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Antioxidative Effects of Mash Beans Depending on Gender and High Fat Intake in a Model Organism

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ABSTRACT

Objective: In the study, the aim was to to determine the effect of Mash bean, an antioxidant source, against oxidative stress due to gender and high fat intake.

Materials and Methods: For this purpose, local and commercial Mash was added to the fatty and non-fatty diet of the model organism (*Drosophila melanogaster*) which were let to grow. Malondialdehyde and glutathione S-transferase (GST) activities were determined in adult females and males.

Results: According to the results, it was determined that Mash bean increased the GST activity of the insect. Feeding with non-commercial mash bean (NC-MB) and high-fat diet decreased lipid peroxidation (LPO) in females, whereas commercial mash bean (C-MB) had opposite effects.

Conclusion: It was concluded that the NC-MB seeds were more successful in preventing LPO than C-MB. In our study, the nutrition of male individuals with a fat diet or a non-fat diet did not change the amount of LPO.

Keywords: *Drosophila melanogaster*, Mash bean, lipid peroxidation, Glutathione-S-transferase activity, high-fat diet

INTRODUCTION

In recent years, the fact is that carbohydrate and fat-rich diets accelerated aging, increased obesity, diabetes, cancer, and cardiovascular diseases (1). Accordingly, the use of local and natural nutrients and medicinally important herbal content have become widespread and the necessity of being scientifically relevant in the prevention, and treatment of diseases has occurred (2). With the widespread use of herbal therapies, the investigation of functional nutrients has become an important research area in vivo studies with short life forms.

Due to excess nutrient intake (Obesity), aging and an increase in cell lipid levels, oxidative stress-induced complications can develop (3). *Drosophila melanogaster* (Meigen) fed with high-fat diet (HFD) is a model organism used in in vivo nutrition studies for obesity (4-6). The oxidant-antioxidant mechanism, fatty tissue, and digestive system of *D. melanogaster* are similar to mammals, and due to its advantages in terms of ease of operation, it is frequently preferred in nutrition studies (7-12). It is known that flies fed with HFD have a significant decrease in amino acid and protein levels leading to oxidation (4). Malondialdehyde (MDA) occurs in the biological systems as a result of increased oxidative stress-induced lipid peroxidation (LPO) (13). MDA is an indicator of LPO, and used to determine the LPO grade. Oxidative damage mediated by free radicals caused by many environmental causes and formed during the conversion of nutrients to energy using oxygen as by-products of the metabolism in the body can be prevented by increasing the antioxidant concentration in the tissues (14-17). In many organisms, Glutathione *S*-transferases (GST) are a family of important detoxification enzymes (18). *Leguminosae* legumes, which are rich in dietary fiber and high nutritional value, show antioxidant activity and its success in preventing oxidation is shown in many studies

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(19-25). Used in many parts of the world and known as mung bean, green gram, urad whole, Mash bean *Vigna radiata* (L.) R. Wilczek is a species belonging to the *Fabaceae* family (26,27). Like other legumes, the high nutritional value of Mash bean is also known, and there are many studies in the literature that determined its antioxidant capacity (28). In spite of this, no study was done to investigate the oxidant-antioxidant effects of Malatya-specific local black Mash bean and commercial Mash bean. In this study, the use of HFD (20% palmitic acid) in the larval period and antioxidant source of Mash bean against oxidative effects on lipid molecules in adult individuals were investigated. The antioxidant effects of non-commercial/local (NC-MB) and commercial (C-MB) mash beans before and after cooking was also compared.

For this purpose, the effect of NC-MB and C-MB was determined in the model organism (*D. melanogaster*) fed with a fat and fat-free diet. In addition to the cooking practices in different products, the possible effect of Mash bean due to fat feeding has been demonstrated.

MATERIALS AND METHODS

Biological Materials

In the study, NC-MB and C-MB (Malatya and Mersin origin) were used (Figure 1). *D. melanogaster*'s strain of wild type adult individuals (in 200 ml bottles) were incubated in the laboratory of Necmettin Erbakan University (25 ± 2°C and 60-70% relative humidity, 12 hours light, 12 hours dark photoperiod). An artificial diet (potatoes, agar, sucrose, dry yeast, ascorbic acid, and nipagin) prepared with mashed potatoes for culture food was used. This nutrient was also used as an experimental diet and as a control food. Food for the insects in the culture bottles was refreshed every 15 days, and the procedures were made completely under aseptic conditions (29).

Experimental Pattern

In this study, for HFD, palmitic acid was dissolved within Tween-80 and added to 20% of the hot control nutrient (culture food) (30). Commercial and local unprocessed NC-MB and cooked Mash beans C-MB were added to *D. melanogaster* diet mixed with the food. Similar to the previous study (31), where the upper and lower limits of use were determined, the plant was triturated (unprocessed and cooked) after being held in the incubator for 24 hours at 60°C, and added to the nutrient at 30-40°C. For the cooking process (32), 25 minutes was applied. Due to being added to the nutrient and feeding of the insect, the powdered beans (0.21 g) were added to 100 ml of warm food after weighing.

All of the experiments were carried out under conditions where the stock culture of insects was grown. From the culture, the female and male individuals (3 virgin/1 male) were taken for the trial design, and they were mated and the eggs were collected after six hours. *D. melanogaster*'s newly hatched larvae (1st stage larvae) were floated in water (taking on blotting paper) as indicated in the experimental pattern, and the larvae were grown up to the adult stage by inoculating on the test nutrients with the fine-tipped brush. Fat containing nutrients were used as a positive control. The larvae of the *D. melanogaster* were grown up to the adult stage with the nutrients indicated in the trial pattern.

Biochemical Analyses

25 female and 25 male adults (33) were used for each trial in the biochemical analysis. Samples were extracted with 1.15% potassium chloride in an ultrasonic homogenizer with a cold homogenization buffer (1.15% potassium chloride, 25 mM dipotassium hydrogen phosphate, 5 mM ethylenediamine tetra acetic acid, 2 mM phenethylylsulphonyl, 2 mm dithiillotenitol, pH: 7.4) at + 4°C. The samples were stored at -80°C until analysis was carried out.

MDA, the final product of LPO, was measured according to the method used by Jain and Levine (34). GST (EC 2.5.1.18) determination was made according to the method developed by Habig et al. (35). Total protein determination was made according to the Folin-Lowry method to calculate the MDA levels and enzyme specific activity from the obtained supernatants (36).

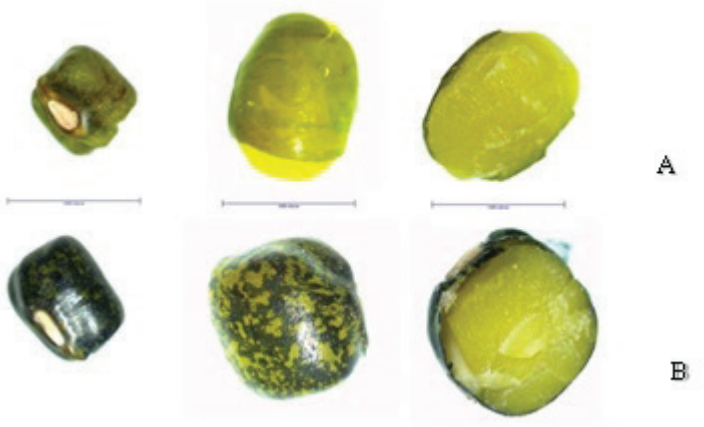

Figure 1. Commercial (A) and non-commercial/local (B) Mash beans (1000 μm).

Table 1. Malondialdehyde (MDA) quantity and glutathione S-transferase (GST) activity in female and male fed with Mash beans

Diets	MDA (nmol/mg protein)		GST (nmol/mg protein/min)	
	Female (Mean* ± S.E)†	Male (Mean* ± S.E)†	Female (Mean* ± S.E)†	Male (Mean* ± S.E)†
Control (0.0§)	0.43±0.22[b]	0.73±0.03[c]	6.03±0.24[a]	14.83±0.52[b]
NC-MB cooked	0.28±0.01[b]	1.60±0.04[d]	31.08±0.76[d]	14.99±0.50[b]
C-MB cooked	0.41±0.01[b]	0.72±0.01[c]	30.04±1.01[d]	4.35±0.26[a]
NC-MB unprocessed	3.44±0.01[e]	2.11±0.04[e]	23.60±0.81[c]	14.53±0.87[b]
C-MB unprocessed	1.77±0.02[d]	4.15±0.05[f]	46.94±0.64[e]	22.94±0.23[c]
NC-MB cooked+ HFD	0.14±0.04[a]	0.43±0.01[b]	10.38±0.84[b]	28.59±0.56[d]
C-MB cooked + HFD	1.5 ±0.10[c]	4.47±0.05[f]	7.79±0.25[a]	12.63±1.09[b]
NC-MB unprocessed + HFD	0.12±0.04[a]	0.41±0.01[b]	11.85±0.35[b]	10.16±0.42[b]
C-MB unprocessed + HFD	3.32±0.12[e]	0.24±0.01[a]	5.44±0.77[a]	30.68±1.00[d]

*Four experiments were tested, and 25 females and 25 males were used in each trial, † Values in the same column with different lowercase letters (a to f) were significantly different by LSD test; b was statistically different from a ($p < 0.05$), c was significantly different from a ($p < 0.01$), d was different from c ($p < 0.05$), e was very significantly different from d ($p < 0.001$), f was very significantly different from e ($p < 0.001$). §Control, NC-MB: Non-commercial/local Mash bean, C-MB: Commercial/local Mash bean, HFD: High-fat diet (20% palmitic acid).

Data Analysis

The data were analysed by the statistics package program (SPSS Inc., Chicago, IL, USA). One-way analysis of variance (ANOVA) was used to evaluate data on MDA quantity and GST activity. "LSD test" was used in comparison of diets because the "Duncan's multiple range test" result was homogeneous. Mann Whitney U test was used to determine the changes occurring between male and female adult stages. The significance of the means was evaluated at the 0.05 probability level.

RESULTS

The amount of LPO formed by cooked NC-MB and C-MB in adult female individuals is similar to the negative control, whereas fat supplementation showed that NC-MB reduced LPO (Table 1). It was determined that the amount of MDA increased in the females fed with unprocessed NC-MB and C-MB, and that the unprocessed fatty NC-MB decreased the amount of MDA (Table 1). It was observed that the GST activity of cooked beans increased by about 5 times compared to the control, and that the use of fat increased GST activity by about 4 times compared to the control (Table 1).

Although the activity of GST increased in the cooked beans, it was determined that the resistance was decreased when used with fat, and non-resistant individuals were formed. However, the use of Malatya-origin cooked beans with fat proved its usefulness as it contained low MDA content and GST activity.

The use of unprocessed beans with fat reduced GST activity by more than half. The beans used with the addition of fat reduced the resistance of females, and the beans used without the addition of fat increased the GST activity.

In male subjects, the amount of MDA between control negative and control positive was similar to the negative control with the use of cooked C-MB, but this ratio was increased by 2-fold in the NC-MB and increased by 4-fold and 8-fold in unprocessed products (Table 1). However, the use of beans together with fat decreases the amount of MDA in male subjects. Nonetheless, the use of unprocessed C-MB seeds with fat or fat-free does not differ much in terms of LPO (Table 1). It was observed that there was an inversely proportional relation to the increasing amount of MDA in males and that GST activity decreased in controls, and individual non-resistance was formed. While the GST activity of the cooked NC-MB was statistically similar to the control, it was observed that GST activity decreased in C-MB. This decline can be said to be due to the low amount of MDA. However, when fat was added, the GST activity of insects that eat NC-MB decreased and the resistance of insects that eat C-MB fatty beans increased (Table 1).

LPO of female and male individuals was compared according to their nutritional contents. Fat diet caused oxidation in both, but the female individuals were more affected (Figure 2). Unprocessed NC-MB increased the amount of MDA in females compared to males. In C-MB, it was observed that males had more LPO. In the cooked NC-MB and C-MB, the amount of MDA in females was less than that in males (Figure 2). It was observed that the addition of fat in unprocessed C-MB caused less oxidation in females, the use of cooked NC-MB with fat increased the amount of MDA of males while the amount of MDA in females was higher in the use of fat C-MB (Figure 2).

The use of Mash bean differed in the antioxidant activity of male and female individuals. The combined use of only fat and cooked NC-MB revealed a statistical similarity in males and fe-

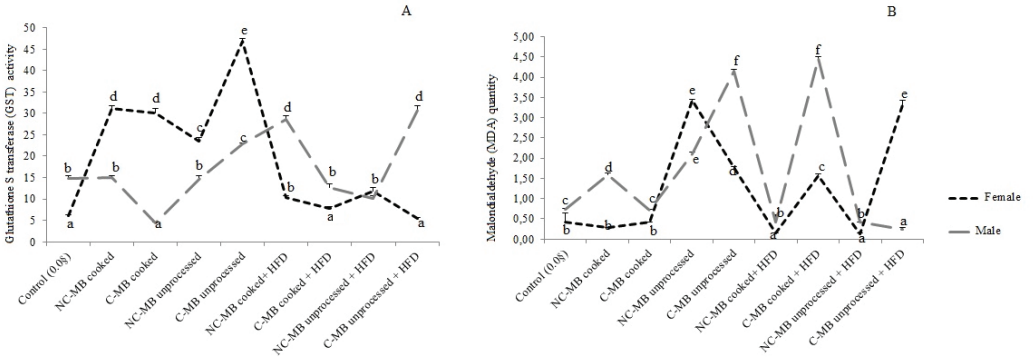

Figure 2. Comparison of A) Glutathione S-transferase (GST) activity and B) Malondialdehyde (MDA) quantity in male and female individuals fed with Mash bean. For each diet, significant differences are indicated by different lowercase letters (a to f) over the line graphs by Mann Whitney U test; b was statistically different from a ($p < 0.05$), c was significantly different from a ($p < 0.01$), d was different from c ($p < 0.05$), e was very significantly different from d ($p < 0.001$), f was very significantly different from e ($p < 0.001$). Control, NC-MB: Non-commercial/local Mash bean, C-MB: Commercial/local Mash bean, HFD: High-fat diet (20% palmitic acid).

males. However, when fat was not involved in the nutrition, it was determined that antioxidant activity made female individuals more resistant than males, and the GST activity of males was more effective in fat use (Figure 2).

DISCUSSION

It is stated that Mash bean is an important component of the intestinal flora due to the high rate of resistant starch in its content (37,38). Mash bean sprout is known to have antimicrobial activity against the gastroduodenal diseases in humans, the immune stimulatory effect of the seed, antioxidant, anti-inflammatory, and anti-diabetic activity, while the mungin protein isolated from the seeds has an antifungal effect (39-45). Globulin-rich, Mash bean, reduces fat accumulation and fatty liver in rodents induced for obesity by HFD (46). Against liver and kidney damage, Mash bean seeds have hepatoprotective and nephroprotective activity (47). It has a protective effect against alcohol-induced liver damage (48). Mash bean, known for its detoxification properties, has been used to reduce the sensation of swelling, superficial infections, acne, eczema, dermatitis and itching (48). It is known that Mash bean soup, cooked in the traditional way in Asian countries, has a protective effect against heat stress (26), lowers LDL cholesterol with its antioxidants, has a protective effect on DNA, and has anti-cancer and liver protective effects (32,49,50).

Mash bean shows anti-lipid peroxidation activity and superoxide anion cleansing activity (28). It is seen in the literature that Mash bean has significant changes in nutritional and anti-nutritional properties by cooking and germinating (51). Mash bean significantly increased antioxidant enzymes such as superoxide dismutase (SOD) and catalase in rats (52). *Vigna mungo* shell extract provides protection against the oxidative stress caused by hydrogen peroxide in DNA and erythrocytes (53).

Similarly, in our study, It was determined that Mash bean increased GST activity of female insects compared to control. Antioxidants in Mash bean reduce inflammation by regulating cholesterol levels, cleaning free radicals, and reversing the damage to blood vessels (32,50). The effect seen in our experiment is thought to be due to this feature. In addition, Vitexin and Isovitexin (43,54,55), which are two types of flavonoids with high free radical cleansing activity in Mash bean, have approximately 70% inhibition of SOD radicals (56) supports our study. In a study of Liyanage et al. (32), it was observed that the cooking process increased the soluble fiber content of Mash bean and its hypocholesterolemic potential in vivo, preserved the hypoglycemic potential, and decreased total phenolic and flavonoid content and antioxidant activity. It is known that male GST activity is better than females because of the significant sex-dependent differences with low basal levels and egg production (57). When the beans were cooked, in male individuals fed with and without fat, continuing the feeding with C-MB decreased the GST activity. Although other studies have suggested that Mash bean can be used in the development of food supplements for patients with hypercholesterolemia, according to our findings, it is necessary to adjust the amount of use according to sex and the way of the feeding of the individual. In addition, the non-commercial or commercial origin of a product or even the conditions of cultivation of herbal products may lead to changes in the antioxidant activity in vivo. As in this study, C-MB seeds showed generally limited antioxidant activity compared to NC-MB seeds. Differently, Wang et al. (58), reported that the cooking process significantly increased the dietary fiber content (dry weight basis) in beans but had no effect on iron, zinc and phytic acid contents. The content of soluble dietary fiber in boiled Mash beans is higher than that of raw Mash beans (59). When these results are considered in terms of digestibility, it has been shown that Mash bean which is added to the nutrient prepared with the mashed potatoes, which provide the carbohydrate and fat intake, provide the insects with more nutritive carbohydrates. Soluble dietary fibers are known to significantly reduce bad (LDL) cholesterol (60). The fact that LPO is lower in cooked Mash beans compared to unprocessed beans in our study is similar to the results of previous studies (cooked LPO <unprocessed LPO). Increased soluble dietary fiber in cooked beans is believed to in-

crease the LPO inhibition of Mash beans. It is also seen that LPO is different depending on gender (30). In this study, Mash bean in female individuals achieved similar results compared to control (excluding NC-MB unprocessed only), whereas in females fed with fat diet, C-MB increased LPO (Table 1). Similar results were found in the study which are related to fat diet and gender (3). In our study, feeding of male individuals with a fat diet and fat-free diet change the amount of LPO. Nutrition with HFD increased MDA, protein carbonyl levels in liver, heart and kidney tissues in obese rats and decreased the activity of GST and paraoxonase enzymes (61). It has been reported in the studies that flies fed with HFD have an increase in triglyceride levels fat similar to that of mammals, and exhibit changes in insulin/glucose homeostasis (62). In our study, besides the use of C-MB and NC-MB Mash beans, the effect of fat and fat-free nutrition was also examined. In the individuals fed with HFD, C-MB generally increased the amount of MDA in comparison to NC-MB and control. This has proven that the local beans were more reliable in terms of consumption compared to the commercial ones. NC-MBs have proven to be more reliable in terms of consumption than C-MBs. As a result, nutrition with HFD increased the amount of MDA in vivo as in other studies. However, non-commercial beans generally used in the fatty diet reduced the amount of MDA produced.

CONCLUSIONS

It is determined that the cooking process applied to the mash bean and its raw (unprocessed powder) usage was effective on the fatty and non-fat diet, and the insect changes the oxidant antioxidant activity depending on the gender. Continuing feeding of the females regardless of the origin of unprocessed Mash beans increased antioxidant activity in a fat-free diet and caused a change in the activity of GST, which is one of the determining factors for resistance. Regardless of the processing, the use of the non-commercial product caused the LPO to fall in the individuals fed with the fat diet. A similar effect was observed in male subjects.

As a result, the fat intake and cooking process in males decreased oxidation, and the opposite was observed in female individuals. However, it was determined that females became more resistant to increasing GST activity. In the comparison of commercial and non-commercial products, NC-MB has been shown to be more useful than C-MB by reducing oxidation. According to these results, it is observed that NC-MB has a positive effect on both insect mechanism and oxidant-antioxidant activity. Thus, the use of the Mash bean as a beneficial nutritional supplement was determined by looking at the effect of our model resembling the vertebrate organisms. It is likely that our study will serve to guide the advancing studies based on nourishment for the correct use of unconsciously used alternative medicine products.

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Conflict of Interest: The authors declare that they have no conflicts of interest to disclose.

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