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Antioxidant potential of barley extract in rats subjected to a high-fat diet

Potencial antioxidante de extrato de cevada em ratos submetidos à dieta hiperlipídica

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Abstract

Antioxidants have the ability to neutralize free radicals produced in the body during lipid oxidation. The objective in this article was to study the effect of the barley extract on lipid oxidation in rats subjected to a high-fat diet. The experiment lasted 67 days. The animals were separated into three experimental groups: standard (P), high-fat diet group (L), and group with high-fat diet supplemented with barley extract (C). The feed intake of L and C groups was the lowest ($p < 0.05$). The treatments did not influence weight gain, organ weight, and the blood parameters measured. However, the levels of malondialdehyde present in the liver tissue were higher in the L group and lower in the P and C groups. Therefore, the results indicated an increased level of lipid peroxidation in the liver of rats subjected to high-fat diet, which was reduced by the consumption of barley.

Keywords: oxidative stress; biological assay; lipid peroxidation.

Resumo

Os antioxidantes têm a capacidade de neutralizar os radicais livres gerados no organismo durante a oxidação lipídica. O objetivo deste artigo foi estudar o efeito do extrato de cevada na oxidação lipídica em ratos submetidos a uma dieta rica em gordura. O experimento durou 67 dias. Os animais foram separados em três grupos experimentais: grupo padrão (P), grupo de dieta rica em gordura (L) e grupo com dieta rica em gordura suplementada com extrato de cevada (C). O consumo de ração dos grupos L e C foi mais baixa ($p < 0,05$). Os tratamentos não influenciaram no ganho de peso, no peso dos órgãos e nos parâmetros sanguíneos avaliados. No entanto, os níveis de malondialdeído presentes no tecido hepático foram maiores no grupo L e menores nos grupos P e C. Portanto, os resultados indicaram um aumento do nível de peroxidação lipídica no fígado de ratos submetidos à dieta rica em gordura, a qual foi reduzida com o consumo de cevada.

Palavras-chave: estresse oxidativo; ensaio biológico; peroxidação lipídica.

1 Introduction

Environmental factors, along with a high-fat diet, cause an imbalance between the antioxidants and pro-oxidant tissue. Thus, high consumption of lipid induces oxidative stress in rat liver (MARCZUK-KRYNICKA et al., 2009).

The exposure to stress leads to the formation of reactive oxygen species (EROS) favoring the oxidative stress, inducing physiological and behavioral changes interfering with the maintenance of homeostasis of an organism, which can cause cell damage and damage to lipids, DNA, proteins, mitochondria, and membranes (LIMA et al., 2006).

Oxidative stress is the major cause of many diseases. Its damage may lead to several diseases and negative effects on the body, such as aging, cancer development of cardiac and degenerative and neurological diseases, as well as changes in serum glucose and lipids (LIMA et al., 2006).

Nutrients with antioxidant properties can neutralize free radicals preventing the loss of cellular integrity (BELING et al.,

2007). Therefore, dietary antioxidants are effective means to limit lipid peroxidation *in vivo*. Recent investigations have been focused on natural molecules to identify consumer concerns about safety and toxicity (GLADINE et al., 2007).

Polyphenols are some examples of these natural components with antioxidant properties found in foods. Phenolic acids, flavonoids, proanthocyanidins, and tannins are polyphenols that may have beneficial effects on human health offering protection against chronic diseases (OVASKAINEN et al., 2008).

Among important food sources of polyphenols are the cereals and a large variety of natural plants. They contribute to the recommendations for a varied diet of fruits, cereals and vegetables (OVASKAINEN et al., 2008). Cereal grains are widely consumed by several people owing to culture and their low cost, high nutritional value, and variety of use. The main cereals grown are oats, wheat, rye, corn, barley, and triticale (PHILIPPI, 2006). Barley (*Hordeum vulgare* L.) ranks fourth in cereal production and consumption in the world (CAIERÃO;

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SPEROTTO, 2006), and there is a growing interest for its use in human food (BAIK; ULLRICH, 2008). Those cereals are rich in antioxidant (HOLTEKJOLEN et al., 2008) and naturally healthy compounds (YALÇIN et al., 2007).

Therefore, the abundant content of phenolic compounds in barley suggests its use as a potential source of antioxidants for disease prevention and health promotion (LIU; YAO, 2007). Similar studies on barley extracts have also shown its antioxidant effect (GIRIWONO et al., 2010). The objective was to study the effect of evaporated hydroethanolic extract of barley on lipid oxidation in rats submitted to a high-fat diet.

2 Materials and methods

2.1 Samples

In order to prepare the extract, we used barley cultivar BRS Lagoa, provided by the Centro de Pesquisa da Embrapa/Trigo Passo Fundo, grown in Ibiaciá/Rio Grande do Sul in 2006.

2.2 Preparation of extract

The extract was obtained by the hydroethanolic extraction on 80%. To each 100 mL of extract, 80 mL of ethanol and 20 mL of water were added. Next, 64 g of barley (previously grounded using a micro-mill) were added in order to obtain a particle size smaller than 1.5 mm. Subsequently, the sample was sonicated for 30 minutes at room temperature ($25\text{ }^{\circ}\text{C} \pm 1$) and filtered through a filter paper of rapid filtration to remove the waste. Subsequently, according to Bezerra (2009), the solvent was evaporated in a rotary-evaporator at $40\text{ }^{\circ}\text{C}$.

2.3 Determination of total phenols

According to Singleton and Rossi (1965), the determination of total phenolic concentration in the barley extract was performed using the Folin-Ciocalteu method.

2.4 Diets

The diets were prepared according to the American Institute of Nutrition (AIN) (REEVES et al., 1993). A standard diet (P) and two hyperlipidemic diets (L and C), were prepared. The latter one added with the barley extract during the animal feeding in the amount of 4 mL/dia/animal. Part of the glycidic fraction was replaced with pork fat achieving 14% of the diet, according to Moraes et al. (2003).

The feed ingredients were mixed and sifted three times for homogeneous and uniform diets. Despite the addition of the antioxidant (TBHQ), the diets were prepared every 10 days and separated into 4 kg packages and kept under refrigeration to avoid possible lipid oxidation.

2.5 Animals and treatments

30 adult male Wistar rats, 50 days old, with initial body weight between 148 to 270 g were used. The animals were separated into three experimental groups; each one with 10

rats: standard (P), high-fat diet group (L), and high-fat diet plus barley extract supplemented group (C).

The animals were placed in individual metabolic cages with water bottle, food bowls, and litter box, and were maintained at room ($21 \pm 3\text{ }^{\circ}\text{C}$) on a 12:12 hours light/dark cycle, with free access to food and water.

The experiment lasted 74 days. During the he first seven days, all animals were fed standard diet for adaptation purpose. During the trial period, the animals were weighed at baseline and then every three days.

In the experimental period of 67 days, the amount of food consumed daily (difference between the original amount given and the leftovers) and the volume of feces excreted were determined. Food efficiency ratio (FER) was calculated by dividing the total weight gain (g) by the total consumption of diet (g).

2.6 Determination of food consumption and digestibility of dry matter, lipids, and proteins

In order to determine the intake of dry matter, ether extract, protein, and dry matter digestibility of lipids and protein, the chemical composition of the feed consumed and feces excreted were determined, according to the techniques described by AOAC (ASSOCIATION..., 1995). For the dry substance determination, feces were collected during the experiment and dried in an oven with air circulation at $60\text{ }^{\circ}\text{C}$ for 72 hours and then cooled, weighed, and ground.

The protein content was determined using the Kjeldahl method, which quantifies the total nitrogen in the sample. The general factor of 6.25 was used to calculate the nitrogen-to-protein conversion factor (ASSOCIATION..., 1995). The determination of lipids was carried out using a Soxhlet apparatus using ether as solvent oil, according to AOAC (ASSOCIATION..., 1995).

2.7 Blood analysis and organ weight

One day after the end of the experiment, the animals were fasted for 12 hours, anesthetized, and euthanized; blood samples were collected by cardiac puncture. The blood collected was centrifuged for 10 minutes at 3,000 rpm to separate the serum, which was kept in a freezer until biochemical analysis of total cholesterol, HDL cholesterol, triglycerides, and total protein and albumin, which was performed using enzymatic colorimetric kits supplied by Doles®, whose methodology was in accordance with the protocol. The proteins in the liver tissue were determined using the method of Lowry et al. (1951). The epididymal fat and other organs were removed and weighed immediately after the rodent's dissection.

2.8 Lipid peroxidation

Lipid peroxidation was determined by the quantification of malondialdehyde (MDA) in the liver of rats, according Ohkawa et al. (1979), to determine the thiobarbituric acid reactive species (TBARS).

2.9 Statistical analysis

The data was submitted to variance analysis (ANOVA), followed by the Tukey's test when appropriate. The differences were considered significant when $p \leq 0.05$.

2.10 Ethics Committee

This research was approved by the Ethics and Animal Welfare Committee from the Universidade Federal de Santa Maria, Santa Maria, RS, Brazil (project # 10/2009 (2) -2010).

3 Results

Total polyphenol content was determined, and a concentration of 0.74 mg of gallic acid equivalent (GAE)/g of barley was found. The extract yield of 30 mL was obtained with 100 mL of solvent hydroethanolic and 64 g of barley. After the extract evaporation (80%), which corresponds to the alcohol fraction, around 6 mL of aqueous extract were obtained. After alcohol evaporation, there was a higher concentration of total polyphenols, although not proportional to the volume reduction, indicating loss of polyphenols during the extract evaporation. The estimated intake of total polyphenols was of 4.32 mg/day/animal.

The food intake and the digestibility of dry substance were lower in the L and C groups ($p < 0.05$). The consumption of lipids and digestibility was greater in rats submitted to a high-fat diet ($p < 0.05$). The food efficiency ratio was also higher in the groups submitted to a high-fat diet ($p < 0.05$) (Table 1).

Weight gain, organ weight, and blood parameters of the animals were similar in the different treatments (Table 2). The determination of protein in the liver tissue presented the following mean \pm SD: 31.77 \pm 7.18 (group P), 27.14 \pm 4.38 (group L), and 31.00 \pm 4.09 (group C).

The levels of MDA, determined by TBARS, increased in the liver tissue of rats fed high-fat diet (L group), and reduced with the consumption of the diet supplemented with barley extract (group C). Therefore, there was an increase in lipid peroxidation (TBARS) and a reduction in these levels in the group fed diet supplemented with barley extract (Figure 1).

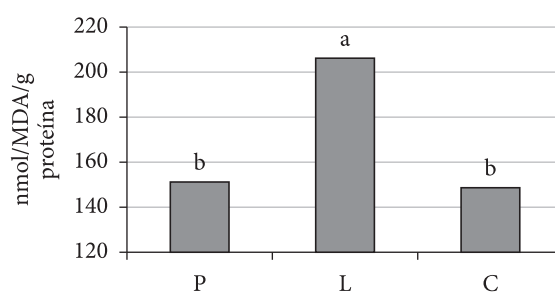


Figure 1. Effect of high-fat diet and supplementation with barley extract on TBARS of liver of rats. The data are expressed as nmol MDA/g protein and represented as average. Values statistically different (^{a,b}) ($p \leq 0.05$) according to the Tukey test. P – standard diet; L - fat diet; C - fat diet supplemented barley extract.

Table 1. Dry matter intake (DMI), dry matter digestibility (DMD), ether extract intake (EEI), ether extract digestibility (EED), protein intake (PI), protein digestibility (PD), and food efficiency ratio (FER).

Variables	P	L	C
DMI (g)	1284.18 \pm 95.10 ^a	1025.42 \pm 96.75 ^b	1013.66 \pm 100.54 ^b
DMD (%)	91.28 \pm 0.65 ^a	89.05 \pm 1.01 ^b	88.91 \pm 1.14 ^b
EEI (g)	56.31 \pm 4.17 ^b	202.57 \pm 19.11 ^a	200.25 \pm 19.86 ^a
EED (%)	95.65 \pm 1.51 ^b	98.53 \pm 0.52 ^a	98.50 \pm 0.78 ^a
PI (g)	172.25 \pm 12.75 ^a	137.54 \pm 12.98 ^b	135.96 \pm 13.49 ^b
PD (%)	90.19 \pm 0.72 ^a	88.67 \pm 1.32 ^b	88.82 \pm 1.52 ^b
FER	0.11 \pm 0.02 ^b	0.14 \pm 0.03 ^a	0.17 \pm 0.03 ^a

Values represent mean \pm S.D. Values statistically different (^{a,b}) between groups ($p \leq 0.05$) according to the Tukey test. P – standard diet; L - fat diet; C - fat diet supplemented barley extract.

Table 2. Gain weight (WGT), liver weight, heart weight, epididymal fat weight (EFW), and blood parameters of animals in different experimental groups.

Variables	P	L	C
WGT (g)	159.46 \pm 29.47	166.19 \pm 42.56	194.53 \pm 38.01
Liver weight (g)	9.43 \pm 1.72	9.48 \pm 1.82	10.64 \pm 2.11
Heart weight (g)	0.99 \pm 0.17	1.01 \pm 0.15	1.03 \pm 0.16
EFW (g)	5.29 \pm 1.03	5.96 \pm 1.38	5.70 \pm 1.25
Total cholesterol (mg/dL)	80.13 \pm 20.73	75.82 \pm 12.26	75.69 \pm 12.09
HDL (mg/dL)	110.49 \pm 53.76	103.57 \pm 38.45	85.10 \pm 17.75
Triglycerides (mg/dL)	47.86 \pm 19.63	45.71 \pm 12.04	49.96 \pm 13.78
Glucose (mg/dL)	82.02 \pm 5.31	83.65 \pm 5.90	82.28 \pm 4.03
Total protein (mg/dL)	6.73 \pm 0.87	6.67 \pm 0.60	6.81 \pm 0.65
Albumin (mg/dL)	3.33 \pm 0.21	3.30 \pm 0.21	3.30 \pm 0.19

Values represent mean \pm S.D. No values statistically different between groups ($p < 0.05$) according to the Tukey test. P – standard diet; L - fat diet; C - fat diet supplemented barley extract.

4 Discussion

Ultrasonic extraction for 30 minutes, using solvent hydroethanolic, (80% v/v) was the method chosen due to its stronger extraction capacity of polyphenols and its low toxicity solvents (acetone and methanol), according to Bezerra's studies (2009). The level of polyphenols present in barley is consistent with that found by Bezerra (2009) (1.02 mg GAE/g) in the same cultivar. Similar amounts of total phenolic were found in sorghum, which varied from 0.46 to 0.76 (mg GAE/g flour), according to the type of extraction used (KAMATH; CHANDRASHEKAR; RAJINI, 2004).

The level of total polyphenols present in the extract evaporated from barley was of 1.08 mg GAE/mL. This value is higher than those found in grape juice and red wine (0.62 and 0.73 mg GAE/mL, respectively) according to Moura (2009).

During the experimental period, the average daily feed consumption in the control group was of 19.17 ± 1.42 g, and in the groups fed high-fat diet it was of 15.30 ± 1.44 g and 15.13 ± 1.50 g (group supplemented with barley extract). The total food consumption and the average daily consumption of animals fed high-fat diet were lower than that of the L group. This is probably due to the fat in the diet, or high-energy, which leads to reduction in food intake and increases satiety (BENELAM, 2009). As can be seen, the extract offered was well accepted, and it did not influence the acceptability of the diet by the rats, whereas no effect on food intake was observed.

Many studies have shown that increased intake saturated fat contributes to body weight gain and increases triglycerides, total cholesterol, and LDL cholesterol, risk factors for cardiovascular diseases (MING et al., 2009). Contrary to the expectations, there was no difference in weight gain between the groups, finding that was also observed by Cherem and Bramosrki (2008). This is probably due to the reduced food intake and digestibility of hyperlipidemic diets, which contributed to reduce the amount of energy absorbed. Moura (2009), working with high-fat diet, did not find changes in total cholesterol levels, triglycerides, total protein, and glucose in the animals' blood although it caused an increase in LDL cholesterol and a decrease in HDL cholesterol.

Similarly, the weight of the liver and heart of the rats were similar among groups and represent on average 2.64 and 0.2% of their respective body weights. These results were similar to those found in the literature, for example in the study conducted by Gladine et al. (2007).

Sources of saturated and unsaturated fats induce oxidative stress in rat liver (MARCZUK-KRYNICKA et al., 2009), as found by Oliveros et al. (2004), when increasing the levels of TBARS tissues of rats fed a diet rich in saturated fat. In the present study, it was observed an increase in the levels of MDA (also determined by TBARS) in the liver of rats fed a diet rich in fat pork.

The TBARS method was used for screening and monitoring lipid peroxidation. Thiobarbituric acid reactive substances are produced during oxidative stress, which is induced by damage to lipids, or lipid peroxidation; MDA is the best known specific TBARS (BEHULIAK et al., 2009).

Furthermore, when compared to others, they are more sensitive and simple. The determination of serum MDA is still the most commonly used test for lipid peroxidation in biomedical sciences since MDA is one of the major aldehydes formed after breakdown of lipid hydroperoxides. Thus, it is considered a good biomarker of oxidative damage caused by free radicals in diseases associated with oxidative stress (MATEOS et al., 2005).

There was a clear decrease in lipid peroxidation and restored antioxidant status in the animals supplemented with barley extract, demonstrated by the low level of MDA in the liver of this animal group (Figure 1). Similar results were also observed by Giriwono et al. (2010) investigating the use of yeast extract of fermented barley.

This effect can be attributed in part to the antioxidant properties of polyphenols (VINSON et al., 2001), which have caught the attention of medical scientists because of their strong ability to scavenge free radicals and break the reaction chain of these radicals *in vitro* and *in vivo* (LIU; YAO, 2007).

Many studies with extracts of several plants have shown reduction of oxidative stress due to their richness in antioxidants such as polyphenols. Vijayakumar et al. (2004), for example, found these effects in black pepper; Gladine et al. (2007) in extracts of rosemary, grape, citrus, and calendula; and Papandreou et al. (2009) in blueberries (*Vaccinium angustifolium*).

5 Conclusions

The addition of 14% of fat pork in the diet of adult Wistar rats reduced food intake consequently reducing dry matter digestibility inhibiting excessive weight gain and blood biochemical parameters decompensation. However, an increase in lipid peroxidation in liver was observed, i.e., a high-fat diet can induce oxidative stress harming the body. However, the phenolic compounds present in barley, evaporated hydroethanolic extract, showed antioxidant activity *in vivo* reducing lipid peroxidation in the animals' liver.

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