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High fat diet and high polyphenols beverages effects in enzymatic and non-enzymatic antioxidant activity

Efectos de dieta con alto contenido de grasa y bebidas ricas en polifenoles en la actividad antioxidante enzimática y no enzimática

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ABSTRACT

Background: High fat diets have been implicated in the generation of reactive oxygen species (ROS). Polyphenols from grapes may reduce ROS and restore oxidative balance. The aim of this study is to investigate the antioxidant properties of high polyphenols beverages associated with a high fat diet in enzymatic and non-enzymatic antioxidant activity.

Material and methods: Fifty female rats were divided into five groups: a) control group (CG) - control diet (4% fat); b) high fat diet group (HFD) - high fat diet (20% fat); c) grape juice group (GJ) - grape juice (15 ml/day) + high fat diet; d) red wine group (RW) - red wine (10 ml/day) + high fat diet; and e) resveratrol solution group (RS) –

resveratrol solution (15 ml/day) + high fat diet. Eight weeks later, the superoxide dismutase, catalase and glutathione peroxidase activities were measured. Superoxide dismutase activity was assayed by measuring the inhibition of adrenaline auto-oxidation, catalase by the decrease rate in hydrogen peroxide and glutathione peroxidase by monitoring the oxidation of nicotinamide adenine dinucleotide phosphate. Non-enzymatic antioxidant activity was assessed by oxygen radical absorbance capacity and DDPH method in the animal's plasma.

Results: GC and GJ presented the lowest glutathione peroxidase activity, pointing to a possible protective effect of grape juice against high levels of ROS ($p < 0.05$). RW increased catalase activity when compared to the RS ($p < 0.05$). Superoxide dismutase activity and non-enzymatic antioxidant plasma activity were similar in all groups.

Conclusion: Grape juice showed to be the most effective in minimizing the deleterious effects of a high fat diet. Resveratrol did not present any benefit and red wine possibly shows a harmful effect due to ethanol content.

Key words: Grape polyphenols. Redox homeostasis. Superoxide dismutase. Catalase. glutathione peroxidase.

RESUMEN

Introducción: las dietas ricas en grasas se han implicado en la generación de especies reactivas del oxígeno (ROS). Los polifenoles de las uvas pueden reducir el ROS y restaurar el equilibrio oxidativo. El objetivo de este estudio es investigar las propiedades antioxidantes de las bebidas ricas en polifenoles asociadas con una dieta rica en grasa en la actividad antioxidante enzimática y no enzimática.

Material y métodos: cincuenta ratas fueron divididas en cinco grupos: a) grupo control (CG) - dieta de control (4% de grasa); b) grupo rica en grasa (HFD) - dieta con 20% de grasa; c) jugo de uva (GJ) - jugo (15 ml/día) + dieta rica en grasas; d) vino tinto (RW) - vino tinto (10 ml/día) + dieta rica en grasas; y e) grupo solución de resveratrol (RS) - solución de resveratrol (15 ml/día) + dieta rica en grasas. Se midieron superóxido dismutasa, catalasa y glutatión peroxidasa. La actividad de superóxido dismutasa para la inhibición de la auto-oxidación de adrenalina, la catalasa por la tasa de disminución

de peróxido de hidrógeno y glutatión peroxidasa monitorizando la oxidación de nicotinamida adenina dinucleótido fosfato. La actividad antioxidante no enzimática se midió por el método de capacidad de absorción de radicales de oxígeno y DDPH.

Resultados: GC y GJ presentaron la menor actividad de glutatión peroxidasa, señalando un posible efecto protector del jugo de uva frente a altos niveles de ROS ($p < 0,05$). RW aumentó la actividad de catalasa en comparación con RS ($p < 0,05$). Superóxido dismutasa y la actividad antioxidante no enzimática fueron similares.

Conclusiones: el jugo demostró ser el más eficaz para minimizar los efectos deletéreos de una dieta rica en grasas. Resveratrol no presentó ningún beneficio y el vino tinto posiblemente muestra un efecto perjudicial debido al contenido de etanol.

Palabras clave: Polifenoles de las uvas. Homeostasis Redox. Superóxido dismutasa. Catalasa. Glutatión peroxidasa.

INTRODUCTION

Diet plays an important role in maintaining human health and the progression of disease states (1,2). A permanent exposure to high saturated fat diets leads to metabolic abnormalities such as obesity development and progression, type II diabetes, cardiovascular diseases and some forms of cancer. It is proposed that the link between a high fat diet and the development of diseases is provided by oxidative imbalance, which is mediated by an increase in reactive oxygen species (ROS) production such as hydrogen peroxide and hydroxyl radical (2).

The generation of free radicals is a continuous and physiological process, playing important biological functions such as cell signaling and defense against micro-organisms. However, the imbalance between the prooxidant and antioxidant systems, with predominance of oxidative reaction, results in oxidative stress (3,4). To limit the intracellular levels of ROS and control the damage occurrence, cells have an important enzymatic defense system composed by endogenous antioxidant enzymes such as glutathione peroxidase (GPx), superoxide dismutase (SOD) and catalase (CAT) (5,6).

CAT catalyzes the conversion of hydrogen peroxide to water, whereas SOD catalyzes the dismutation of superoxide to hydrogen peroxide. GPx reduces lipid hydroperoxides to their corresponding alcohols and free hydrogen peroxide to water (7).

Unbalanced diets can affect cellular oxidative responses. During a high saturated and/or transaturated fat diet, the production of free radicals is increased and, due to a compensatory response to maintain the redox homeostasis, an antioxidant enzyme also increases (8,9).

Epidemiological and prospective studies have demonstrated the role of poliphenolic rich foods such as fruits, nuts and vegetables as non-enzymatic antioxidants. These components, obtained through diet and/or supplementation, can act in synergy with enzymatic antioxidants, controlling oxidative stress (3,10-12).

Red grape derivatives, due to their high pholyfenols levels, present a major role in scavenging free radicals. It has been reported that regular intake of red grape juice and its derivatives, such as wine, are associated to anti-inflammatory activity, lower expression and activity of antioxidants enzymes as CAT and GPx and reduced lipid peroxidation, oxidative damage and apoptotic cell death (3,10-12). Therefore, this study was undertaken to determine the effects of a daily intake of phenolic-rich beverages obtained from red grape on antioxidants enzymes concentrations of rats fed a high-fat diet (HFD).

MATERIALS AND METHODS

Animals

The study was conducted in the Experimental Nutrition Laboratory of the Department of Nutrition and Dietetics, School of Nutrition Emilia Jesus Ferreiro at the Federal Fluminense University (LabNE-UFF). It was approved by the Brazilian Society of Science in Laboratory Animals (SBCAL) of the Federal Fluminense University, according to the guidelines of the Brazilian College on Animal Experimentation (protocol n° 473).

Fifty female *Rattus novergicus Wistar albino*, all adults (90 days), weighing 200 ± 20 g obtained at the LabNE-UFF were housed in plastic cages in a controlled environment (24 °C ± 2 °C, with a 12 h daylight cycle), with free access to food and water. The experiment lasted for 8 weeks.

The animals were randomly divided into five groups (n = 10/group):

1. Control group (CG): fed a control diet (4% of total calorie intake from fat) based on the American Institute of Nutrition Recommendations for adult-rodents (AIN 93M) (13).

2. High fat diet group (HFD): fed high fat diet (20% of total calorie intake from fat).

3. Grape Juice group (GJ): fed a high fat diet (20% of total calorie intake from fat) and received red grape juice (15 mL/day).

4. Red wine group (RW): fed a high fat diet (20% of total calorie intake from fat) and received red wine (10 mL/day).

5. Resveratrol solution group (RS): fed a high fat diet (20% of total calorie intake from fat) and resveratrol solution (15 mL/day).

Table I show the formulation of ingredients for the chow and the chemical composition of the high fat and control diet.

Samples collection and preparation

At the end of the experiment, all animals were subjected to vaginal smear procedure to identify the stage of the estrous cycle.

The rats were fasted for 6h prior to sacrifice and anesthetized with ketamine chloride (90 mg/kg) and xylazine hydrochloride (10 mg/kg). Their blood was collected by cardiac puncture into tubes with EDTA. The plasma was separated by centrifugation at 3000 rpm (20 minutes; 4 °C) and its samples were stored at - 70 °C for biochemical analysis.

Antioxidants enzymatic activity

Glutathione peroxidase (GPx), superoxide dismutase (SOD) and catalase (CAT) activities

To determine the SOD, CAT and GPx activities, plasma was used. The SOD activity was assayed by measuring the inhibition of adrenaline auto-oxidation as absorbance at 480 nm (Beckman Spectrophotometer mod DU 640; Fullerton, CA, USA) (14). The CAT activity was measured by the rate of decrease in hydrogen peroxide absorbance at 240 nm (15). The GPx activity was measured by monitoring the oxidation of nicotinamide adenine dinucleotide phosphate (NADPH) at 340 nm in the presence of H_2O_2 (16). The total protein content in the plasma was determined by the Bradford method.

The specific activity of SOD and CAT are expressed as U/mg protein. GPx is expressed as K/gHb/s (17).

Plasma ORAC method

The oxygen radical absorbance capacity (ORAC) assay was conducted to measure the peroxyl radical-scavenging activity in plasma using a method previously reported by Prior et al. (18).

Briefly, 100 µL plasma were mixed with 200 µL ethanol and 100 µLMilli-Q water. The organic phase containing the lipophilic antioxidants was extracted twice with hexane, followed by evaporation with nitrogen. The residue was diluted with 0.7% bcyclodextrin and acetone. The hydrophilic residue was mixed with 400 lL 0.5 M perchloride acid and centrifuged at 2500 g for 5 min at 20 °C. The upper phase was diluted in 75 mM phosphate buffer (pH 7.4). The Trolox standard solutions were prepared at concentrations ranging from 6.25 to 100 lM. The Multi-Detection microplate reader (Synergy HT, Bio-Tek Instruments Inc., Winooski, VT) was programmed to record the fluorescence of the diluted samples (25 lL) every minute after the incubation of the samples with 150 lL 40 mM fluorescein in 75 mM phosphate buffer, pH 7.4, and addition of 25 lL AAPH (153 mM in 75 mM phosphate buffer, pH 7.4) for 60 min. The area under the curve of the fluorescence decay was calculated using Gen5 software. The antioxidant activity was measured four times for each plasma sample, and results are expressed as mmolTrolox equivalents/g.

Plasma DPPH method

The percentage of antioxidant activity (aa%) of each substance was assessed by DPPH free radical assay. The measurement of the DPPH radical scavenging activity was performed according to the methodology described (19). The plasma was reacted with the stable DPPH radical in an ethanol solution. The reaction mixture consisted on adding 0.5 mL of plasma, 3 mL of absolute ethanol and 0.3 mL of DPPH radical solution 0.5 mM in ethanol. The changes in color were read [absorbance (abs)] at 517 nm after 100 min of reaction using a uV-ViS spectrophotometer (du 800; Beckman coulter, Fullerton, CA, USA). The mixture of ethanol (3.3 mL) and plasma (0.5 mL) served as blank. The control solution was prepared by mixing ethanol (3.5 mL) and dPPH radical solution (0.3 mL). The scavenging activity percentage (aa%) was determined according to Mensor et al. (20).

Statistical analysis

The data were expressed as mean ± standard deviation. The student´s *t* test was applied to establish differences within the group (before *versus* after).

For means of comparison among the groups, analysis of variance (ANOVA one-way) and Duncan post-test was used. For data correlation, Pearson's correlation was used. The assumption of normality (Gaussian distribution) was verified by Kolmogorov-Smirnov tests to support the use of the statistical methods described above. The analyses were performed using Graphpad Prism for Windows.

RESULTS

Three specific enzymes involved in the antioxidant endogenous defense mechanisms have been analyzed. Additionally, the antioxidant activity in beverages and plasma were also evaluated.

The SOD activity is shown in figure 1. All groups showed similar SOD activity (CG 9.01 ± 2.96; HFD 11.00 ± 2.59; GJ 10.23 ± 1.63; VT 10.34 ± 2.89; SR 9.19 ± 1.07 U/mg protein).

The CAT activity in HFD (5.40 ± 1.85 U/mg protein), GJ (4.11 ± 1.39 U/mg protein), RW (6.82 ± 1.5 U/mg protein), RS (2.86 ± 1.49 U/mg protein) were similar to CG (2.98 ± 1.28 U/mg protein), as can be observed in figure 2.

Although there was no significant difference in the CAT activity between animals that received a high fat or a control diet, it is evident that this diet, associated with high polyphenol beverages, increased the enzimatic activity in 125% in GJ, 82% in RS, 196% in HFD and 273% in RW when compared to CG.

Additionally, the CAT activity increased 138% after exposure to red wine when compared to the resveratrol solution ($p < 0.05$).

The glutathione peroxidase is shown in figure 3. The GJ presents (63.20 ± 12.8 K/gHb/s) similar enzymatic activity to CG (14.6 ± 8.21 K/gHb/s), showing that grape juice was effective in controlling oxidative stress mediated by high fat diet. Compared to GJ, enzymatic activities were 85% higher in HFD (117.34 ± 15.21 K/gHb/s), 257% in SR (225.57 ± 24.42 K/gHb/s) and 162% in RW (165.00 ± 20.04 K/gHb/s).

In the other groups, there was an increase in the GPx activity, possibly demonstrating a greater need of GPx in the neutralization of EROS from the high fat diet (117.34 ± 15.21 K/gHb/s), red wine (165.00 ± 20.04 K/gHb/s) and/or resveratrol solution (225.57 ± 24.42 K/gHb/s) ($p < 0.0001$). Comparing all groups to CG, an enzimatic activity

increase of 431% in GJ, 803% in HFD, 1.160% in RW and 1.573% in RS can be observed. Using DPPH (CG 59.20 ± 4.47; HFD 60.43 ± 4.84; GJ 52.78 ± 11.71; RW 59.33 ± 13.06; RS 67.19 ± 2.77 K/gHb/s) or ORAC method (GC 45.70 X 10^6 ± 4.56 X 10^6; HFD 44.43 X 10^6 ± 2.03 X 10^6; GJ 46.89 X 10^6 ± 4.90 X 10^6 RW 55.52 X 10^6 ± 1.09 X 10^6 RS 55.28 X 10^6 ± 1.22 X 10^6 µmolET/g), there was no statistical difference in plasma antioxidant activity of animals treated with high fat diet or high fat diet associated with polyphenol-rich beverages when compared to control group, as seen in figures 4 and 5. Correlations were observed between activities of CAT and GPx enzymes ($r = 0.47$, $p = 0.029$) and ORAC/GPx in HFD group ($r = 0.9823$, $p = 0.0177$). No other associations were observed.

DISCUSSION

High fat diets are associated with increased oxidative stress, lipid peroxidation and inflammation (2,12). To counterbalance the production of EROS (superoxide anion and hydroxyl radical), SOD, CAT and GPx are produced naturally and daily, conferring protection against damage to macromolecules such as DNA, lipids and proteins (21). Non-enzymatic antioxidants act synergistically to those enzymes. High levels of oxidative stress are directly related to several pathologies and metabolic disorders, reducing the life expectancy of individuals (2,22).

According to AIN-93M (13) recommendations, the daily consumption of lipid for adult rats should be 4%. The dietary model adopted in this study was a high fat diet containing 20% saturated fat, being this concentration five times higher than the recommended.

Some reports in literature have shown an over activity of antioxidant enzymes when there is an increase in saturated fats from diet, maintaining cellular steady-state (2,4). High fat diets increase fatty acid oxidation for energy production. Additionally, the mitochondria produces more H_2O_2 when oxidizing fatty acids than pyruvate derived from glycolysis (4).

However, in this study, the SOD and CAT activities had similar results when comparing control and treated groups. Only GPx activity was affected by high lipid intake, presenting greater activity in groups that received high fat diet. It must be noted that, in this study, 20% of saturated fat in the diet were used, but others studies found in

the literature have used higher amounts of saturated fats (40-70%). Possibly, such dietary models provide a more prooxidative and EROS-rich environment, justifying an expressive increase in the SOD and CAT activity (23,24).

Although there was no statistical difference between treated and control groups in relation to the CAT activity, an important percentage difference was noted. The RW group presented three times more CAT activity than the CG, followed by the HFD, RS and GJ group.

Corroborating with other studies assessing high fat diets and GPx (25,26), groups that received a high fat diet presented a higher GPx enzymatic activity than CG. As previous studies show, high fat diet upregulates genes related to glutathione metabolism and the largest increase in GPx activity makes the cell more adapted to tolerates high concentrations of O_2 - and H_2O_2 (25).

Observing the correlation between ORAC/GPx in HFD groups, possibly the enzymatic and non-enzymatic systems are recruited to neutralize and/or minimize the damages mediated by the high fat diet, since GPx is strongly associated with lipid peroxidation as previous reported (4,26,27).

Evaluating the CAT and GPx activities, a correlation between these enzymes was expected to be found, as demonstrated in the literature. The coordination of these enzymes is crucial for the correct redox balance in cellular environment. Probably, both enzymes act in synergy, presenting the same function: convert H_2O_2 to H_2O and O_2. However, GPx is the one with the higher specificity when it comes to lipid peroxidation as demonstrated by Rindler et al. (4).

Dietetic polyphenols derived from grapes, such as resveratrol, catechin and epicatechin, are known by their antioxidant and scavenging properties against a wide range of free radicals (7,28-30). Some experimental studies suggest that treatment with resveratrol or polyphenol-rich foods/beverages, as grape juice and red wine, maintain the properly enzymatic activity of SOD, CAT and GPx (25,31).

Nevertheless, in the present study, no changes were observed in enzymatic activity of SOD and CAT in groups that received polyphenol-rich beverages.

In GPx activity, GJ presented enzymatic activity equal to CG. Similar to other studies that reported a better control of oxidative stress after grape juice ingestion (32) GC and GJ groups presented the lowest GPx activity, pointing to a possible protective

effect of grape juice against high levels production of EROS induced by a high fat diet consumption. The positive result may be explained by a complex matrix of polyphenols found in grape juice, which include proanthocyanidins, ellagic acid, kampferol, myricetin, quercetin, malvidin, peonidine, cyanidin and catechin and resveratrol which act in synergy, amplifying the response in the maintenance of redox homeostasis (33).

On the other hand, red wine, despite being a source of polyphenols, including resveratrol, presents ethanol. Due to its deleterious prooxidative effect, ethanol and its main metabolite, acetaldehyde, can counteract or nullify the protective effects of polyphenols (5). Experimental studies using alcoholic beverages (wines, spirit or gin) with high fat diets show a significant increase in the production of mitochondrial H_2O_2 and free radical reactions, producing alkoxyl and hydroxyl free radicals, being positively associated with increased lipid peroxidation, with consequent alteration of the enzymatic activity (23,34,35).

No differences in the SOD activity was noticed in RW group when compared to all groups. A study in humans using high fat diet associated with wine consumption did not find any variation in the SOD activity (36). Another study with 80 animals submitted to treatment with ethanol, water, wine and non-alcoholic wine also did not show alterations under this enzyme (37).

Comparing the difference between CAT in RW and RS group, wine consumption increased the enzymatic activity. The presence of ethanol in RW provides a more oxidative environment, leading to a higher activity when compared to the group that received resveratrol supplementation. Additionally, the consumption of ethanol led to an important percentage increase of the CAT activity (273%) when compared to all groups. Similar to our findings, the above mentioned experimental study using 80 animals also found an increase in CAT activity in groups that had chronic alcohol consumption (37).

Corroborating with the above mentioned results, GPx activity in RW group was higher than in CG, GJ and HFD groups. As was previously mentioned, ethanol and high saturated fat from diet act as prooxidants, possibly leading to a high hepatic production of EROS. Previous studies mentioned that cytochrome P450, NADPH reductase and NADPH oxidase are involved in this process, due to the inducibility of these enzymes after chronic consumption of alcoholic beverages, altering the redox

status and recruiting larger amounts of GPx to maintain the oxidative balance (31,34,35).

Resveratrol supplementation, although appearing to be a promise in controlling oxidative stress, in this experimental model does not bring any benefit.

Observing the CAT and SOD activities, there were no differences between CG and RS groups. However, in the GPx activity, RS showed the highest activity when compared to the remaining groups. Animals that received higher doses of resveratrol solution and/or supplementation are prone to form higher concentrations of EROS through two distinct routes: higher expression of genes related to glutathione metabolism in response to an increased lipid peroxidation, and prooxidant activity of resveratrol when used alone at very high doses, as demonstrated in previous studies (38,39).

It is worth to observe that different sources of nutrients, as the natural antioxidants present in high polyphenols beverages used in this study, should not necessarily affect all enzymatic activicty from redox system. Antioxidant enzymes respond independently to different radicals and it can not be expected that these enzymes respond similarly (40).

In short, analyzing the antioxidant activity related to the enzymatic system, in the experimental model adopted by this study, resveratrol did not demonstrate any effect or benefit, wine may act as a prooxidant due to the ethanol content and grape juice seems to minimize the effects of a high fat diet.

Non-enzymatic system is constituted by a great variety of antioxidant substances, which can have endogenous or dietetic origin. From food sources, those that stand out most are vitamins, minerals and phenolic compounds such as vitamin A and C, selenium and zinc, lutein, resveratrol, catechins, among others (5).

The use of polyphenol-rich beverages and the mega dose of resveratrol do not seem to influence non-enzymatic antioxidant activity measured by DPPH and ORAC method, although they present an influence on CAT and GPx activity. It must be noted thatpolyphenols, in this experimental model, acted in enzymatic system but without changes in the non-enzymatic system.

CONCLUSION

In this experimental model, grape juice showed to be the most effective in minimizing the deleterious effects of a high fat diet, possibly due to a set of bioactive compounds that acted synergistically. Resveratrol did not presented any benefits and red wine demonstrates a possible harmful effect due to ethanol presence.

Thus, the use of natural matrices or food products, instead of wine and supplements, may represent a more reliable and effective alternative in nutritional and therapeutic approaches.

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Table I. Ingredients used for formulation of control and high fat diets (g/100 g chow)

Ingredients	*Control*	*High fat*
Casein*	14.0	14.0
Starch	62	46.07
Soybeanoil	4.0	-
Lard	-	20
Celulose	5.0	5.0
Vitaminmix[1]	1.0	1.0
Mineralsmix[2]	3.5	3.5
B-colin	0.25	0.25
L-cystine	0.18	0.18
Sugar	10.0	10.0
Total	100	100

*% protein in casein = 92.5% protein/100 g casein; [1]Vitaminmix (mg/kg dieta): retynilpalmitate 2.4, cholecalciferol 0.025, benadionasodiumbisulfite 0.8, biotin 0.22, cyanocobalamin 0.01, riboflavin 6.6, thiaminehydrochloride 6.6 and tocopherolacetate 100; [2]Mineralsmix (g/kg dieta): coppersulphsate 0.1, ammoniummolybdate 0.026, sodiumiodate 0.0003, potassiumchromate 0.028, zinco sulfate 0.091, calciumhydrogenphosphate 0.145, iron sulfate 2.338, magesium sulfate 3.37, manganese sulfate 1.125, sodiumchloride 4, calciumcarbonate 9.89 and potassiumdiidrogenophosphate 14.75.

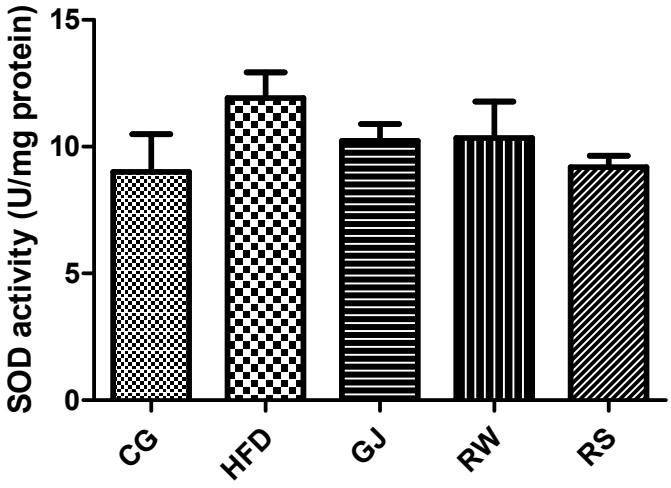

Fig. 1. Superoxide dismutase activity (measurements of plasma superoxide dismutase activity from all experimental groups: a) control group (CG) - control diet (4% fat); b) high fat diet group (HFD) - high fat diet (20% fat); c) grape juice group (GJ) - received 15 ml/day grape juice + high fat diet; d) red wine group (RW) - received 10 ml/day red wine + high fat diet; and e) resveratrol solution group (RS) - received 15 ml/day resveratrol solution + high fat diet.

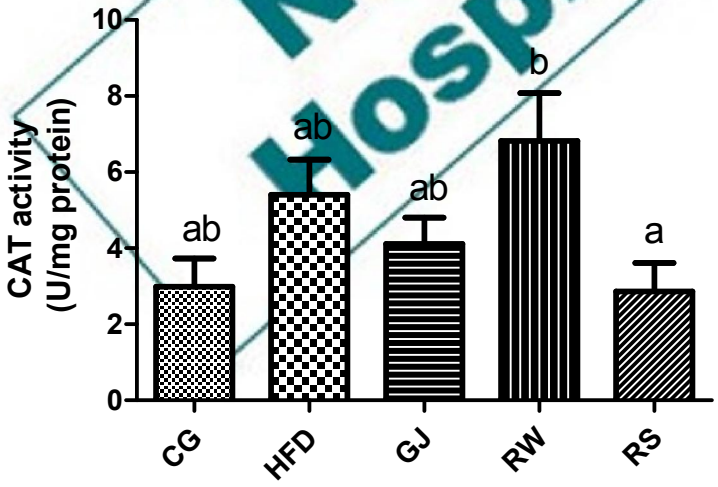

Fig. 2. Catalase activity (measurements of catalase (CAT) activity from all experimental groups. Different letters represent statistically differences between groups ($p < 0.020$); a) control group (CG) - control diet (4% fat); b) high fat diet group (HFD) - high fat diet (20% fat); c) grape juice group (GJ) - received 15 ml/day grape juice + high fat diet; d)

red wine group (RW) - received 10 ml/day red wine + high fat diet; and e) resveratrol solution group (RS) - received 15 ml/day resveratrol solution + high fat diet).

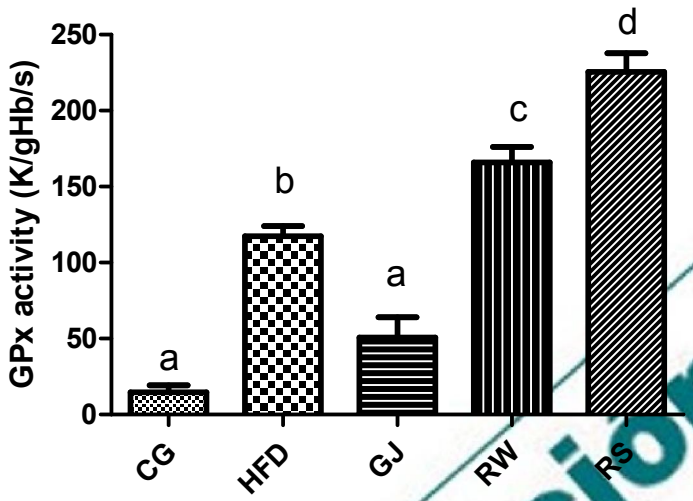

Fig. 3. Gluthatione peroxidase activity (measurements of gluthatione peroxidase (GPx) activity from all experimental groups. Different letters represent statistically differences between groups ($p < 0.0001$); a) control group (CG) - control diet (4% fat); b) high fat diet group (HFD) - high fat diet (20% fat); c) grape juice group (GJ) - received 15 ml/day grape juice + high fat diet; d) red wine group (RW) - received 10 ml/day red wine + high fat diet; and e) resveratrol solution group (RS) - received 15 ml/day resveratrol solution + high fat diet).

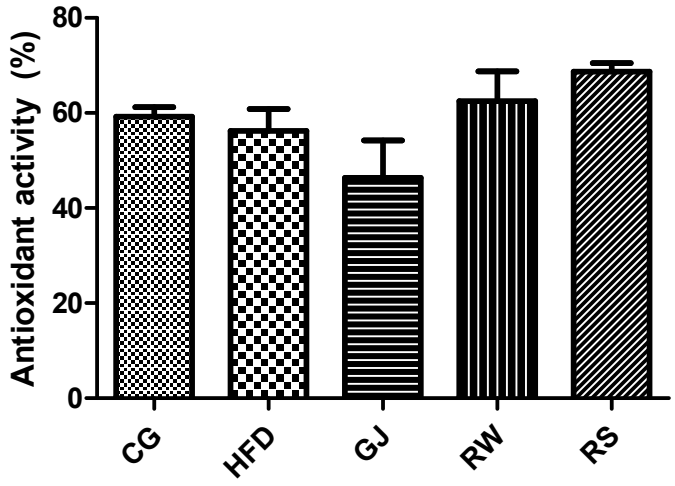

Fig. 4. DPPH Plasma antioxidant activity (plasma antioxidant activity from all experimental groups using DPPH. Control group (CG) - control diet (4% fat); high fat diet group (HFD) - high fat diet (20% fat); grape juice group (GJ) - received 15 ml/day grape juice + high fat diet; red wine group (RW) - received 10 mL/day red wine + high fat diet; resveratrol solution group (RS) - received 15 ml/day resveratrol solution + high fat diet).

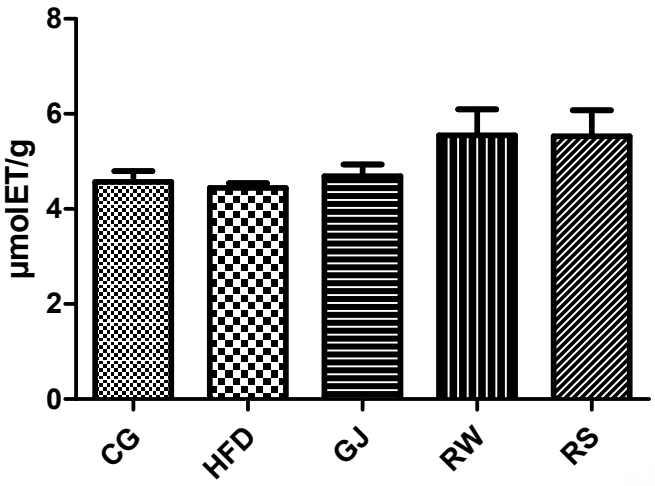

Fig. 5. ORAC plasma antioxidant activity (plasma antioxidant activity from all experimental groups using ORAC. Control group (CG) - control diet (4% fat); high fat diet group (HFD) - high fat diet (20% fat); grape juice group (GJ) - received 15 ml/day grape juice + high fat diet; red wine group (RW) - received 10 ml/day red wine + high fat diet; resveratrol solution group (RS) - received 15 ml/day resveratrol solution + high fat diet).