Effect of *Amaranthus cruentus* seeds on oxidative status in plasma and selected tissues of rats fed with high doses of fructose

Paweł PAŚKO[1], Henryk BARTOŃ[1], Paweł ZAGRODZKI[1,2], Shela GORINSTEIN[3], Zofia ZACHWIEJA[1], Maria FOŁTA[1], Mirosław KROŚNIAK[1] , Małgorzata GAWLIK[4], Maciej GAWLIK[4]

[1]Department of Food Chemistry and Nutrition, School of Medicine, The Jagiellonian University, 9 Medyczna Street, 30-688 Kraków, Poland

[2]Henryk Niewodniczański Institute of Nuclear Physic, Department of Nuclear Physical Chemistry, 152 Radzikowskiego Street , 31-342 Kraków, Poland

[3]Department of Medicinal Chemistry and Natural Products, School of Pharmacy, The Hebrew University - Hadassah Medical School, Jerusalem 91120, Israel

[4]Department of Toxicology, School of Medicine, The Jagiellonian University, 30-688 Kraków, Poland

Abstract

Oxidative stress plays an important role as a mediator of damage produced by fructose. This work was designed to investigate the effect of amaranth seeds on oxidative stress in plasma, hearts, kidneys and pancreas of fructose-administered rats. Fructose administration (310g/kg fodder for 5 weeks) caused oxidative damage that was manifested by the increase in plasma malondialdehyde (MDA) and by the decrease in the enzymatic antioxidant capacity in plasma and selected tissues. Co-administration of amaranth seeds (310 and 155g/kg fodder) restored the activities of some enzymes. It also influenced the oxidative stress as was evidenced by decreasing MDA and increasing FRAP in plasma, and the activities of antioxidant enzymes (erythrocyte superoxide dismutase – eSOD, catalase – CAT, glutathione peroxidase – GPX). The findings demonstrate that amaranth seeds, in dose dependent manner, can act as a moderate protective agent against fructose-induced changes in rats by reducing lipid peroxidation and by enhancing the antioxidant capacity.

Key words: amaranth seeds, malondialdehyde, catalase, glutathione peroxidase, supeoxide oxidase

Pseudocereals such as amaranth seeds (AM) contain remarkable amounts of antioxidant phytochemicals including phenols, flavonoids, anthocyanins, fat-soluble vitamins, fatty acids, squalene and other compounds (non-enzymatic antioxidants) which exert a protective mechanism against the oxidative stress and damage of tissues.

On the other hand, it is well known that the administration of fodders enriched with fructose to rats induces oxidative stress leading to insulin resistance, hypertriglyceridemia, heart disease and obesity (1). Therefore, in our work the experimental model with addition 31% of fructose was applied in aim to induce oxidative stress.

A number of oxygenated compounds are produced during the attack of free radicals against membrane lipoproteins and polyunsaturated fatty acids (PUFA). Malondialdehyde (MDA) is one of the aldehydes produced during this attack from PUFA. MDA may be an indicator of oxidative stress, as its plasma concentration increases in accordance with the rate of free-radical processes. The antioxidative system enables transformation of oxygen reactive forms into inactive and harmless compounds. The antioxidant enzymes produced in the body provide an important defense against free radical. Superoxide dismutase (SOD), glutathione

peroxidase (GPX) and catalase (CAT) are the most important antioxidant enzymes. SOD can selectively scavenge superoxide radicals by catalyzing its dismutation to hydrogen peroxide and molecular oxygen. Other antioxidative enzymes GPX and CAT serve to decompose hydrogen peroxide to water.

The aim of this study was to assess the influence of fructose addition (31%) and addition of amaranth seeds to fodder on the antioxidant status in selected rats' tissues and plasma.

Material and Methods

Plant material: Amaranth seeds (*Amarantus cruentus*) were cropped in eastern Poland.

Animals and diets. Groups of 6 male Wistar rats (body weight 245.7±2.8 g) were kept for 5 weeks. The groups of animals were combined into pairs: in every pair one group was fed a diet enriched with amaranth seeds, without fructose, while the second group was fed with the fodder enriched with amaranth seeds, in which 31% of starch was replaced with fructose. The fodder of control group contained corn starch instead of fructose (C; CF). Water and food intake and rats' body weight changes were monitored in the course of the experiment. Diets were formulated according to a following scheme: (compounds in constant amounts, g/kg fodder): casein 200, rapeseed oil 50, chalk 28, calcium monophosphate 29, lecithin 10, sodium chlorate 3, cellulose 50, mixture of vitamins and microelements 10 (Premix LPM, BASF, Poland); amaranth seeds were added in varied amounts, depending on the type of diet: the first group - 310 g/kg fodder (subgroups: AMH, AMHF), the second group – 155 g/kg fodder (subgroups: AML, AMLF).

Samples collection. The material used in MDA, FRAP and SOD analysis, were rats' plasma. Blood samples were taken from abdominal aorta under general anaesthetic with tiopenthal via intraperitoneal. The organs were isolated and stored at –20°C. All organs were frozen immediately after the animal was sacrificed. Before the analysis, the samples were defrozen and homogenized in phosphate buffered saline pH=7.4. In all tissues, the activity of GPX and CAT were determined.

Measurement of MDA levels. Determination of MDA levels was based on the coupling of MDA (7). Results were expressed as μmol/L plasma.

Determination of FRAP activity. FRAP (Ferric Reducing Ability of Plasma) assay was conducted at 37°C and pH 3.6. Ferric (Fe^{3+}) to ferrous (Fe^{2+}) ion reduction causes formation of intensive blue colored ferrous-tripyridyl-s-triazine complex with absorbance maximum at 593 nm. Absorbance was measured after 60 minutes and was proportional to the combined ferric reducing/antioxidant power of the antioxidants in plasma (2).

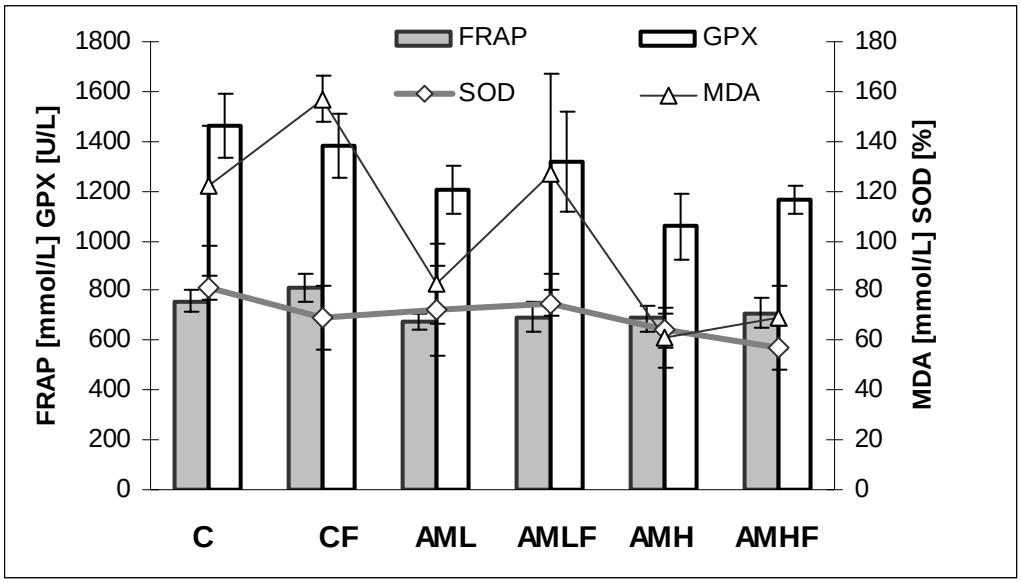
Measurement of antioxidant enzymes activity. Catalase (CAT, EC 1.11.1.6) activity was determined using the kinetic method by Aebi. Glutathione peroxidase (GPX, EC 1.11.1.19) activity was determined by the modified method of Paglia and Valentine. Superoxide dismutase (SOD, 1.15.1.1.) was determined in red blood cells (7).

Statistical Analysis. Kruskal-Wallis test was applied to check for any differences between different groups of animals. Differences with $p<0.05$ were considered to be statistically significant.

Results and Discussion.

The studies confirmed the disadvantageous effect of the administered dose of fructose upon the antioxidative system in rats' plasma. In comparison with control group (C), the fructose caused in CF group statistically significant increase in MDA level ($p<0.05$) testifying to intensified lipid peroxidation. That group also experienced a decrease by 5.6% of GPX activity in plasma, 14.5% of SOD activity in red blood cells (SODE) and increase by 7.2% of FRAP activity in plasma (changes not statistically significant) in comparison with the C group. The administration of amaranth in lower dosage did not protect plasma against peroxidation (MDA increased $p<0.05$). We also observed increase of GPX, FRAP in plasma and SODE but these changes were not statistically significant. The administration of amaranth in higher dosage protected plasma against peroxidation (MDA decreased $p<0.05$), and we observed significant decrease in SODE activity and increase in FRAP and GPX activity in plasma (still not significant) (Fig. 1).

Fig. 1 Antioxidant status of rats evaluated in plasma and erythrocytes



The values are medians±SD for 6 rats per group. C-control group, CF- control group +31% fructose, AML – lower dose of amaranth seeds, AMLF – lower dose of amaranth seeds + 31% fructose, AMH - higher dose of amaranth seeds, AMHF dose of amaranth seeds +31% fructose; MDA in mmole/L, FRAP in mmole/L, GPX in U/L; SOD in %.

There are no reports on the influence of amaranth seeds upon the antioxidative status in animals. It results from our studies that more affective is the supplementation with amaranth in higher dose. The administration of vitamin E caused increased activity of SOD (3) but amaranth seeds had a disadvantageous effect on the SODE activity. Perhaps, significant decrease in SODE activity could be associated with high content of methionine in amaranth seeds compared to normal diet, as this effect was observed previously, when administration of amino acid lowered the concentrations of Cu and Zn (4). In CF group fructose decreased not significantly the activities of CAT and GPX in kidneys (Tab. 1), and similar effect was previously observed (6). Only in rats with lower dosage of seeds fructose induced significant decrease in activity of GPX ($p<0.03$), which suggests that this dosage was not enough to prevent kidney against stress. The administration of amaranth seeds (AMHL) with fructose increased the CAT activity and decreased GPX activity in kidneys (not

significantly). There were also evidences demonstrating nephroprotective effect of polyphenolic in several experimental models associated with oxidative stress. Polyphenolic compounds, which are present in amaranth seeds have been shown to attenuate the renal dysfunction and improve the morphological renal cytoarchitecture (6).
In comparison with C group, fructose in hearts caused only not significant increase in CAT activity and not significant decrease in GPX activity. The administration of amaranth seeds (AMH) with fructose did not influence GPX activity but decreased CAT activity ($p<0.006$) and the levels of these both enzymes were significantly higher in this group than in C or CF. In the second group (AML) fructose did not cause significant changes in the activity of both enzymes. In comparison with C group, fructose in pancreases caused significant decrease in CAT activity ($p<0.04$) and not significant decrease in GPX activity. The administration of amaranth seeds (AMH) with fructose increased significantly GPX activity ($p<0.01$) and CAT activity (not significantly).

Table 1. Antioxidant enzymes activity in different tissues of rats.

GROUPS	KIDNEY		HEART		PANCREAS	
	GPX	CAT	GPX	CAT	GPX	CAT
C	0.32±0.06	19.8±3.4	0.33±0.05	3.6±0.5	0.36±0.06	1.5±1
CF	0.29±0.09	16.6±4.2	0.30±0.04	3.9±0.8	0.34±0.05	0.8±0.3
AML	0.36±0.26	22.5±2.2	0.29±0.08	6.8±0.9	0.21±0.07	0.26±0.25
AMLF	0.26±0.03	21.3±3.4	0.27±0.14	5.0±2.1	0.20±0.02	0.38±0.2
AMH	0.29±0.14	16.4±3.3	0.37±0.05	13.5±2.8	0.20±0.02	0.4±0.4
AMHF	0.33±0.03	20.6±4.5	0.36±0.06	5.2±3.1	0.32±0.07	0.9±0.5

The values are medians±SD of 6 rats per group. GPX - activity of glutathione peroxidase – GPX [U/10 mg protein], CAT- activity of catalase [U/g protein], C - control group, CF- control group +31% fructose, AML – lower dose of amaranth seeds, AMLF – lower dose of amaranth seeds + 31% fructose, AMH - higher dose of amaranth seeds, AMHF – higher dose of amaranth seeds +31% fructose.

In conclusion: The administration of amaranth seeds reduced peroxidation of lipids and changed the activity of antioxidative enzymes in plasma and selected organs in dose dependent manner. Heart tissue had less antioxidant enzyme activity compared to the liver (5), therefore it may be more sensitive to prooxidative damage. Our results suggest that amaranth seeds could be a good additives to the common diet and could for some extent protect heart against free radicals.

References

1. Basciano H., Federico L., Adeli K. Fructose, insulin resistance and metabolic dyslipidemia. Nutrition & Matabolism, 2005, 2, 5.
2. Benzie I.F. Strain J.J. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. Anal. Bioch,1996, 15, 70-76.
3. Błaszczyk I., Grucka-Mamczar E., Kasperczyk S., Brikner E. Influence of fluoride on rat kidney antioxidant system: effect of methionine and vitamin E. Biol. Trace Elem. Res. 2008, 121, 51-59.
4. Patra R.C., Swarup D. Effect of antioxidant ascorbic acid, l-methionine or a tocopherol alone or along with chelator on cardiac tissue of lead –treated rats. Veterinarski Archiv. 2004, 74, 235-244.

5. Saravanan R., Pugalendi V. Impact of ursolic acid on chronic ethanol-induced oxidative stress in the rat heart. Pharmacological Reports, 2006, 58, 41-47.
6. Wongmekiat O., Leelarugrayub N., Thamprasert K. Beneficial effect of shallot extract on cyclosporine nephrotoxicity in rats. Food Chem Tox. 2008, 46, 1844-1850.
7. Zagrodzki P., Joniec A., Gawlik M., Gawlik M., Krośniak M., Fołta M., Bartoń H., Paśko P., Chłopicka J., Zachwieja Z.: High fructose model of oxidative stress and metabolic disturbances in rats. Part I. Antioxidant status of rats' tissues. Bull Vet Inst Pulawy 2007, 51, 407-412.