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Antioxidant Enzyme Activity in Rat Liver and Kidney Related to Coix Intake

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Abstract

The effects of dietary Coix *(lacryma-jobi)* water extract on the antioxidant enzyme activity in the liver and kidney of Sprague-Dawley rats were studied. Forty-five rats were fed for 3 weeks with either control diet or experimental diets that contain either Coix water extract or Coix water residue. Twenty percent of the carbohydrate was replaced with Coix water residue by dry weight in the water residue diet, while distilled water was replaced by Coix water extract to make a pellet-form diet in the Coix water extract diet. The levels of glutathione, glutathione-peroxidase, and glutathione-S-transferase activities in liver and kidney were measured. It has been found that glutathione, glutathione peroxidase, and glutathione-S-transferase enzyme activities from liver and kidney of the rats were enhanced in the group fed with Coix water extract.

Key words: Coix, oxygen free radicals, glutathione, glutathione related enzymes

INTRODUCTION

Coix *(lacryma-jobi)* is an annual plant, which is popular in rural areas of South Korea. Coix seeds consist of water 10.4%, crude protein 21.3%, crude fat 3.7%, starch 61.1%, fiber 2.0 and ash 2.3% (1). Coix has been used as a food source for humans and livestock in Korea. Coix seeds, of the gramineous plant Coix *lacryma-jobi L. var. ma-yuen Stapf*, have been used in oriental medicine as a diuretic, analgesic, antispasmodic and hypoglycemic agent. Physiologically active substances that have recently been isolated were found to possess antiphlogistic and antitumor promoting activities (2). It was also found that Coix contained high levels of cystine and a trypsin inhibitor that was similar to the soybean Bowman-Birk trypsin inhibitor (3). In addition, it is suspected that some components other than the non-proteinous and defatted components in Coix *lacryma* seeds may contribute to activation of macrophages through induction of NO for biostatic activity (4).

Oxygen free radicals are continuously generated in cells during oxidative metabolism (5). Antioxidant defense systems have also co-evolved with aerobic metabolism to counteract the oxidative stress from free radicals. However, the antioxidant defense system can be inundated by free radicals to cause damage in DNA, RNA, lipids and proteins, finally leading to cancer (6). Therefore, consuming extra antioxidants through food or drinks would be very critical in the defense system against oxidative stress.

The liver and kidney are critical organs in maintaining homeostasis in the body (7). The liver is constantly exposed to environmental contaminants. The liver, positioned between the intestinal tract and the rest of the body, plays a critical role in maintaining the body's metabolic homeostasis. The liver is the first organ to encounter environmental contaminants as well as the waste products of bacteria entering the portal blood, since venous blood from the stomach and intestines flows into the liver via the portal vein before the general circulation. The environmental contaminants transported to the liver via venous blood are subjected to extensive metabolism in the liver. Therefore, sound function of the liver is of supreme importance. The kidney plays a principal role in excreting metabolic wastes and regulating extracellular fluid volume, electrolyte composition, and acid-base balance. Therefore, protecting these two vital organs from oxidative stress is very important in maintaining overall homeostasis.

In this research, we investigated the effects of Coix water extract and Coix residue on antioxidant enzyme activities in experimental animals' liver and kidney. Results of this study may be useful to determine the antioxidant activities of dietary Coix.

METHODS AND MATERIALS

Animals and diets

Forty-five male Sprague-Dawley rats with body weights ranging from 160 to 180 g were divided into 3 groups: control, Coix water extract, and Coix water residue groups. Animals were fed with control and experimental diets for 3 weeks after 1 week of a basal diet. The control diet was modified from AIN-76. The Coix water residue diet was formulated by replacing 20% of carbohydrates with Coix water residue from the control diet and the Coix water extract diet had the same diet as the control group but the distilled water was used to make a pellet form. The Coix water extract was

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prepared by soaking 2 kg of Coix in 2 liters of distilled water for 10 hours. Specific contents of each diet is presented in Table 1.

Sample collection and analysis

During the experiment, all animals were weighed every other day and food intake was measured daily (Table 2). At the end of the test, all animals were anesthetized with ether and sacrificed by decapitation followed by removal of organs. The removed organs were blotted-dry, weighed and stored at -70°C until analysis.

Glutathione (GSH) assay

Half a gram of liver and kidney was homogenized using Polytron PT 120 (Kinemtatica. Switzerland) in 3 ml of 5% trichloroacetic acid for 10 sec., and centrifuged at 10,000× g for 5 min at 4°C. The obtained supernatant fraction was analyzed for total GSH by the enzymatic method of Tietze (8).

Glutathione-peroxidase (GSH-Px) activity

GSH-Px activities were assayed as described by Paglia and Valentine (9) with slight modifications. GSH-Px activities were determined by measuring absorbance and expressed as micromoles of NADPH oxidized per minute per milligram of protein.

Glutathione-S-transferase (GST) activity

GST activities were assayed as described by Habig et al. (10) with slight modifications. Sample and reference cuvettes were read for five minutes in a dual-beam spectrophotometer set at 340 nm. GST activities were expressed as nanomoles of 1-chloro-2,4-dinitrobenzene conjugate formed in a minute per milligram of protein. Protein was measured by the method of Bradford (11).

Statistical analysis

The statistical significance in antioxidant enzyme activities of Coix water extract and Coix water residue from each tissue was assessed using one-way ANOVA at a significance level of 0.05, followed by application of the Tukey test using Systat 8.0 (SPSS Inc. Chicago, IL, USA).

Table 1. Food composition of experimental groups

Ingredient	Control	Coix water extract	Coix residue
Casein	20	20	20
DL-Methione	0.3	0.3	0.3
Corn oil	10	10	10
Mineral mixture	3.5	3.5	3.5
Vitamin mixture	1.0	1.0	1.0
Choline bitartrate	0.2	0.2	0.2
Cellulose	5	5	5
Rice powder	60	60	40
Coix residue	0	0	20
Total	100	100	100
Coix water extract	0 liter	2 liter	0 liter
Distilled water	2 liter	0 liter	2 liter

Table 2. The body weight changes and feed efficiency ratios (FER) of rats fed Coix water extract and Coix residue for 3 weeks

	Weight gain (g/3 wks)	Feed intake (g/3 wks)	FER
Control	72.8±20.8[a]	604.8±67.2[a]	0.12
Coix water extract	64.8±23.8[a]	623.7±31.5[a]	0.10
Coix residue	58.7±20.5[a]	621.6±57.8[a]	0.09

Values are means±*SD* for 15 animals per group. Means not sharing a common superscript within a column are significantly different at a significance level of 0.05.

RESULTS

Rats in the control group gained the greatest amount of weight (72.8 g) over 3 weeks of the test period, followed by rats in the Coix water extract (64.8 g) and in the Coix residue (58.7 g) groups, even though there was no statistical significance (Table 2). Also, there was no significant difference in feed intake, which ranged from 604.8 to 323.7 g. Feed efficiency ratios, which was calculated by dividing weight gain by feed intake, of control, Coix water extract, and Coix residue groups were 0.12, 0.10, and 0.09, respectively.

The weights of the liver, kidney, and heart were not significantly different among the groups, which ranged from 4.54 to 4.84, 1.9 to 2.1, and 1.15 to 1.28 g, respectively (Table 3). However, weights of the spleen between control and Coix residue groups differed significantly. The spleens from the Coix residue group weighed the most, 0.73±0.18 g, followed by Coix water extract group, 0.67±0.2 g, and the control group, 0.55±0.15 g.

GSH content, GSH-Px and GST activities were always higher in the liver than in the kidney (Fig. 1). GSH content in the rat liver showed statistically significant differences among groups. GSH content of the liver of the control group (5.98± 0.58 μmol/g tissue) was the lowest among the three groups and significantly different from contents of the Coix water extract (8.12±0.45 μmol/g tissue) and Coix residue groups (7.32±0.26 μmol/ g tissue). A statistical significance was not found between the Coix water extract and Coix residue groups. Contrary to liver GSH level, there was no significant difference in the kidney GSH content among three groups. Kidney GSH contents of the control, Coix water extract and Coix residue groups were 2.49±0.15, 2.58±0.12, and 2.54±0.18 μmol/g tissue, respectively.

GSH-Px activities were 15.76±0.45, 17.16±1.48, and 15.18±1.80 μmol/min/mg protein, respectively for the control, Coix water extract, and Coix residue groups and they were

Table 3. The weights of different organs from test groups

	Liver (g)	Kidney (g)	Spleen (g)	Heart (g)
Control	4.75±0.8[a]	2.0±0.1[a]	0.55±0.15[a]	1.20±0.3[a]
Coix water extract	4.54±0.7[a]	1.9±0.8[a]	0.67±0.2[ab]	1.15±0.7[a]
Coix residue	4.84±0.9[a]	2.1±0.9[a]	0.73±0.18[b]	1.28±0.9[a]

Values are mean±*SD* for 15 animals per group. Means within a column not sharing a common letter are significant different ($p<0.05$) by Tukey test.

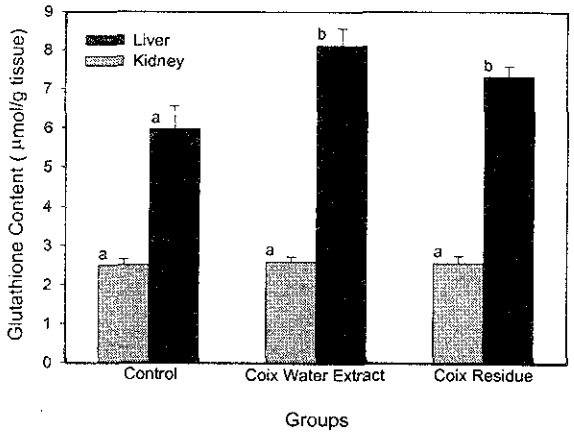

Fig. 1. The amount of glutathione in the liver and the kidney. The bars with same letters are not statistically different in the same organ at a significance level of 0.05.

not statistically different (Fig. 2). In the kidney, GSH-Px activity was the highest in the Coix water extract group, 8.91 ± 0.49 μmol/min/mg protein, which was significantly different from the control, 3.34 ± 0.36 μmol/min/mg protein, and from the Coix residue group, 4.29 ± 0.41 μmol/min/mg protein. In addition, GSH-Px activity of the control group was not significantly different from that of the Coix residue group.

Levels of GST in the liver showed statistically significant difference among the groups (Fig. 3). The Coix residue group was found to have the lowest activity of GST (241.65 ± 18.17 nmol/min/mg protein), followed by the control group, 603.05 ± 18.17, and Coix water extract group, 731.18 ± 46.12 nmol/min/mg protein. The GST level in the control and Coix water extract groups were not significantly different. In the kidney, the Coix water extract group (217.81 ± 24.37 nmol/min/mg protein) also showed the highest level of GST activity among three groups, followed by Coix residue (134.67 ± 35.76 nmol/min/mg protein) and control groups (127.34 ± 19.29 nmol/min/mg protein). In addition, kidney GST level of Coix water extract group was significantly different from the other two groups.

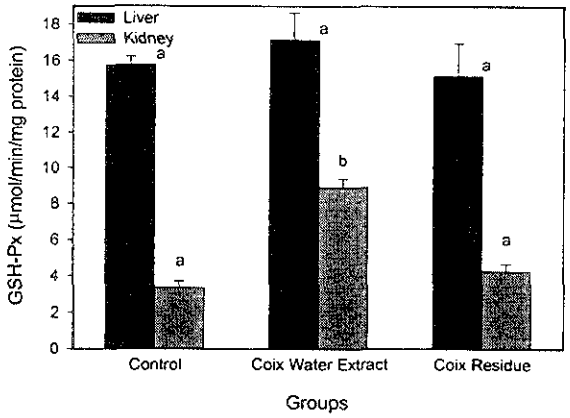

Fig. 2. The activity of glutathione-peroxidase in the liver and the kidney. The bars with same letters are not statistically different in the same organ at a significance level of 0.05.

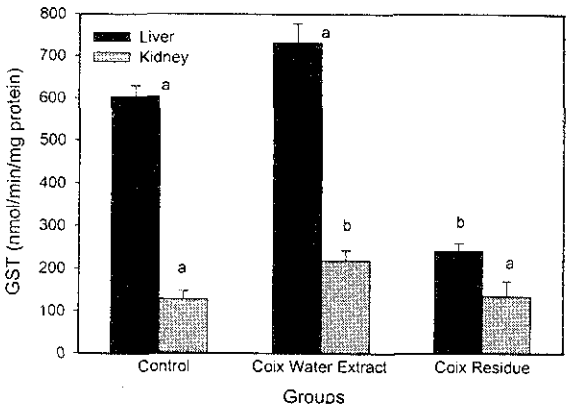

Fig. 3. The activity of glutathione-S-transferase in the liver and the kidney. The bars with same letters are not statistically different in the same organ at a significance level of 0.05.

DISCUSSION

Free radicals, molecules or molecular fragments with one or more unpaired electrons in its outer orbital are constantly formed in the body during normal oxidative metabolism by cytochrome P450 or other enzymes (12). Also, xenobiotics can cause the formation of free radicals. For instance, xenobiotics such as paraquat, benzo(a)pyrene and doxorubicin can accept an electron from reductases to give rise to radicals. These radicals are able to transfer the extra electron to molecular oxygen resulting in the formation of a superoxide anion free radical ($\cdot O_2$). A superoxide anion free radical can be transformed to a hydroxy free radical ($\cdot OH$) by superoxide dismutase and the Fenton reaction. Free radicals can also be formed by homolytic bond fission. For instance, carbon tetrachloride (CCl_4) is, by electron transfer from cytochrome P450 or the mitochondrial electron transport chain, biotransformed to the trichloromethyl free radical ($Cl_3C\cdot$) that can react with $\cdot O_2$ to form the trichloromethyl peroxy radical ($Cl_3COO\cdot$) (13).

These free radicals can lead to oxidative damage in the body, including endogenous depurination of DNA, and DNA strand breakage, which may eventually cause cancer (14). The free radicals can also cause peroxidative damage to cell membranes, inactivation of sulfurhydryl-containing enzymes, and cross-linking of integral proteins. It has been known that oxidative stress due to free radical burden can cause damages in various organ systems including the liver, kidney, cardiovascular, nervous, and pulmonary systems. However, this damage can be prevented by the action of antioxidants like GSH. GSH through GST can conjugate with free radicals making them inert. Subsequently conjugates can be excreted via bile or further transformed to mercapturic acid and excreted with urine. GSH-Px also plays a significant role in detoxifying peroxy free radicals (15). Therefore, activity of antioxidants is critical in the internal defense system against oxidative stress.

The levels of GSH, GST, and GSH-Px were significantly

higher in the liver than in the kidney. These may be attributable to high oxidative metabolism in the liver. The toxic substances absorbed in the intestine are transported to the liver through the hepatic portal vein, and are subjected to oxidative metabolism including phase I and II. In phase I metabolism, functional groups are added to substrates, giving them higher polarity, while biosynthetic reactions occur in phase II metabolism, facilitating excretion of the contaminants through bile or urine. Therefore, more antioxidant titer is required in the liver than any other organs.

Given that weight gains both in Coix water extract and Coix residue groups were lower than that of the control group, Coix affects the weight gain in rats (16). However, this may be attributable to the fact that extra fiber or starch are added to the diet in the feed processing, given that the feed intakes are similar in all three groups.

In both Coix water extract and Coix residue groups, GSH contents were significantly higher than the control, suggesting that consuming Coix helps cope with oxidative stress because GSH is used in many metabolic reactions to detoxify detrimental substances. Besides GSH conjugation that was previously described, GSH can play a significant role in detoxifying environmental contaminants in the body. That is, GSH can be used in metabolizing some organochlorine pesticides such as DDT and aldrin (17). Also, GSH can bind with epoxides, which are toxic metabolic products of phase I metabolism in the smooth endoplasmic reticulum. By binding with epoxides, biologically active epoxides turn to inert GSH conjugates, which may possibly be excreted through bile to the feces or through the kidney to the urine (18). Since the GSH was found at high concentration in the livers of Coix water extract and Coix residue fed rats, it is obvious that Coix consumption boosted the GSH level in the organ. This is very important in defending the system against the oxidative stress because the oxidative metabolism by cytochrome P450 is very active in the liver, which may render more free radical formation at higher concentration than other organs which have lower activities of cytochrome P450. It has been shown that dietary GSH enhanced metabolic clearance of peroxidized lipids and decreased their net absorption. It was also reported that consuming the foods high in GSH reduced the risk of oral and pharyngeal cancers by approximately 50% (19). Thus, it is very clear that Coix consumption alleviates the risk caused by oxidative stress. Between the two Coix groups, Coix water extract group showed slightly higher GSH content. There was, however, no statistical difference in the GSH content between the two groups. Thus, it seems the different formulations used in this research does not produce significant differences in *in vivo* GSH levels.

GSH-Px protects cell membranes from lipid peroxidation by reducing H_2O_2 and other hydroperoxides to water. Hydrogen peroxide is formed as a normal enzymatic product of numerous oxidases present in cell cytosol, microsome, peroxisomes and mitochondria, since hydrogen peroxide is used in phagocytosis, ethanol and methanol oxidation, and thyroid hormone production (20). GSH-Px content was also much higher in the than in kidney reflecting higher oxidative metabolism in the liver. GSH-Px level in the liver was not different among the groups, suggesting that detoxifying peroxide free radicals to yield water will not be much affected by different levels of Coix intake. GSH-Px level in the kidney of Coix water extract fed rats was significantly higher than that of the other groups. In fact, GSH-Px content in the Coix residue group was higher than that of the control group, suggesting there may be a biological significance, even though statistical significance was not found.

GSTs are a family of enzymes catalyzing conjugation of GSH with electrophilic and potential alkylating agents and glutathione conjugation is the first step in mercapturic acid formation (21). Hence these enzymes play an important role in detoxifying xenobiotics and their reactive metabolites, which can be generated by metabolic activation under influence of microsomal cytochrome P450 containing a mixed function oxidase system (22). These enzymes increase the rate of glutathione conjugation by deprotonating GSH to yield GS. However, glutathione conjugation can also occur nonenzymatically in significant amounts. GST content was also much higher in the liver than in the kidney, which again reflected higher oxidative metabolism in the liver. Unlike GSH activities in the organs, GST levels showed different trend between different organs. That is, GST level of the liver in the Coix residue group was significantly lower than those in the other two groups, while GST level was highest in Coix water extract group followed by the control and Coix residue groups. A peculiar thing is GST content in the liver is significantly lower in the Coix residue group. It is not known what is lowering the liver GST content in the Coix residue group. However, as described previously, glutathione conjugation can occur without the action of GST. Therefore, the effect of low GST level in the liver on glutathione conjugation would not be as significant as it would appear in GST activity. That is, glutathione conjugation in the liver is still mainly affected by GSH content.

CONCLUSION

Liver glutathione level in rats fed Coix diets was significantly higher than that of the control group. Therefore, it is highly suspected that consuming Coix in any form may help defend against oxidative stress which may be imposed naturally or chemically. Since a human body is exposed to multiple toxic agents, consuming functional food such as Coix will help lower the risk of cancer or other illness considerably which may be caused by the imbalance between antioxidants and free radicals.

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