Nutrición Hospitalaria

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In vivo assessment of the protection conferred by β-glucans from Pleurotus ostreatus against the harmful effects of acrylamide intake (Part I)

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Evaluación in vivo *de la protección que confieren los β-glucanos de* Pleurotus ostreatus *frente a los efectos nocivos de la ingestión de acrilamida (Parte I)*

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ABSTRACT

Introduction: acrylamide is formed in food through Maillard's reaction during thermal processing, and has been shown to be neurotoxic in humans, and a possible carcinogen. Studies have shown that β-glucans from *Pleurotus ostreatus* have diverse biological properties such as antioxidant and anticancer activities.

Objective: the aim of this work was to evaluate the protective effect of β-glucans from *Pleurotus ostreatus* against the harmful effects of acrylamide consumption in mice.

Methods: β-glucans were obtained by alkaline-acid hydrolysis of *Pleurotus ostreatus,* and the content was characterized by liquid chromatography. To evaluate the effect of β-glucans on the expression of glutathione, Balb/c mice were used, and 4 test groups were established. All groups were fed normally, and the groups treated with acrylamide were administered the compound intragastrically at a concentration of 50 μg/mL; β-glucans were administered at a concentration of 50 μg/mL.

Results: mice exposed to acrylamide showed a marked variation in the activity of glutathione enzymes in the liver. Significant differences ($p < 0.05$) were only found in the expression of glutathione transferase, which was increased almost 3 times in the group treated with β-glucans as compared with the control group, and 1.5 times as compared with the group treated with acrylamide.

Conclusions: the results show that β-glucans could act by increasing the activity of enzymes involved in xenobiotic detoxification, thus protecting the biological system against the harmful effects caused by acrylamide intake.

Keywords: Protection. Acrylamide. β-glucans. Enzyme activity. Toxicity.

RESUMEN

Introducción: la acrilamida se forma en los alimentos a través de la reacción de Maillard durante el proceso térmico, y ha demostrado ser neurotóxica en humanos y un posible carcinógeno. Algunos estudios han demostrado que los β-glucanos de *Pleurotus ostreatus* tienen diversas propiedades biológicas, como actividades antioxidantes y anticancerígenas.

Objetivo: el objetivo de este trabajo fue evaluar el efecto protector de los β-glucanos de *Pleurotus ostreatus* contra los efectos nocivos por consumo de acrilamida en ratones (prueba *in vivo*).

Métodos: los β-glucanos se obtuvieron por hidrólisis ácido-alcalina de *Pleurotus ostreatus* y su contenido se caracterizó por cromatografía líquida. La oxidación de los lípidos se evaluó mediante el método de TBARS, y para evaluar el efecto de los β-glucanos en la expresión de glutatión se usaron ratones Balb/c, y se establecieron 4 grupos de prueba. Todos los grupos fueron alimentados normalmente; a lo grupos tratados con acrilamida, esta se les administró intragástricamente en una concentración de 50 µg/ml, y los β-glucanos en una concentración de 50 µg/ml.

Resultados: en el presente trabajo, los ratones expuestos a acrilamida mostraron una marcada variación en la actividad de las enzimas de glutatión determinadas en el hígado. Solo se encontraron diferencias significativas ($p < 0,05$) en la expresión de glutatión-transferasa, que aumentó casi 3 veces en el grupo tratado con β-glucano en comparación con el grupo de control, y 1,5 veces con respecto al grupo tratado con acrilamida.

Conclusiones: los resultados muestran que los β-glucanos podrían

actuar como agentes antioxidantes que protegen el hígado contra el estrés oxidativo causado por la ingesta de acrilamida.

Palabras clave: Protección. Acrilamida. β-glucanos. Actividad antioxidante. Toxicidad.

INTRODUCTION

In April 2002 the National Food Authority of Sweden published a study in which the presence of a carcinogen was reported for the first time in experimental animals, identified as acrylamide. The main mechanism for its formation in foods is through the Maillard reaction between reducing sugars (such as glucose or fructose) and the amino-terminus of asparagine during thermal processing (T > 120 °C) (1).

Thermally processed products are consumed in large proportions by the general population, since heat treatments increase the range of colors, flavors and aromas, and also favor the development of the desired sensory characteristics, thus offering tastier foods to consumers. However, warming also favors chemical reactions that could promote a reduction in the content of beneficial compounds, or even the formation of potentially toxic ones such as acrylamide (2,3).

The metabolism of acrylamide has been investigated in toxicokinetic studies in humans, rats, and mice. After ingestion, acrylamide is rapidly absorbed and distributed throughout the body. It can be found in many organs such as the thymus, liver, heart, brain, and kidneys (4), as well as in the human placenta (5) and in breast milk (6), being easily transferable to the fetus or the newborn. Acrylamide can be oxidized by cytochrome P4502E1 to epoxide glycidamide (2,3-epoxypropionamide) (7,8) or conjugated to glutathione (GSH) in order to be more easily eliminated in the urine (7).

One of the most important enzymatic complexes for the detoxification of acrylamide is the enzymatic complex of glutathione S-transferases (GST), which conjugates activated xenobiotics with glutathione. Thus, the induction of this enzyme complex could improve the detoxification and excretion of potentially harmful compounds. GST is generally involved in the detoxification of activated xenobiotics such as acrylamide, which is considered a highly reactive α,β-carbonyl compound (9). Studies have shown that the intake of xenobiotics in the diet affects GST levels, and consequently elimination (10). On the other hand, some studies have shown the ability of vegetables such as garlic, celery, and members of the Brassicaceae family, as well as fungal and yeast-derived β-glucans, to modulate GST levels (9,11-13).

β-glucans are glucose polymers found in the cell wall of cereal grains, particularly oat and barley, and of fungus such as *Pleurotus ostreatus,* which is a popular and important edible mushroom commercially cultivated worldwide due to the biological properties attributed to it (13). Therefore, the objective of this study was to evaluate the protection conferred by β-glucans from *Pleurotus ostreatus* against the harmful effects of acrylamide consumption *in vivo*.

METHODS

Obtention of β-glucans from *Pleurotus ostreatus*

The fungus was obtained from the culture collection belonging to the Industrial Microbiology Laboratory. Mature fungi were thoroughly washed in distilled water, then dried and disinfected with ethanol (70% v/v) before extracting the β-glucans present in the cell wall.

β-glucans were obtained by the method described by Fleet and Manners (14), with some modifications made by Catley (15). After harvesting the fungus was milled to fractionate the fruiting body, which contains the β-glucans. Briefly, 1 g of fungus extract (pulverized) was put in contact with 10 mL of NaOH (4% w/v). The tubes were placed in a water bath at

80 °C for 4 hours. Subsequently, they were centrifuged at 5000 rpm for 20 min. The supernatant (alkali-soluble glucan-mannan) was separated from the insoluble glucan-chitin fraction (precipitate). The alkali-soluble fraction was separated from the insoluble fraction and 5 mL of CH_3COONa (2 M) were added. The samples were kept at 75 °C in a water bath. Subsequently, 100 mL of ethanol (96% v/v) were added and allowed to stand for 12 hours under refrigeration at 4 °C, after which time they were centrifuged at 5000 rpm for 20 minutes — the precipitate was discarded and the supernatant, which contains the β-glucans, was recovered.

The insoluble alkali material was re-extracted to recover the β-glucans by adding to the precipitate 5 mL of NaOH (6% w/v) at 80 °C for 3 hours. Then the samples were centrifuged at 5000 rpm for 20 minutes to separate the sediment from the supernatant. The supernatant was treated with 5 mL of CH_3COONa (2 M), and incubated at 75 °C for 2 hours. Subsequently, 100 mL of ethanol (96%) were added, allowing the samples to stand for 24 hours at 4 °C. The supernatants were pooled and passed to a dialysis membrane (Spectra/Pro, 12,000 to 14,000 MWCO). The samples were dialyzed at 4 °C for 24 hours with constant agitation at 70 rpm in a shaking incubator (Labtech), changing the solution of phosphate buffer (50 mM), pH 7.4 plus NaCl (0.2 M), every 8 hours. Finally, the dialyzed concentrate was ultrafiltered using a membrane of 3 kilodaltons MW (Spectra Por), and the fractions obtained were lyophilized and stored in freezing until their use.

Intake of acrylamide and administration of β-glucans

The experiment was performed with Balb/c male mice previously acclimated to and housed in plastic cages, and handled according to the Official Mexican Standard NOM-062-ZOO-1999. During the period of the experiment these mice were kept under conditions of 12:12 hours of light/darkness at a temperature of 25 °C, and were provided with water

and feed *ad libitum*. For the evaluation of this part of the experiment 4 groups of mice were set up as depicted in table I. The group administered acrylamide and β-glucans was made to assess the effect of β-glucans upon the harmful effects of acrylamide, and the groups provided with β-glucans or with acrylamide alone were used to assess whether these compounds themselves had any effect on enzyme activity; the control group received none of the above. The amount of acrylamide and β-glucan was determined according to an estimate of the intake reported in other studies and adjusted at a concentration of 50 µg/kg of body weight (BW) for acrylamide, and at adose 50 mg/kg per day of β-glucan.

Determination of glutathione-S-transferase, glutathione reductase, and glutathione peroxidase

To evaluate this part of the experiment the mice in the 4 experimental groups were sacrificed by guillotine cutting, and had their liver immediately removed. This was homogenized in Tris-HCl buffer (50 mM, pH = 8) containing sucrose (0.25 M) and phenylmethylsulfonyl fluoride (PMSF) as protease inhibitor; fat was removed by homogenizing using cold centrifugation at high speed (4 °C, 10,000 g, 30 min) and filtration. The filtrate thus obtained was used for the determination of glutathione-S-transferase, glutathione reductase and glutathione peroxidase.

Commercial kits (Sigma Aldrich) were used to determine the activity of these enzymes. These kits provide the necessary reagents for a rapid measurement of glutathione transferase, glutathione reductase and glutathione peroxidase levels in tissue extracts, as well as in red cells and blood plasma.

Statistic analysis

A one-way analysis of the variance was used with the Tuckey test using a confidence level of 95% to evaluate the differences between treatments.

RESULTS

β-glucans from *Pleurotus ostreatus*

β-glucans were obtained by alkaline extraction and subsequent ultrafiltration from the whole body of mature and fresh fungi, from which 3.4 grams of extract equivalent to 6.3% of whole fungus were obtained. This extract had a concentration of β-glucans of 80%, determined by HPLC and calculated with a calibration curve constructed with different concentrations of β-glucan.

Determination of glutathione-S-transferase, glutathione reductase, and glutathione peroxidase

Figure 1 shows the activity of the enzyme glutathione peroxidase. This enzyme had a greater activity in the control group (0.188 U/mL) as compared to the other groups evaluated. In the groups treated with β-glucans, with acrylamide + β-glucans, and with acrylamide an enzymatic activity of 0.120 U/mL, 0.113 U/mL, and 0.111 U/mL, respectively, was recorded and no significant differences ($p < 0.05$) were observed between them.

On the other hand, with respect to the enzyme glutathione reductase, the group in which the highest activity of this enzyme was observed was the group treated with acrylamide + β-glucans (2.484 U/mL); however, no significant differences between groups were observed because the control group had an activity of 2.407 U/mL, and the group treated with acrylamide alone had an activity of 2.395 U/mL; finally, the group with the lowest activity observed was the group treated with β-glucans alone, where activity was 1.885 U/mL. Glutathione reductase activities in the evaluated groups are depicted in figure 2.

Finally, regarding glutathione transferase, the group in which the highest activity of this enzyme was observed was the group treated with acrylamide + β-glucans (1.834 μmol/mL/min); in figure 3, in the group

treated with acrylamide and β-glucans enzymatic activity may be seen to increase in a statistically significant fashion when compared to the groups treated only with acrylamide or with β-glucans separately, and even when compared to the control group. That is an indication of the protection that β-glucans may offer by increasing the activity of the enzyme involved in the detoxification of acrylamide and other xenobiotics.

DISCUSSION

The family of enzymes that includes glutathione S transferase represents the main and most important group of enzymes involved in the phase-II metabolism of xenobiotics. This group of enzymes exhibits peroxidase activity and catalyzes the reduction of organic hydroperoxides. It has been shown that induction of these enzymes may occur as a result of the activity of both natural and synthetic agents, which represents a promising chemoprotective strategy. Studies in animals have shown that the levels of antioxidant enzymes depend on the availability of antioxidants in the diet, which can be acquired through consumption of vegetables such as garlic, celery, brassicas, and/or components of some organisms such as β-glucans from fungi and yeasts (16). It has been reported that the activity of many enzymes is reduced in the plasma and different organs because of acrylamide. In the present work the mice exposed to acrylamide showed a marked variation in the activity of glutathione-related enzymes as determined in the liver. It has been shown that the enzyme glutathione peroxidase offers protection against oxidation, and that organic compounds such as β-glucans could increase the activity of certain enzymes; however, our results indicate that this was not the case, since in the group treated with acrylamide there was no increase in the enzymatic activity of glutathione peroxidase, and in no group were significant differences observed regarding its activity. On the other hand, the enzyme glutathione reductase is very important

since it is responsible for the reduction of glutathione, which is important in the reduction of the reactive oxygen species that cause oxidation. In relation to this enzyme, the group in which the highest activity was observed was the group treated with acrylamide + β-glucans (2.484 U/mL); however, no significant differences between groups were observed because the control group had an activity of 2.407 U/mL, and the group treated with acrylamide had an activity of 2.395 U/mL; finally, the group with the lowest activity observed was the group treated with β-glucans only, with an activity level of 1.885 U/mL. The fact that no significant differences were found between the groups treated with β-glucans could be an indicative of the fact that β-glucans do not contribute to the increase seen in the activity of glutathione reductase, and therefore in the detoxification of acrylamide. Glutathione reductase activity in the evaluated groups is represented in figure 2. In 2011, Jayakumar et al. (17) reported that the enzymatic activity of glutathione reductase was reduced in the heart of Wistar rats treated with carbon tetrachloride; in addition, no significant differences were found between this group and the group treated with an extract of the fungus *Pleurotus ostreatus.* However, previously in 2010 the same group of researchers reported that the administration of an extract of the fungus *Pleurotus ostreatus* restored the activity of the enzyme glutathione reductase in elderly rats (18), and in the present work the administration of β-glucans from this fungus had no effect on the activity of the enzyme glutathione reductase. As there were no significant differences in activity between said group, the control group, and the group treated with acrylamide and glucans, it is possible that the null increase in this enzyme be due to extensive metabolism of acrylamide, which has been pointed out by Cui-Li et al. (19), who observed a similar behavior in Wistar rats treated with N-nitrosodiethylamine.

As regards the enzyme glutathione transferase, it is responsible for catalyzing the conjugation of reduced glutathione with a xenobiotic,

which helps to protect cells and tissues against oxidative stress. In a study by Dagett et al. (20), they found that the activity of GST in the kidney increased from 1.3 times at 12 hours after a single injection to 4.2 times the control values after 7 days from the first of three daily injections of lead acetate. In the present work we found that the activity of GST was higher in the group 1 treated with acrylamide, and this activity was recorded in the liver, although it has been reported that the activity of many enzymes such as transaminases have been inhibited by acrylamide in the plasma and in different organs such as the liver and testes, this being due to the cellular damage caused by toxic substances, which is often accompanied by an increase in the permeability of the cell membrane, causing loss of enzymes through it (21).

CONCLUSIONS

The best increase in enzymatic activity was recorded for glutathione transferase, which is an enzyme responsible for catalyzing the conjugation of reduced glutathione with a xenobiotic. It suggests that this polysaccharide may be useful for protection against the deleterious effects caused by acrylamide intake; however, further studies are required in order to elucidate the precise mechanism whereby β-glucans may be protective against acrylamide.

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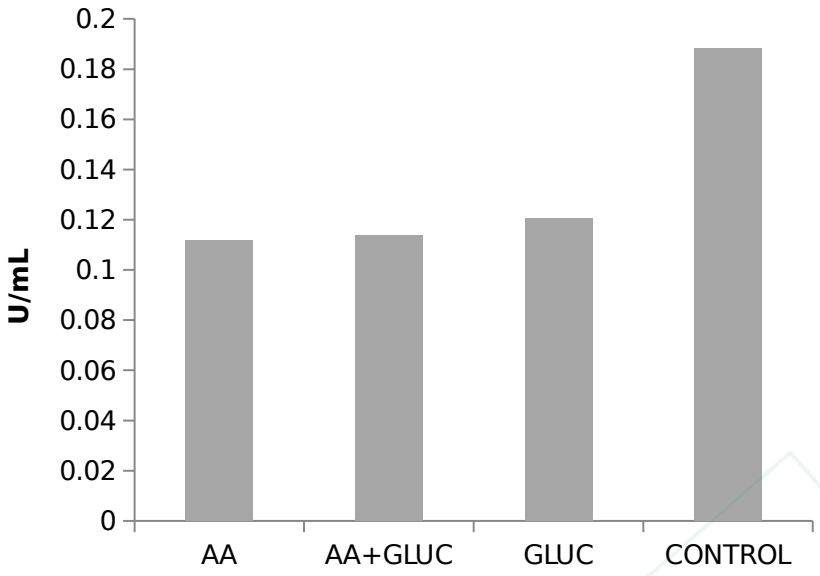

Fig. 1. Glutathione peroxidase activity in mice liver.

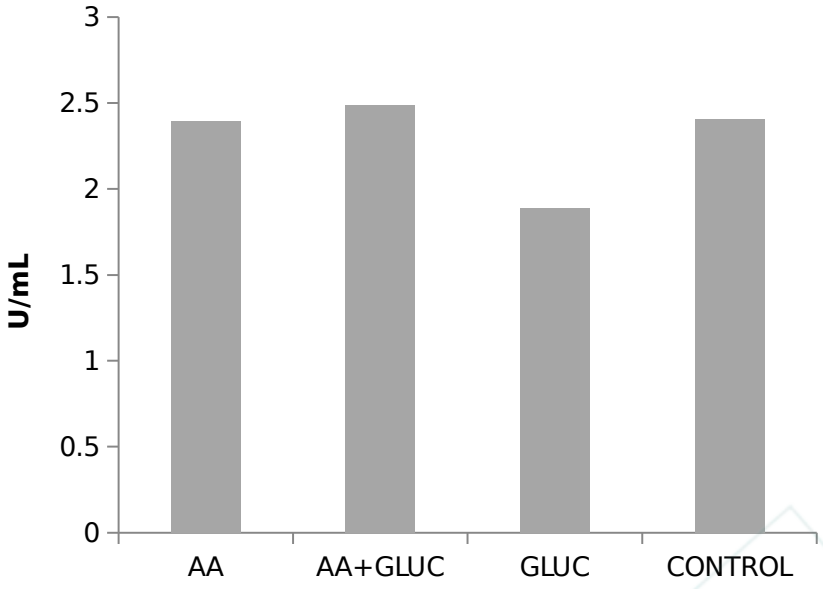

Fig. 2. Glutathione reductase activity in mice liver.

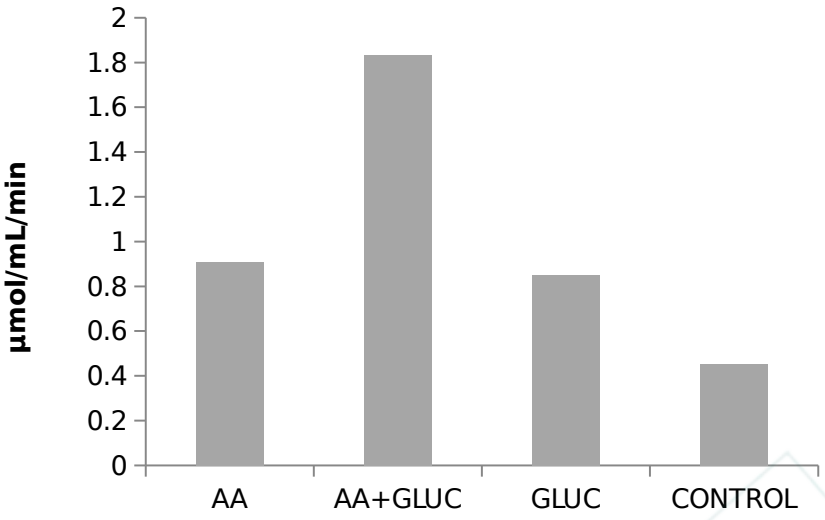

Fig. 3. Glutathione transferase activity in mice liver.

Table I. Distribution of experimental groups

Experimental groups	*Treatments*
Group 1	Food + acrylamide
Group 2	Food + acrylamide + β-glucans
Group 3	Food + β-glucans
Group 4	Control, acrylamide- and β-glucan-free