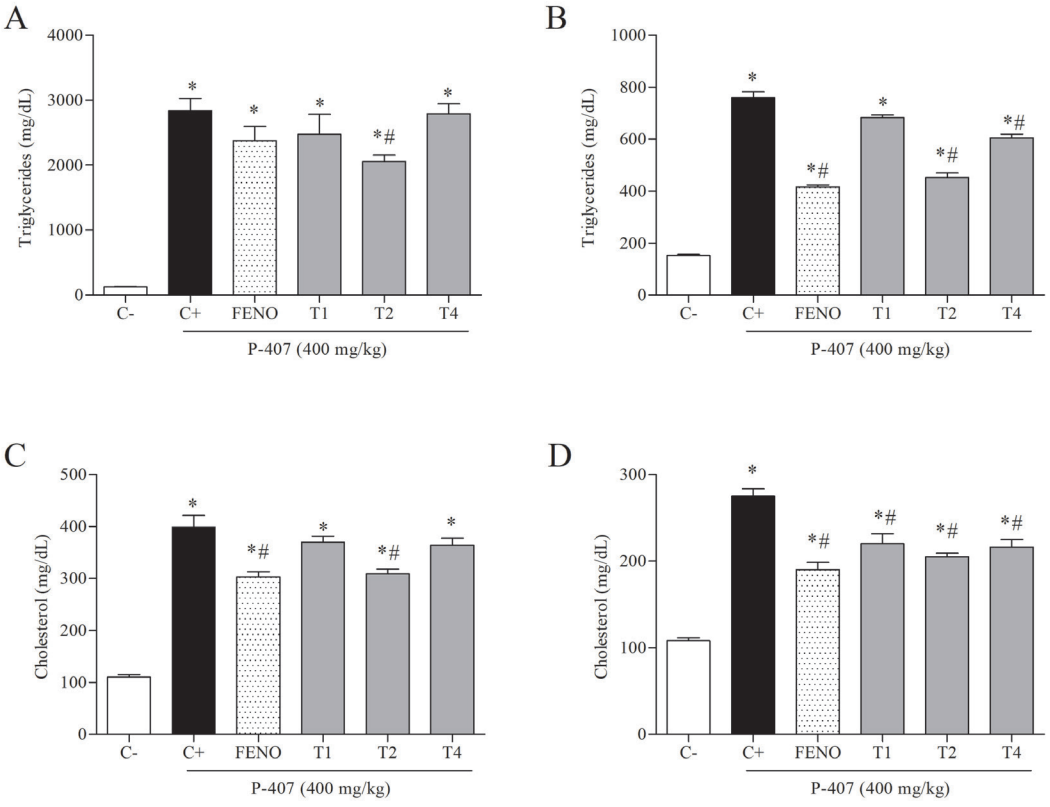

FIGURE 1 - Plasma triglycerides and cholesterol after treatment with tyramine. (A) Plasma triglycerides after 24h of treatment; (B) Plasma triglycerides levels after 48h of treatment; (C) Plasma cholesterol after 24h of treatment; (D) Plasma cholesterol levels after 48h of treatment. C- – Negative control treated with water; C+ – positive control induced with polaxamer-407 (P-407); T1 – Animals treated with tyramine 1 mg/Kg; T2 – tyramine 2 mg/Kg; T4 – tyramine 40 mg/Kg; FENO – Fenofibrate. Results are shown as mean ± S.E.M. *p < 0.05 vs. C-; #p < 0.05 vs. C+.

TABLE II - Biochemical parameters of rats after consecutive daily treatment for 28 days with tyramine (T) or drinking water (CN). The results of the experimental groups (n = 10) were expressed as mean ± standard error of the mean (EPM), where CN = negative control, T10 = tyramine 10mg/Kg, T20 = tyramine 20mg/Kg, T40 = tyramine 40 mg/Kg. "a" represents p <0.05 in comparison with the control (ANOVA with Tukey's post-test)

	C-	C+	FENO	T1	T2	T4
Glucose (mg/dL)	170.8 ± 4.8	184.7 ± 6.5	188.0 ± 7.2	188.2 ± 6.2	187.3 ± 7.5	189.5 ± 11.1
ALT (U/L)	19.5 ± 1.0	**30.5 ± 3.9***	**31.1 ± 2.3***	**32.1 ± 3.5***	**33.0 ± 1.6***	**33.2 ± 1.0***
AST (U/L)	63.2 ± 1.0	**84.7 ± 2.1***	**80.2 ± 4.1***	**89.3 ± 2.6***	**83.8 ± 2.3***	**78.8 ± 3.9***

Subtitle: C- – Negative control treated with water; C+ – Positive control induced with polaxamer-407 (P-407); T1 – Animals treated with tyramine 1 mg/Kg; T2 – Tyramine 2 mg/Kg; T4 – Tyramine 40 mg/Kg; FENO – Fenofibrate. ALT – Alanine aminotransferase; AST – Aspartate aminotransferase. Results are shown as mean ± S.E.M. ANOVA with Tukey post-test; *p <0.05 vs. C-.

Lipid peroxidation in liver tissue of mice with poloxamer-407-induced dyslipidemia was assessed indirectly by analyzing TBARS (Figure 2A). Results showed that P-407 induction was able to increase TBARS levels in C+ group (41.8 ± 2.6 ng/mg) in comparison with the C- group (23.6 ± 3.8 ng/mg). All groups treated with tyramine showed a significant reduction in lipid peroxidation similar to those treated with fenofibrate.

Regarding the determination of nitrite levels in poloxamer-407-induced hyperlipidemic mice livers (Figure 2B), the induction led to a significant increase in hepatic nitrite levels in C+ group (4.02 ± 0.37 µmol/g) when compared to C- (2.61 ± 0.06 µmol/g). In treated animals, a significant reduction was observed only in the T2 group (2.78 ± 0.11 µmol / g).

The measurement of superoxide dismutase (SOD) activity in poloxamer-407-induced hyperlipidemic mice livers is shown in the Figure 2C. The results indicated that induction of dyslipidemia occurred concurrently to a significant decrease in enzyme activity in C+ group (0.1834 ± 0.0099 U/g) compared to C- (0.2417 ± 0.0121 U/g). The treatment with tyramine (2 mg/Kg) was able to completely recover this decrease, with results like C- group (0.2428 ± 0.0038 U /g).

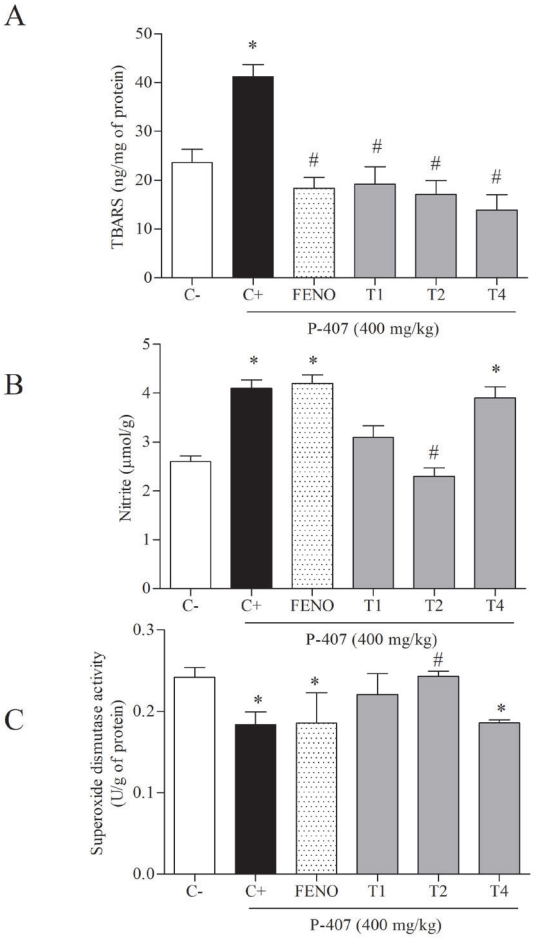

FIGURE 2 - Effects of tyramine on oxidative parameters in mouse livers. (A) Thiobarbituric acid reactive substances (TBARS) levels; (B) Nitrite levels; (C) Superoxide dismutase (SOD) activity. C- – Negative control treated with water; C+ – positive control induced with polaxamer-407 (P-407); T1 – Animals treated with tyramine 1 mg/Kg; T2 – tyramine 2 mg/Kg; T4 – tyramine 40 mg/Kg; FENO – Fenofibrate. Results are shown as mean ± S.E.M. *p < 0.05 vs. C-; #p < 0.05 vs. C+.

Effects of tyramine on diet-induced hyperlipidemic mice

The dyslipidemia induced diet was able to almost double the plasma TC concentration in C+ group (200.3 ± 11.69 mg/dL) after two weeks of induction, in comparison with C- (91.63 ± 4.39 mg/dL), which received conventional feed.

After one month of treatment, tyramine treatment reduced TC concentration compared to C+ (198.0 ± 4.97 mg/dL) on groups T1 (170.4 ± 5.84 mg/dL), T2 (170.0 ± 5.87 mg/dL) and T4 (172.8 ± 7.93 mg/dL). Treatment with reference drug, simvastatin, showed a similar result (168.0 ± 5.35 mg/dL). Mean cholesterol in group C- was 125.8 ± 2.77 mg/dL, as shown in Figure 3A.

After the second month of treatment, there was a reduction in plasma cholesterol in groups T1 (233.3 ± 7.28 mg/dL) and T2 (240.0 ± 11.91 mg/dL) compared to C+ (291.0 ± 1 5.08 mg/dL), simvastatin also showed a similar reduction (239.0 ± 15.43 mg/dL). On the other hand, in the T4 group no effect (262.0 ± 10.5 mg/dL) was observed. Mean cholesterol in group C- was 118.5 ± 5.32 mg/dL as shown in Figure 3B.

TG concentrations were decreased in groups submitted to specific diet: C+ (117.0 ± 11.7 mg/dL), T1 (95, 83 ± 2.86 mg/dL), T2 (86.83 ± 6.50 mg/dL), T4 (109.7 ± 1.95 mg/dL) and simvastatin (SIMV - 91.67 ± 9.72 mg/dL) when compared to C- (159.7 ± 16.19 mg/dL), as shown in Figure 3C. Interestingly, T1, T2 and SIMV showed decreases of 18.1%, 25.8% and 21.6%, respectively compared to C+.

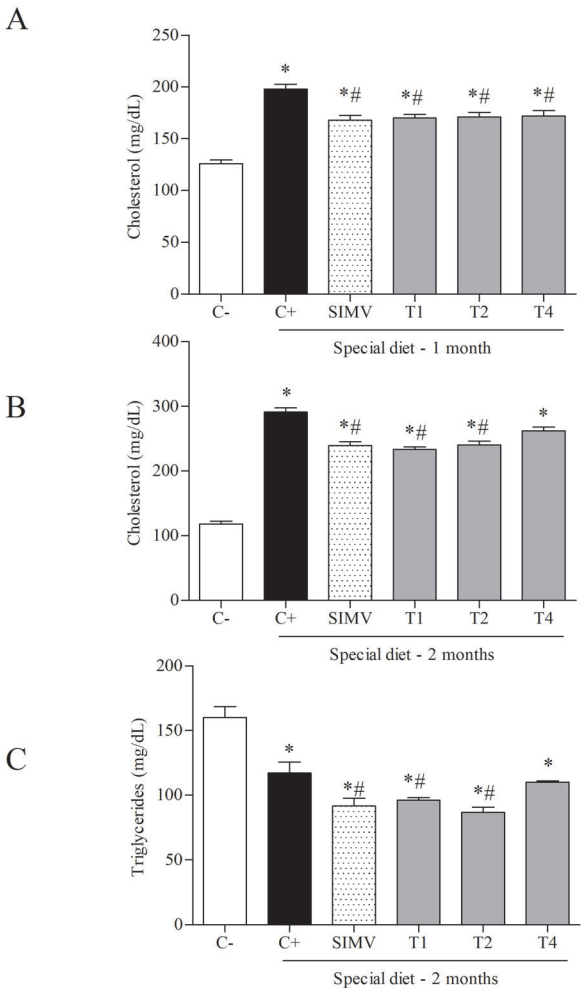

FIGURE 3 - Effects of tyramine after hypercholesterolemic diet. (A) Plasma cholesterol after 1 month of diet; (B) Plasma cholesterol after 2 months of diet; (C) Plasma triglycerides after 2 months of diet. C- – Negative control exposed to normal diet; C+ – positive control exposed to hypercholesterolemic diet; T1 – Animals treated with tyramine 1 mg/Kg; T2 – tyramine 2 mg/Kg; T4 – tyramine 40 mg/Kg; SIMV – Simvastatin. Results are shown as mean ± S.E.M. *p < 0.05 vs. C-; #p < 0.05 vs. C+.

Effects of tyramine on diet-induced obesity

The hypercaloric feed used was able to induce obesity, evidenced by an increase in the body mass of C+ (50.13 ± 1.84 g) after fifteen weeks, in comparison to C- (42.13 ± 0.74), representing an increase of 15.96% (Figure 4A). Tyramine promoted a significant response in reducing body mass of animals treated with tyramine at 5 mg/L (T1) (43.88 ± 0.77 g) and 10 mg/L (T2) (42.13 ± 0.48 g), as well as sibutramine 50 mg/L (SIB) (43.63 ±

1.16 g). This reduction corresponded to 12.46%, 15.96% and 12.97%, respectively.

Concomitant to weight gain, a significant increase in abdominal adipose tissue deposition on C+ (2.70 ± 0.29 g) was observed, in comparison to C- (0.77 ± 0.08 g) (Figure 4B). A reduction was observed in groups treated with T1 (1.38 ± 0.18 g), T2 (1.42 ± 0.17 g) and SIB (1.57 ± 0.23 g), corresponding to a reduction of 48.9%, 47.40% and 41.8%, respectively.

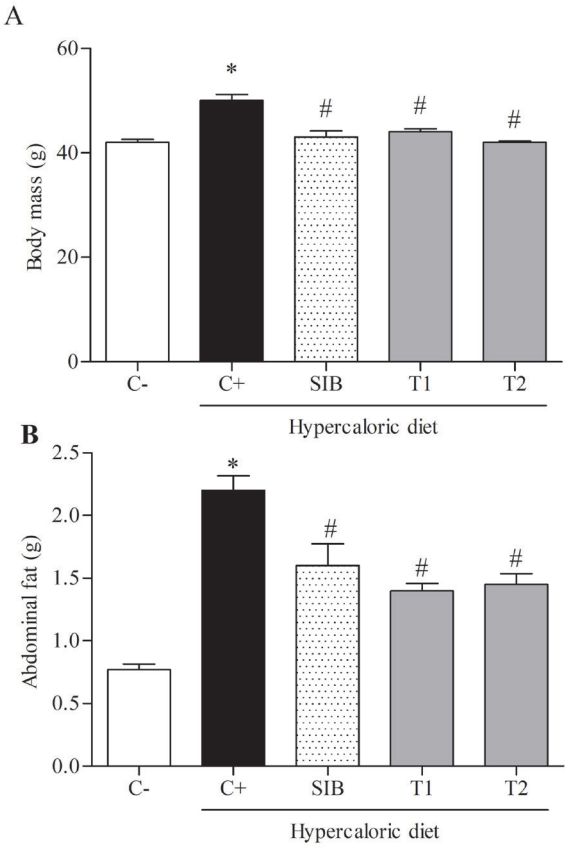

FIGURE 4 - Effects of tyramine on body weight after 15 weeks of a high caloric diet. (A) Body mass; (B) Abdominal fat. C- – Negative control exposed to normal diet; C+ – positive control exposed to hypercaloric diet; T1 – Animals with tyramine 5 mL/L in drinking fountain; T2 – tyramine 10 mL/L; SIB – Sibutramine. Results are shown as mean ± S.E.M. *p < 0.05 vs. C-; #p < 0.05 vs. C+.

The consumption of high caloric feed caused a significant increase of 84.70% in plasma glucose concentration (218.1 ± 18.17 mg/dL) when compared to C- (118.0 ± 7, 12 mg/dL). T1 (164.80 ± 11.13 mg/dL) and T2 (161.40 ± 10.62 mg/dL) significantly reduced GLU concentration by 24.44% and 26.00%, respectively. Sibutramine (206.60 ± 10.86 mg/dL), as expected, had no effect on this parameter (Figure 5A).

Regarding plasma cholesterol levels, animals in the C+ group had TC concentrations significantly higher than C- (180.8 ± 3.43 mg/dL vs. 113.1 ± 3.36 mg/dL), representing an increase of 59.86%. T1 (157.4 ± 4.47 mg/dL), T2 (159.9 ± 2.12 mg/dL) and SIB (160.0 ± 3.87 mg/dL) significantly reduced this parameter (Figure 5B). The consumption of hypercaloric feed was not able to induce changes on plasma TG: C- (86.88 ± 4.04 mg/dL), C+ (83.25 ± 6.78 mg/dL), showing similar values compared to treated groups: SIB (84.38 ± 5.04 mg/dL), T1 (81.13 ± 6.25 mg/dL) and T2 (80.00 ± 6.44 mg/dL) (Supplementary Material).

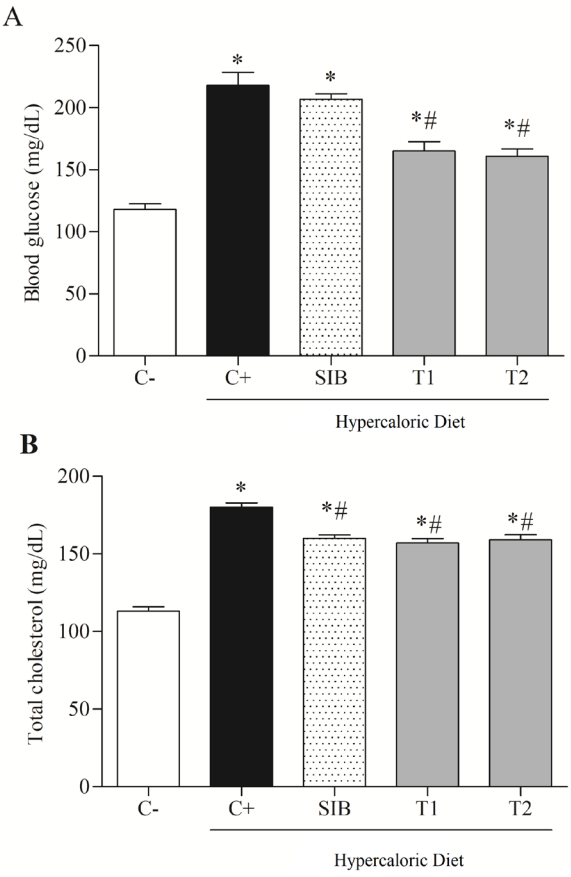

FIGURE 5 - Effects of tyramine on biochemical parameters after 15 weeks of a high caloric diet. (A) Blood glucose; (B) Plasma cholesterol. C- – Negative control exposed to normal diet; C+ – positive control exposed to hypercaloric diet; T1 – Animals with tyramine 5 mL/L in drinking fountain; T2 – tyramine 10 mL/L; SIB – Sibutramine. Results are shown as mean ± S.E.M. *p < 0.05 vs. C-; #p < 0.05 vs. C+.

Determination of hormones in animals submitted to high caloric diet

The consumption of the hypercaloric diet induced an insulin increase in C+ group (1.18 ± 0.23 ng/dL) when compared to C- (0.34 ± 0.01 ng/dL). Tyramine treatment at concentrations of 5 mg/L (0.46 ± 0.04 ng/dL) and 10 mg/L (0.44 ± 0.02 ng/dL) were able to significantly reduce insulin concentration when compared to C+. SIB (0.80 ± 0.21 ng/dL) showed no significant reduction (Figure 6A).

Plasma concentrations of adiponectin were reduced by consumption of high caloric diet, with concentrations in C+ (0.83 ± 0.02 ng/mL) lower than those in C- (1.00 ± 0.02 ng/mL) (Figure 6B). Tyramine-treated groups at concentrations of 5 mg/L (1.02 ± 0.02 ng/mL) and 10 mg/L (0.96 ± 0.04 ng/mL) were able to increase the concentrations of this hormone, similar to sibutramine (0.99 ± 0.03 ng/mL). Ghrelin determination showed no difference between the groups (Supplementary Material).

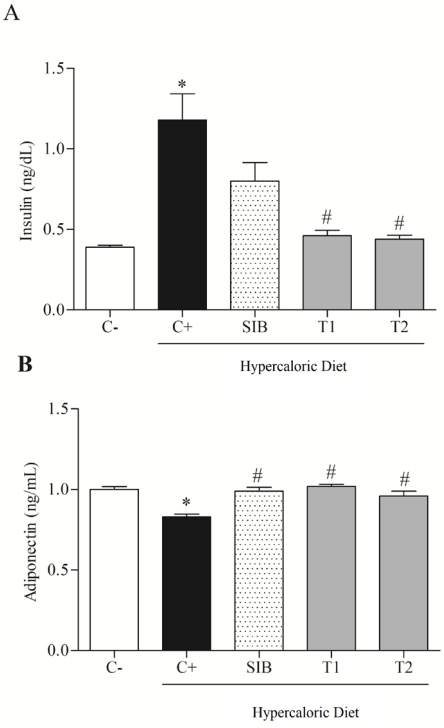

FIGURE 6 - Effects of tyramine in hormones after 15 weeks of a high caloric diet. (A) Insulin; (B) Adiponectin. C- – Negative control exposed to normal diet; C+ – positive control exposed to hypercaloric diet; T1 – Animals with tyramine 5 mL/L in drinking fountain; T2 – tyramine 10 mL/L; SIB – Sibutramine. Results are shown as mean ± S.E.M. *p < 0.05 vs. C-; #p < 0.05 vs. C+.

DISCUSSION

This study demonstrates that tyramine has relatively low acute toxicity based on OECD protocols. When analyzing repeated-dose toxicity, the treatment did not cause any death, and the animals treated with tyramine appeared healthy and showed no signs of toxicity, despite some slight biochemical alterations such as increase in plasma glucose, GGT and urea. It was observed that tyramine improved the biochemical parameters in hyperlipidemic mice, such as triglycerides and cholesterol, with results comparable to fenofibrate. These effects were concomitant to its activity on oxidative parameters in mice livers, resulting in reducing lipid peroxidation, nitrite accumulation and increasing superoxide dismutase activity. Additionally, after hypercholesterolemic diet, tyramine reduced plasma cholesterol concentration, similarly to simvastatin. Tyramine also promoted a significant response in reducing body mass after hypercaloric diet-induced obesity like sibutramine, reducing the abdominal adipose tissue deposition, blood glucose, plasma cholesterol and resistance to insulin in addition to increasing plasma concentration of adiponectin.

Several groups have continuously invested substantial resources in research and development for new treatments of dyslipidemia (Ahn, Choi, 2015), mainly because hypolipidemic drugs have been associated with several side effects. Currently available drugs commonly cause disorders such as hyperuricemia, diarrhea, nausea, myositis, gastric irritation, flushing, dry skin and abnormal liver function (Tarantino *et al.*, 2017), highlighting the importance of bioprospecting substances from biodiversity with low toxicity and significant hypolipidemic effects.

In toxicity study, tyramine modified GGT activity, an enzyme present in the epithelial cells that revert to bile ducts and is also useful in the evaluation of hepatobiliary function (Visentin *et al.*, 2018). However, this change was considered discrete once other liver parameters were not changed. It is also known that GGT induction may occur in absence of liver damage, as reported with some therapeutic drugs, such as phenobarbital and phenytoin (Lippi *et al.*, 2008).

Additionally, tyramine probably did not cause any kidney damage, except an increase in plasma urea. Tyramine can act as a neurotransmitter, promoting release of norepinephrine (Meck *et al.*, 2003; Iepsen *et al.*, 2018). Norepinephrine is an important neurotransmitter responsible for activating sympathetic nervous system, which promotes increased reabsorption of salt and water in addition to urea, creatinine, calcium, uric acid and bicarbonate by proximal tubule (Jackson, Zhang, Cheng, 2017; Matthews *et al.*, 2017). Previous work also found no evidence of tyramine in renal toxicity studies (Til *et al.*, 1997). Morin *et al.*, (2002) uses doses of tyramine similar to those used in the present study with a different model, related to insulin sensitivity. The present study showed the effect of tyramine on the reduction of obesity serum lipids, in addition to the toxicity study, justifying the use of these concentrations.

The present work used a hypertriglyceridemia model using P-407. The injection results primarily in the inhibition of TG degradation due to inhibition of lipoprotein lipase (LPL), the major enzyme responsible for hydrolysis of plasma lipoproteins containing TG. In addition, a compensatory activation of 3-hydroxy,3-methylglutaryl (HMG)-CoA reductase is partially responsible for the elevation of total cholesterol in P-407-treated animals (Johnston, 2004; Subramaniam *et al.*, 2011). It is important to note that tyramine decreased triglyceride levels in animals induced with P-407 and hypercholesterolemic diet.

All these effects are associated with cholesterol metabolism, corroborating with our data. According to a study performed by Cho *et al.*, (2011), tyramine derivatives feruloyltyramine and coumaroyltyramine isolated from the root bark of *Lycium chenese* demonstrated hypochlolesterolemic and antioxidant effects in obese mice. These effects were produced by inhibiting liver microsomal HMG-CoA reductase and acyl-CoA:cholesterol acyltransferase (ACAT) activity. These findings were associated with significant delay in LDL oxidation accompanied by lower levels of TBARS (Cho *et al.*, 2011).

In this context, many studies described that tyramine and related molecules can reduce serum lipids. Chang *et al.*, (2017) demonstrated the inhibitory effect of *Solanum nigrum* tyramine-rich polyphenol extracts on the expression of the HMG-CoA reductase enzyme *in vitro* and inhibition of lipogenesis in obese mice. Zhang *et al.*, (2016) and Hafez *et al.*, (2017) showed the same effect of *Forsythia suspense* (Thunb.) and Spinach roots and flowers, respectively, causing reduction of serum glucose in diabetic mice. In our study, when animals were submitted to hypercholesterolemic diet, tyramine treatment had, quantitatively, a similar effect like that of simvastatin, a statin that inhibits enzyme HMG-CoA reductase, which decreases cholesterol synthesis and causes a positive feedback on the expression of LDL receptors in peripheral tissues, in addition to decreasing atherosclerotic risk (Valentovic, 2007).

Moreover, the present work showed that tyramine presented compatible effects with fenofibrate, a reference drug that regulates blood lipid levels through its agonistic effect on peroxisome proliferator receptor alpha (PPARα) signaling (Razavian *et al.*, 2014). This data is complementary to another study that affirms that tyramine mimics insulin action in the adipocytes differentiation (Subra *et al.*, 2003). It is described that enzymes such as monoamine oxidase (MAO) oxidizes biogenic amines like tyramine and generates hydrogen peroxide (H_2O_2). This ROS stimulates glucose transport in adipocytes as a result of PI3-kinase activation and GLUT4 translocation (Subra *et al.*, 2003).

Still in this context, in present study, the hypercaloric diet-induced obesity model increased plasma glucose, which was reduced after treatment with tyramine. In addition, tyramine reduced plasma concentration of insulin, indicating a decrease in insulin resistance in these animals. It is noted that current clinical antidiabetic drugs, such PPARγ agonists, have some serious side effects, making it necessary to find alternative agents. It was demonstrated that tyramine-derivatives compounds from *Lycium barbarum* and *L. chinense*, phenylethylamine-based phytochemicals, present a good docking scores and binding on *in silico* models and PPARγ gene induction with cell-based assay (Yalamanchili *et al.*, 2020).

Obesity is the excess of body fat in an amount that determines damage to health. A person is considered obese when his Body Mass Index (BMI) is greater than or equal to 30 kg / m^2 and the normal weight range varies

between 18.5 and 24.9 kg / m^2. Individuals who have a BMI between 25 and 29.9 kg / m^2 are diagnosed with overweight and may already have some losses with excess fat (Apovian, 2016). Therefore, although obesity is not characterized only by isolated weight gain, with important comorbidities, the hypercaloric diet model used in this study seeks to mimic a typical condition of obesity.

Several studies have sought to clarify the relationship between metabolic disorders and redox imbalance. A study demonstrated that N-acetylcysteine, an anti-inflammatory antioxidant, attenuates programmed susceptibility to obesity and insulin resistance in a high fat diet. The treatment normalized weight gain and decreased white adipose tissue plus oxidative stress, improved lipidome and prevented leptin resistance. Furthermore, the treatment improved glucose and insulin tolerance, reduced leptin plus insulin and increased adiponectin (Charron *et al*., 2020). In this context, another study demonstrated that a polysaccharides-rich *Lycium barbarum* extract, source of tyramine derivatives, was able to restored blood lipid and upregulate levels of adiponectin., This phenomenon occurred through anti-inflammatory mechanisms by reducing the expression of interleukin-6 (IL- 6), tumor necrosis factor-alpha (TNF-α) and AMP-activated protein kinase (AMPK) (Liao *et al*., 2019).

Indeed, the redox balance is closely related to lipids metabolism, interfering with function of organs like the liver. An *in vitro* study showed that N-trans-feruloyltyramine from garlic presented prominent antioxidant activity, providing protective effects against H_2O_2-induced oxidative damages in hepatocytes and maintaining the integrity of mitochondria (Gao *et al*., 2019). However, in the present study, tyramine was unable to revert changes in transaminase levels after induction with P-407. The enzymes ALT and AST are considered good indicators of liver function and can be applied as predictive biomarkers for possible toxicity. Generally, damage to the hepatic parenchyma causes an elevation of both enzymes in blood (Siemionow *et al*., 2016).

It is described that the P-407-induced atherosclerosis model leads to LDL oxidation as result of a severe hypercholesterolemic state (Johnston, Korolenko, Sahebkar, 2017). Abnormally high lipid peroxidation and simultaneous decline of antioxidant defense mechanisms can cause damage to cellular organelles, which leads to oxidative stress (Ince *et al*., 2017). These events can be assessed by TBARS analysis. In the present study, a decrease in lipid peroxidation was observed after treatment with tyramine, indicating that tyramine causes a blockage of oxidative stress.

TBARS reaction is an indirect method to evaluate lipid peroxidation, which occurs, partially, due to the production of peroxynitrite ($ONOO^-$). In the present study, tyramine prevented the increase of nitrite levels in the liver, which is an indication of antioxidant activity. Nitric oxide (NO), an important physiological regulator of vascular homeostasis, is implicated in pathophysiology of atherosclerosis. NO produced by endothelial nitric oxide synthase (e-NOS) induces expression of SOD in muscle layer of vessel and extracellularly, reducing levels of available O_2 and, consequently, production of $ONOO^-$ (Malekmohammad, Sewell, Rafieian-Kopaei, 2019). Moreover, in the presence of atherosclerotic plaque, activated macrophages produce O_2, leading to elevated expression of inducible isoenzyme (i-NOS) and production of high concentrations of NO. The upregulation of these parameters contribute to tissue damage through chronic inflammation, resulting in an increase of the severity of atherosclerosis (Ponnuswamy *et al*., 2012; Gimbrone, García-Cardeña, 2016).

All these observations corroborate to findings of our work related to oxidative stress analysis. The induction of dyslipidemia with P-407 led to hypertriglyceridemia and consequently hypercholesterolemia, which favored the oxidation of cholesterol represented by lipid peroxidation. The peroxidation was caused, in part, due to the $ONOO^-$ formation, related to the nitrite accumulation. The process of inducing dyslipidemia decreases SOD activity, which increases oxygen concentration, resulting in creation of an oxidative environment and a cascade of events. Tyramine, due to its antioxidant activity, seems to impair the redox unbalance, revealing to be a great candidate for preventing cardiovascular risk associated with dyslipidemia.

It is important to highlight that overall effects observed in T2 group were better than those observed in T4 group that consists of animals treated with higher concentration. Tyramine probably exerts an effect known as hormesis, which is a dose-response phenomenon, characterized by

low-dose stimulation and high-dose inhibition. Numerous investigations have revealed that there is no single hormetic mechanism (Calabrese, Baldwin 2003; Franco, Navarro, Martínez-Pinilla, 2019). Further studies should be performed to evaluate the pharmacokinetics of this process.

Altogether, data presented in this study consolidates the relationship between antioxidant activity and effects of tyramine against dyslipidemias. With data presented in this work, further studies are encouraged to understand the aspects related to pharmacokinetic, pharmacodynamics, toxicological and clinical outcomes of tyramine use. However, it is worth mentioning that this article is a preliminary study, emphasizing the need for more in-vitro and in-vivo tests.

CONCLUSION

The results of this study suggest that tyramine has a low toxicity profile in rodents. It can reduce plasma triglycerides and cholesterol levels. Tyramine showed this phenomenon due to its antioxidant effect as evidenced by reduction in lipid peroxidation, nitrite accumulation and increase in superoxide dismutase activity. Tyramine probably exerts an effect known as hormesis, which is a dose-response phenomenon characterized by low-dose stimulation and high-dose inhibition. Such data may be used to develop new antilipidemic drugs that can be helpful in the protection against cardiovascular diseases. However, further studies should be conducted in rodents and non-rodents to better understand the pharmacodynamic and pharmacokinetic properties of tyramine.

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DECLARATION OF INTEREST STATEMENT

The authors declared that they do not have anything to disclose regarding conflict of interest with respect to this manuscript.

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ETHICAL APPROVAL

This study was approved by Animal Research Ethics Committee of Federal University of Ceará (N° Protocol 24/2010)

CONTRIBUTORSHIP

TMFM, RRPPBM and TLS: wrote the manuscript; TMFM, TLS, JMF and DFS: planned the project; TMFM, MBD, TSM and MGRQ: collected data; TMFM, MBD, TSM, EPM and MLF: performed the experiments; TLS, RRPPBM, ODLP, FCF, TLS and MGRQ: analyzed, interpreted data and critically reviewed the manuscript. All authors reviewed and edited the manuscript and approved the final version of the manuscript.

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SUPPLEMENTARY MATERIAL

DIETS FORMULATION AND COMPOSITION
Rat, mouse, and hamster feed - NUVILAB CR-1 AUTOCLAVABLE BALANCED FEED COMPOSED OF: Ground whole corn, wheat bran, soybean meal, calcium carbonate, dicalcium phosphate, sodium chloride, amino acid, vitamin mineral premix.

WARRANTY LEVELS PER KILOGRAM OF PRODUCT:

Humidity (max.)	120 g/Kg
Crude Protein (min)	220 g/Kg
Etheric Extract (min.)	40 g/Kg
Mineral Material (max.)	90 g/Kg
Fibrous Matter (max.)	70 g/Kg
Calcium (min.- max.)	(10 - 14 g/Kg)
Phosphorus (min.)	8,000 mg/Kg

SUPPLEMENTATION PER Kilo, NOT LESS THAT: VITAMINS: Vitamin A 25,500 IU; vitamin D3 2,100 IU; vitamin E 60.00 IU; vitamin K3 12.50 mg; vitamin B1 14.40 mg; vitamin B2 11.00 mg; vitamin B6 12.00 mg; vitamin B12 60.00 mcg; niacin 60.00 mg; pantothenic acid 112.00 mg; folic acid 6.00 mg; biotin 0.26 mg; choline 2,400 mg. MINERALS: Sodium: 2,700 mg, Iron 50,00 mg; zinc 60.00 mg; copper 10.00 mg; iodine 2.00 mg; manganese 60.00 mg; selenium 0.05 mg; cobalt 1.50 mg, fluorine (max) 80 mg/kg. AMINO ACIDS: methionine 5,000 mg; Lysine 14 g. ADDITIVES: BHT 100.00 mg. ENERGY CONTENT: 17.03 kJ/g.
Source: Manufacturer information.

HYPERCHOLESTEROLEMIC DIET: Conventional diet added with 10% coconut oil (*Cocus nucifera*), 1% powdered cholesterol and 0.1% powdered cholic acid, as previously standardized (Wilson *et al*., 2007). To the conventional food previously crushed was added the oil, in which the cholic acid and cholesterol (previously dissolved in alcohol 97 °) had been dissolved initially under agitation and temperature of 80 ° C. These components were mixed in an industrial mixer until completely homogenized. Then, 2% carmellose was added in sufficient quantity to promote agglutination of the feed.

HYPERCALORIC DIET: The hypercaloric diet was produced with conventional feed, peanuts, milk chocolate and sweet biscuits in a 3: 2: 2: 1 ratio, previously standardized by Estadella *et al*., (2004). All components were crushed and mixed in an industrial mixer until completely homogenized. Then, the mass obtained for making the pellets was extruded in order to leave it in the shape of the conventional ratio. Energy content: 21.40 kJ/g (Melo *et al*., 2009).

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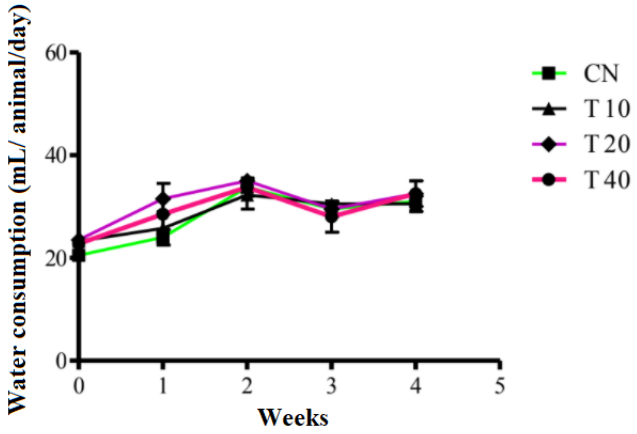
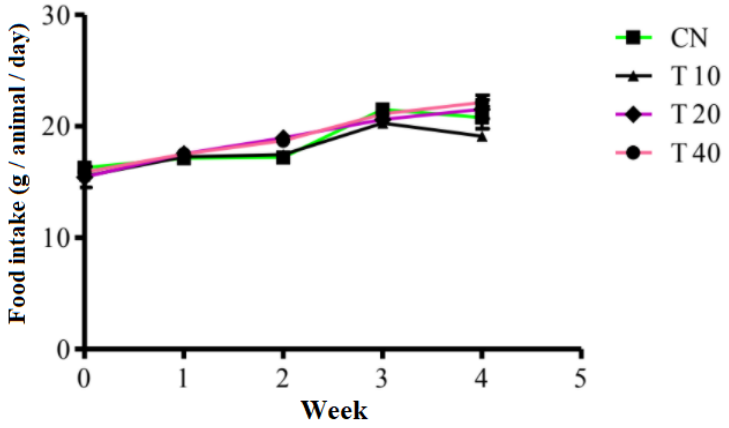
TOXICOLOGICAL ANALYSIS

TABLE I - Initial and final weights of the five mice in which tyramine was administered at concentrations of 175 mg/Kg, 550 mg/Kg, 2000 mg/Kg, 2000 mg/Kg, and 2000 mg/Kg according to OECD 425, as well as observation (30 minutes, 1 hour and 4 hours after administration) as to the appearance of signs of toxicity and mortality; NST = no signs of toxicity

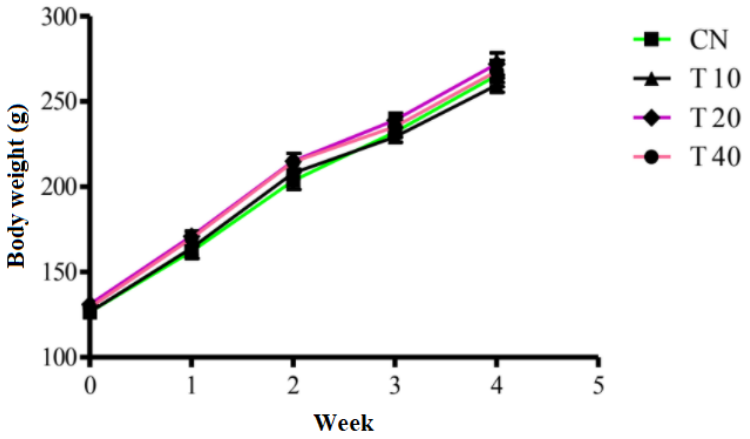
Animal	**1**	**2**	**3**	**4**	**5**
Dose (mg/Kg)	175	550	2000	2000	2000
Initial weight (g)	31	31	27	29	32
Final weight (g)	34	33	29	32	33
Observations 30 min	NST	NST	Piloerection	Piloerection	Piloerection
Observations 1 h	NST	NST	NST	NST	NST
Observations 4 h	NST	NST	NST	NST	NST
Mortality	No	No	No	No	No



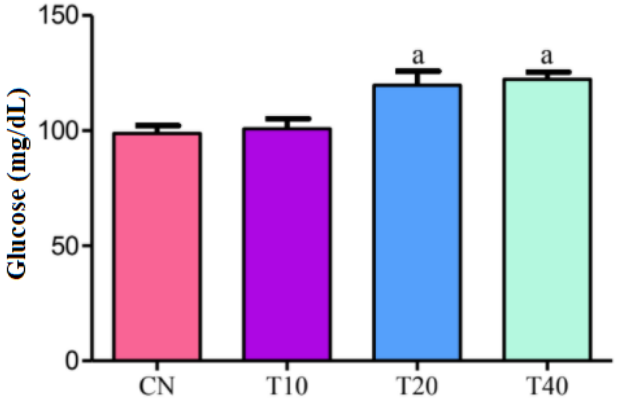
GRAPH 01 - Weekly water consumption (mL/ animal/day) per animal in the experimental groups treated for 28 days with water (CN), tyramine 10mg/Kg (T10), tyramine 20mg/Kg (T20), ortyramine 40mg/Kg (T40). The results of the experimental groups (n = 10) were expressed as mean ± standard error of the mean (SEM).



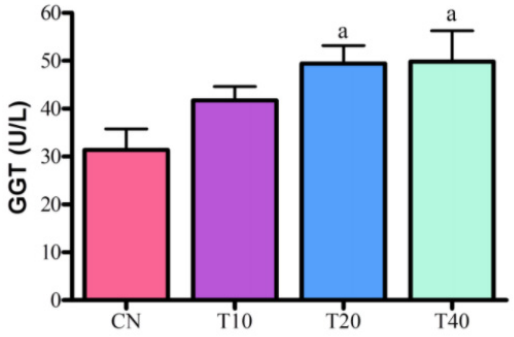
GRAPH 02 - Weekly feed intake (g/animal/day) of the experimental groups treated for 28 days with water (CN), tyramine 10mg/Kg (T10), tyramine 20mg/Kg (T20), ortyramine 40mg/Kg (T40). The results of the experimental groups (n = 10) were expressed as mean ± standard error of the mean (SEM).



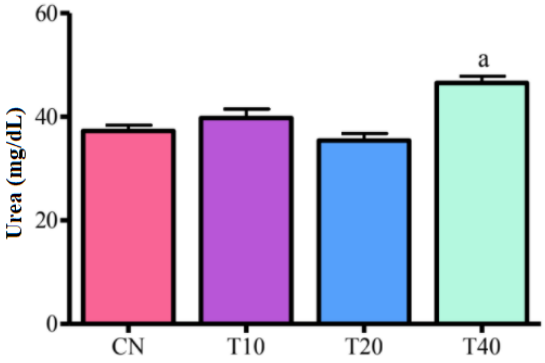
GRAPH 03 - Average body mass gain of experimental groups treated for 28 days with water (CN), tyramine 10mg/kg (T10), tyramine 20mg/kg (T20), or tyramine 40mg/kg (T40). The results of the experimental groups (n = 10) were expressed as mean ± standard error of the mean (SEM).



GRAPH 4 - Plasma glucose concentration (mg/dL) of animals treated for 28 days with water (CN), tyramine 10 mg/kg (T10), tyramine 20 mg/kg (T20), or tyramine 40 mg/kg (T40). The results of the experimental groups (n = 10) were expressed as mean + standard error of the mean (SEM). "a" represents $p < 0.05$ in comparison with the control (ANOVA, Tukey).



GRAPH 5 - Plasma activity of gamma-glutamyl transferase (GGT - U/L) of animals treated for 28 days with water (CN), tyramine 10 mg/Kg (T10), tyramine 20 mg/Kg (T20) or tyramine 40 mg/Kg (T40). The results of the experimental groups (n = 10) were expressed as mean + standard error of the mean (SEM). "a" represents $p < 0.05$ in comparison with the control (ANOVA, Tukey).



GRAPH 6 - Plasma concentrations of urea (mg/dL) of animals treated for 28 days with water (CN), tyramine 10 mg/kg (T10), tyramine 20 mg/kg (T20) or tyramine 40 mg/kg (T40). The results of the experimental groups (n = 10) were expressed as mean + standard error of the mean (SEM). "a" represents p <0.05 in comparison with the control (ANOVA, Tukey).

TABLE II - Biochemical parameters of rats after consecutive daily treatment for 28 days with tyramine (T) or drinking water (CN). The results of the experimental groups (n = 10) were expressed as mean ± standard error of the mean (EPM), where CN = negative control, T10 = tyramine 10mg/Kg, T20 = tyramine 20mg/Kg, T40 = tyramine 40 mg/Kg. "a" represents p <0.05 in comparison with the control (ANOVA with Tukey's post-test)

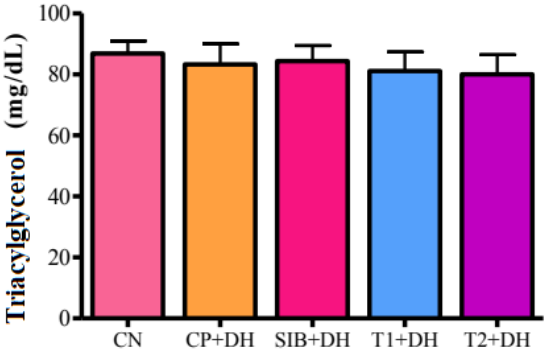
Biochemical parameters	Experimental Groups			
	CN	T10	T20	T40
Glucose (mg/dL)	98.8 ± 3.34	100.8 ± 4.43	**119.7 ± 6.1**[a]	**122.3 ± 3.1**[a]
Triglycerides (mg/dL)	94.60 ± 5.71	129.0 ± 12.5	132.7 ± 9.9	116.6 ± 11.8
Total cholesterol (mg/dL)	77.10 ± 2.30	77.70 ± 2.43	71.20 ± 3.5	72.50 ± 2.31
AST (U/L)	69.30 ± 4.27	62.60 ± 4.01	60.50 ± 2.02	57.60 ± 1.61
ALT (U/L)	34.30 ± 1.44	33.10 ± 0.86	37.10 ± 1.97	36.80 ± 1.26
GGT (U/L)	31.39 ± 4.40	41.74 ± 2.88	**49.42 ± 3.7**[a]	**49.84 ± 6.4**[a]
Alkaline phosphatase (U/L)	500.0 ± 21.7	459.4 ± 13.99	442.5 ± 10.97	467.10 ± 17.12
LDH (U/L)	120.40 ± 13.4	116.0 ± 10.9	83.56 ± 5.21	92.25 ± 7.93
Amylase (U/L)	771.30 ± 6.2	775.30 ± 1.2	774.60 ± 2.3	769.00 ± 1.7
Total Proteins (g/dL)	5.53 ± 0.09	5.45 ± 0.05	5.39 ± 0.05	5.53 ± 0.04
Albumin (g/dL)	3.75 ± 0.04	3.74 ± 0.04	3.72 ± 0.04	3.80 ± 0.05
Creatinine (mg/dL)	0.59 ± 0.01	0.58 ± 0.01	0.61 ± 0.02	0.58 ± 0.01
Uric acid (mg/dL)	0.51 ± 0.05	0.52 ± 0.04	0.46 ± 0.05	0.38 ± 0.02
Urea (mg/dL)	37.30 ± 1.06	39.80 ± 1.66	35.40 ± 1.37	**46.50 ± 1.33**[a]

TABLE III - Blood count of rats after consecutive treatment for 28 days with tyramine (T) or drinking water (CN). The results of the experimental groups (n = 10) were expressed as mean ± standard error of the mean (EPM), where CN = negative control, T10 = tyramine 10mg / Kg, T20 = tyramine 20mg / Kg, T40 = tyramine 40 mg / Kg. "a" represents p <0.05 in comparison with the control (ANOVA with Tukey's post-test)

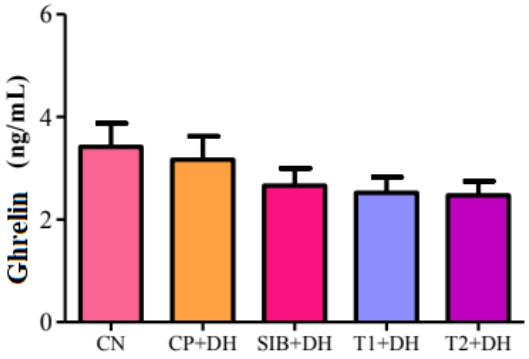
Biochemical parameters	Experimental Groups			
	CN	T10	T20	T40
Red blood cells (10^6/µL)	7.12 ± 0.08	7.46 ± 0.10	7.38 ± 0.08	7.36 ± 0.10
Hemoglobin (g/dL)	14.01 ± 0.12	14.38 ± 0.18	14.12 ± 0.11	14.26 ± 0.16
Hematocrit (%)	43.84 ± 0.32	45.44 ± 0.59	45.09 ± 0.39	45.59 ± 0.50
MCV (fL)	61.71 ± 0.33	60.34 ± 0.50	61.15 ± 0.29	61.97 ± 0.30
HCM (pg)	19.71 ± 0.11	19.10 ± 0.30	19.18 ± 0.14	19.37 ± 0.09

TABLE III - Blood count of rats after consecutive treatment for 28 days with tyramine (T) or drinking water (CN). The results of the experimental groups (n = 10) were expressed as mean ± standard error of the mean (EPM), where CN = negative control, T10 = tyramine 10mg / Kg, T20 = tyramine 20mg / Kg, T40 = tyramine 40 mg / Kg. "a" represents p <0.05 in comparison with the control (ANOVA with Tukey's post-test)

Biochemical parameters	Experimental Groups			
	CN	T10	T20	T40
CHCM (g / dL)	31.94 ± 0.10	31.66 ± 0.24	31.37 ± 0.12	31.41 ± 0.14
Leukocytes (10^3/µL)	8.95 ± 0.32	8.49 ± 0.45	8.96 ± 0.27	8.87 ± 0.40
Platelets (10^3/µL)	1068.0 ± 30.1	931.7 ± 37.5	937.9 ± 48.3	992.80 ± 39.6



GRAPH 7 - Triacylglycerol plasma concentration (mg / dL) of the negative control (CN), positive control (CP), sibutramine (SIB), tyramine 1 (T1) and tyramine 2 (T) groups at the end of the 15th week of consumption of the hypercaloric diet (DH) by groups CP, SIB, T1 and T2 and treatment with water, sibutramine 50 mg / L, 5 mg / L and 10 mg / L, respectively. The results of the experimental groups (n = 8) were expressed as the mean ± standard error of the mean (EPM) (ANOVA - Tukey's posttest).



GRAPH 8 - Ghrelin plasma concentration (ng / mL) of the negative control (CN), positive control (CP), sibutramine (SIB), tyramine 1 (T1) and tyramine 2 (T) groups at the end of the 15th week of consumption of the hypercaloric diet (DH) by groups CP, SIB, T1 and T2 and treatment with water, sibutramine 50 mg / L, 5 mg / L, and10 mg / L, respectively. The results of the experimental groups (n = 8) were expressed as mean ± standard error of the mean (EPM) (ANOVA - Tukey posttest).

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