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Effect of amaranth seeds in diet on oxidative status in plasma and selected tissues of high fructose-fed rats ☆

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ABSTRACT

The aim of this study was to assess the influence of amaranth seeds in different doses, under conditions of oxidative stress induced by dietary fructose, on antioxidant status of selected rat tissues, erythrocytes and plasma. Fructose administration caused oxidative stress that was manifested by the increase in plasma malondialdehyde and by the decrease in the enzymatic antioxidant activity. Co-administration of amaranth seeds influenced the oxidative stress, as was evidenced by decreasing malondialdehyde in plasma and changing the activities of antioxidant enzymes (erythrocyte superoxide dismutase, catalase, and plasma glutathione peroxidase). Our findings demonstrate that amaranth seeds can act as a moderate protective agent against fructose-induced changes. Our results suggest that the antioxidative system of plasma, heart and lungs is more efficient when amaranth seeds are present in the diet.

1. Introduction

Several studies found that the administration of fodders enriched with fructose to rats induced oxidative stress leading to hypertriglyceridemia, insulin resistance and obesity (Tappy, Le, Tran, & Paquot, 2010). It was demonstrated that in rats fed with high-fructose diet, the concentration of free radicals was three times higher than in the control group. Moreover, fructose addition brought about lowering of vitamin E level, which could be the reason of body defence impairment against free radicals. The consumption of fructose in humans has been increasing for long time, but there is little evidence that fructose could influence carbohydrate and lipid metabolism, which is associated with metabolic abnormalities in humans and animals (Isganaitis & Lustig, 2005; Tappy et al., 2010).

A number of oxygenated compounds are produced during the attack of free radicals against membrane lipoproteins, proteins and polyunsaturated fatty acids (PUFA). One of them is malondialdehyde (MDA) which can be used as an indicator of oxidative stress, as its concentration in plasma increases as the result of free-radical processes. The antioxidative system enables transformation of reactive oxygen species (ROS) into inactive and harmless compounds. Natural antioxidant enzymes [superoxide dismutase (SOD), glutathione peroxidase (GPX) and catalase (CAT)] provide primary defence against ROS. SOD can selectively scavenge a superoxide radical by catalysing its dismutation to hydrogen peroxide and molecular oxygen, while GPX and CAT serve to decompose hydrogen peroxide to the unreactive species.

In vitro studies indicated that dietary antioxidants can protect against oxidative damage in some tissues (Farombi, Hansen, Ravn-Haren, Møller, & Dragsted, 2004; Pasko et al., 2010). Amaranth seeds (*Amaranthus cruentus*) contain significant amounts of antioxidant phytochemicals, including flavonoids, phenolic acids, squalene, fat-soluble vitamins, fatty acids, trace elements and other compounds with antioxidant properties (Tikekar, Ludescher, & Karwe, 2008; Paśko et al., 2009). The antioxidant potential of compounds of pseudocereal seeds, however, has not been fully investigated in an animal model or in humans. Until now, to the best of our knowledge, only one study related to the influence of amaranth on antioxidative status in animals has been published (Escudero, Zirulnik, Gomez, Mucciarelli, & Gimenez, 2006).

In recent years the determination of oxidative status in plasma and selected tissues has become one of common laboratory

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methods in experimental models, as it can yield valuable information with respect to the hierarchy of different enzymatic and non-enzymatic responses in tissues, as well as to various pro- or antioxidative nutritional factors. It is possible because usually only several grams, or even lesser amounts, of individual animal organ are required for such analysis. The aim of this study was to assess the influence of amaranth seeds in different doses, under conditions of oxidative stress induced by dietary fructose, on antioxidant status of selected rat tissues, erythrocytes and plasma.

2. Methods and materials

2.1. Plant material

Amaranth seeds (*Amaranthus cruentus*) were cropped in the eastern Poland (Tomaszów Lubelski).

2.2. Preparation of rat fodders

Diets were formulated according to a scheme given in Table 1. The main diet constituents (cornstarch, fructose and amaranth seeds) were added in varied amounts, depending on the type of diet. The amounts of the common compounds were given in the legend to Table 1. In our previous papers we found amaranth extract contained the following compounds with antioxidant properties: gallic acid (440 mg/kg dw), *p*-hydroxybenzoic acid (8.5 mg/kg dw), and vanillic acid (15.5 mg/kg dw) (Pasko et al., 2009; Paśko, Sajewicz, Gorinstein, & Zachwieja, 2008).

2.3. Animals

Male Wistar rats (mean weight 245 ± 7 g) were purchased from the Animal House of Jagiellonian University. The rats were housed in metal–plastic cages (three animals per cage) and kept in an air-conditioned animal-room at a temperature of 22 ± 2 °C, with a relative humidity of 50 ± 5%. The animal-room was on a 12 h daily lighting-period cycle and the rats were kept for 5 weeks. The rats had unlimited access to fodder and tap water. The protocols for animal experiments were approved by the Animal Experimentation Committee of Jagiellonian University.

2.4. Sample collections and analytical procedure

Blood samples were taken from aorta under general anaesthesia following intraperitoneal thiopental injection. The organs were isolated and stored at −20 °C. The material used in analysis was rats' plasma for MDA, FRAP (Ferric Reducing Ability of Plasma) and GPX3, and erythrocytes for SOD (eSOD). In all tissues, the activity of cytosolic glutathione peroxidase (GPX1) and CAT were determined as well. The samples of organs (ca. 1 g) were homogenised in 5 ml ice-cooled phosphate buffer pH 7.4 before analysis and centrifuged at 6000*g* for 15 min at 4 °C. Parameters (FRAP, MDA, catalase, glutathione peroxidase, superoxide dismutase) and methods of their determination were essentially the same as in our previous paper (Zagrodzki et al., 2007).

Table 1
Fodder ingredients (g/kg).

Group	Name of group	Corn starch	Fructose	Material
C	Control group[a]	620	0	–
CF	Control group[a] + fructose	310	310	–
AMH	Amaranth seeds	310	0	310
AMHF	Amaranth seeds + fructose	0	310	310
AML	Amaranth seeds	465	0	155
AMLF	Amaranth seeds + fructose	155	310	155

Each fodder contained (in g/1000 g): 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 – vitamins and minerals).

[a] With additional amount of potassium (K_2SO_4 – 3.5 g) and magnesium (MgO – 0.7 g) in cost of cellulose content.

2.5. Statistical procedure

Values are given as mean ± standard deviation (SD). The statistical analysis of biochemical parameters were conducted using the Statistica 6.1 PL software (StatSoft Inc.). A type of distribution for analysed variables was determined by chi-square test. A variance homogeneity was analysed using Hartley test. In order to compare mean values "one way ANOVA" test was applied. The critical significance level was set as $p < 0.05$. Kruskal–Wallis test was applied to check for statistical evaluations of antioxidant parameters, followed by Dunn post hoc test.

3. Results

3.1. Body weight, fodder and water consumption

The effects of fructose and amaranth seeds on body weight of rats were summarised in Table 2. Final weight did not differ within the pairs of groups. Mean food intake decreased after 5 weeks in non-fructose-fed rats by 8.5–9.5%, whereas in fructose-fed rats (F) it decreased by 7.4–22.5% following the amaranth content (see Table 2). The mean water intake generally decreased, but no clear rule could be formulated for this effect. There were no statistically significant differences between groups.

3.2. Antioxidant status parameters

In comparison to the control group (C), the fructose caused significant increase in MDA level by 15% in the control group with fructose (CF) group. In the aforementioned group, we observed insignificant effects: decrease by 6% of GPX3 activity in plasma, 14% activity of SOD in red blood cells (eSOD) ($p < 0.08$) and increase (9%) of FRAP in plasma in comparison to the C group (Fig. 1).

MDA was decreased significantly by addition of amaranth in both dosages, $p < 0.01$ and $p < 0.001$ for low and high level respectively. We also observed significant decrease in the GPX3 activity in both doses of amaranth seeds ($p < 0.01$). After addition of a lower dose of amaranth seeds fructose increased FRAP value by 13% ($p < 0.01$), while in a higher dose this effect was attenuated. eSOD activity was decreased in case of higher amaranth dose by 21% ($p = 0.03$), while lower dose did not affect eSOD (Fig. 1).

The effects of fructose and amaranth seeds administration on the GPX1 and CAT activity in tissues were summarised in Table 3 while the symbolic summary of enzymatic changes in all organs was presented in Table 4.

4. Discussion

Until now most of the studies on diets enriched in fructose have been focusing on effects induced by very high content of dietary fructose. In animal studies fructose was a source of 45–66% of the energetic value of fodder and in human studies the value even rose to 90% (Hellerstein, 2002). In our study, the addition of 31% fructose was applied in order to induce an oxidative stress.

Weight did not differ within the pairs of groups of animals (Table 2). The same effect was observed in similar experiment (Girard et al., 2006) when the rats received 60% fructose in their diet.

Table 2
Weight of rats and fodder and water intake.

Group	Weight Initial	Weight gain (g)	Weight gain (%)	Fodder intake 1st week (g/day)	Change (5th week) (%)	Water intake 1st week (g/day)	Change (5th week) (%)
AMH	252.3 ± 7.1	63.8 ± 12.0	25.3 ± 4.8	16.0	−9.5	18.4	4.1
AMHF	249.6 ± 10.5	66.3 ± 7.5	26.6 ± 3.7	19.9	−22.5	21.3	−5.1
AML	250.3 ± 11.3	68.8 ± 9.3	27.6 ± 4.5	16.7	−9.3	17.5	−8.3
AMLF	250.2 ± 10.3	61.2 ± 11.5	24.4 ± 3.7	16.1	−11.5	16.0	10.4
C	237.8 ± 4.2	85.2 ± 23.9	36.0 ± 10.6	18.1	−8.5	16.8	−1.2
CF	234.5 ± 8.0	70.5 ± 23.0	30.2 ± 10.4	17.1	−7.4	18.2	−14.6

Values are given as mean ± SD for six animals. C: control group; CF: control group with 31% of fructose; AML: group fed diet with amaranth seeds (155 g/kg fodder); AMLF: group fed diet with amaranth seeds (155 g/kg fodder) and with 31% of fructose; AMH: group fed diet with amaranth seeds (310 g/kg fodder); AMHF: group fed diet with amaranth seeds (310 g/kg fodder) and with 31% of fructose.

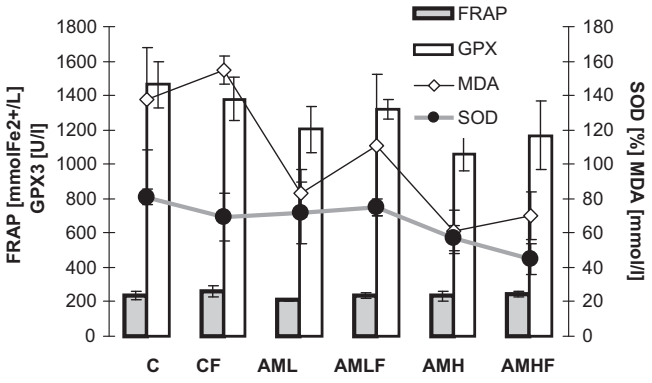

Fig. 1. Antioxidant status of rats evaluated in plasma and erythrocytes*.*. C: control group; CF: control group with 31% of fructose; AML: group fed diet with amaranth seeds (155,360 g/kg fodder); AMLF: group fed diet with amaranth seeds (155 g/kg fodder) and with 31% of fructose; AMH: 361 group fed diet with amaranth seeds (310 g/kg fodder); AMHF: group fed diet with amaranth seeds (310 g/kg 362 fodder) and with 31% of fructose 363.

Busserolles, Gueux, Rock, Mazur, and Rayssiguier (2002) showed that feeding rats with a diet containing 34% fructose induced oxidative stress. In our study, the fructose in the CF group caused significant increase in MDA level, in comparison to the control group, testifying to intensified lipid peroxidation. The administration of amaranth seeds decreased MDA level. This observation, with amaranth seed treatment in both doses, suggests lowered lipid peroxidation against oxidative stress induced by fructose. Similar effects were observed in rats treated with blackcurrant juice (Farombi et al., 2004).

We observed a significant effect of fructose increasing FRAP only in the lower amaranth dose group. However, the same trend was observed in the other groups. It is known that over half of FRAP activity arises from antioxidant activity of uric acid. The observed changes in FRAP can thus be interpreted as a result of increased concentration of uric acid caused by fructose in all groups.

We also observed an insignificant increase in the GPX3 activity in groups fed amaranth seeds with fructose, in comparison to animals fed amaranth seeds without fructose (Fig. 1). In another study, activity of GPX3 in serum was increased by a particular Chinese medicinal formula when the oxidative stress was induced by acute ischaemic myocardial injury (Qin et al., 2009). As FRAP is considered to reflect the cumulative action of all antioxidants present in plasma, no apparent changes in FRAP could be connected with medium antioxidant activity of amaranth seeds in comparison to other good antioxidant plant sources, which induced the increase of total antioxidant capacity of serum (Qin et al., 2009).

Amaranth seeds in higher doses showed decreasing effect on the eSOD activity (Fig. 1), in contrary to vitamin E, and another antioxidant compounds which caused increases in SOD activity (Bouderbala, Lamri-Senhadji, Prost, Lacaille-Dubois, & Bouchenak, 2008; Błaszczyk, Grucka-Mamczar, Kasperczyk, & Brikner, 2008). Perhaps a decrease in SOD activity could be associated with high content of methionine in amaranth seeds compared to a normal diet. This can be tentatively deduced from other studies (Patra & Swarup, 2004) as the administration of methionine reduced the concentration of Cu and Zn in the heart. Possibly the same changes can play a similar role in red blood cells, because the majority of erythrocyte superoxide dismutase is a zinc- and copper-dependent enzyme.

Fructose did not modify GPX1 and CAT activity in hearts, as compared with group C. Amaranth seeds at both doses caused an increase in CAT activity, especially strong (225%) in the higher dose. These results show that the presence of fructose in fodder co-administration of amaranth seeds preserved changes in the activities of the examined enzyme in our study. A similar observation was made by Saravanan and Pugalendi (2006) when the oxidative stress was induced by chronic administration of alcohol, or by isoprenaline (Kumar & Anandan, 2007) – strong stress inducers – which further caused decreasing of activity of antioxidant enzymes. Other studies by the same authors have shown that during oxidative stress, the addition of some plants rich in antioxidants compounds, could improve activities of free radical scavenging enzymes. Bouderbala et al. (2008) observed changes in activity of both aforementioned enzymes in hearts, after *Ajuga iva* treatment. This plant enhanced GPX1 activity in hypercholesterolemic rats in comparison to the untreated group, but did not influence CAT activity in this organ. Increased activity of enzymatic antioxidants (especially CAT activity), observed in our study, demonstrates that amaranth seeds added to rats' fodder in the higher dose can improve protection of heart against free radicals attack. Saravanan and Pugalendi (2006) concluded from their studies that heart tissue exhibited lower antioxidant enzyme's activities in comparison to the liver. Subsequently, the same authors suggested that the heart may be less vulnerable to oxidative damage when additional protection is induced by ursolic acid.

The antioxidant/oxidant balance of the liver was not influenced significantly by fructose. A similar effect was observed in hypercholesterolemic rats (Bouderbala et al., 2008). This result suggests that liver has a stronger capacity than blood or other organs to reduce fructose-induced lipid peroxidation.

Administration of amaranth seeds in both doses caused significant decrease in activity of CAT and increase of GPX1 activity. Bouderbala et al. (2008) observed the same effect in CAT activity after *Ajuga iva* treatment and explained it as the stimulation of the liver CAT activity against oxidation. Regarding our results, this stimulation can be assigned to significant increase in GPX activity. Oxidative damage in liver caused by the strong inducer of oxidative stress, carbon tetrachloride studied by Mehmetcik, Özdemirler, Kocak-Toker, Cevikbas and Uysal (2008) were found to be attenuated by artichoke extract. This effect can be associated with hepatoprotective effect of this plant against oxidative stress.

Table 3
Effect of fructose and amaranth seeds in different doses on glutathione peroxidase (GPX1) and catalase (CAT) activity (U/g protein) in selected organs.

	C	CF	AML	AMLF	AMH	AMHF
Heart						
GPX1	34 ± 11.1	33.3 ± 6.9	28.8 ± 8.2	30 ± 8.9	37.4 ± 5.1	38.1 ± 6.2
CAT	4.2 ± 1.5	3.9 ± 0.8	6.6 ± 0.9[a]	5.7 ± 2.1	13.7 ± 2.8[b]	5.9 ± 3.1
Liver						
GPX1	11.7 ± 0.8	12.7 ± 2.5	36.9 ± 4	35.2 ± 11.1	100.5 ± 71	89.1 ± 62
CAT	116 ± 11.3	121 ± 16.9	85.8 ± 13.2	89.2 ± 12.7	79.7 ± 18.7	84.9 ± 20.7
Kidney						
GPX1	31.1 ± 6.2	32.3 ± 9.7	48 ± 26.4	25.8 ± 3.5	36.3 ± 14.1	34.4 ± 3.7
CAT	16.8 ± 6.3	21.2 ± 6.7	22.5 ± 2.2	22.3 ± 3.4	17.5 ± 3.3	19.8 ± 4.5
Testis						
GPX1	26.8 ± 2.2	26.3 ± 3.9	15.6 ± 6.6[a]	15.3 ± 6.1	29.3 ± 10.2	19.3 ± 2.9
CAT	1.3 ± 0.2	1.7 ± 0.3[c]	0.9 ± 0.3[a]	0.9 ± 0.3[d]	1.8 ± 0.4	1.3 ± 0.4
Lungs						
GPX1	29.1.±5.1	25.3 ± 3.7	35.9 ± 16.7	30.9 ± 4	36.6 ± 16.7	41 ± 18.1
CAT	3.5 ± 1	3.8 ± 0.6	11.3 ± 3.1[e]	10.9 ± 2.2	14.3 ± 5.4[f]	13.6 ± 4
Pancreas						
GPX1	36.3 ± 6.6	34 ± 5.5	22.5 ± 7.8	21.3 ± 2.9	21.1 ± 2	33.5 ± 7.6[g]
CAT	2.3 ± 1.1	1.6 ± 0.5	0.4 ± 0.3	1.1 ± 1.2	06 ± 0.5	0.8 ± 0.5
Spleen						
GPX1	61.6 ± 4.4	63.9 ± 5	33 ± 12.1[e]	39.5 ± 7.4	22.3 ± 5.6[f]	26.8 ± 7.2
CAT	6.9 ± 2.1.	8.1 ± 1.8	22.2 ± 6[e]	26.4 ± 10.5	8.7 ± 1.4	10.2 ± 2.6

Values are given as mean ± SD for six animals. C: control group; CF: control group with 31% of fructose; AML: group fed diet with amaranth seeds (155 g/kg fodder); AMLF: group fed diet with amaranth seeds (155 g/kg fodder) and with 31% of fructose; AMH: group fed diet with amaranth seeds (310 g/kg fodder); AMHF: group fed diet with amaranth seeds (310 g/kg fodder) and with 31% of fructose.

[a] C vs. AML, $p < 0.05$.
[b] C vs. AMH, $p < 0.05$.
[c] C vs. CF, $p < 0.05$.
[d] C vs. AMLF, $p < 0.05$.
[e] C vs. AML, $p < 0.001$.
[f] C vs. AMH, $p < 0.001$.
[g] AMH vs. AMHF, $p < 0.01$.

Table 4
Influence of fructose, amaranth seeds in lower and higher doses on activity of GPX and CAT in organs.

	Influence of fructose		Influence of amaranth in lower dose		Influence of amaranth in higher dose	
	GPX1	CAT	GPX1	CAT	GPX1	CAT
Heart	○	○	○	↑	○	↑
Liver	○	○	↑	↓	↑	↓
Kidneys	○	○	○	○	○	○
Testis	○	↑	↓	↓	○	○
Lungs	○	○	○	↑	○	↑
Pancreas	○	○	↓	↓	↓	↓
Spleen	○	○	↓	↑	↓	○

↑, increasing of enzyme activity; ↓, decreasing of enzyme activity; ○, no influence on enzyme activity.

Fructose did not modify GPX1 and CAT activity in kidneys. On the contrary, Błaszczyk et al. (2008) showed significant decrease of CAT and GPX activity when the oxidative stress was induced by sodium fluoride. A similar effect was observed in streptozotocin-induced diabetic rats (Kaleem, Asif, Ahmed, & Bano, 2006). Furthermore, our findings demonstrate insignificant increasing trend in activity of both enzymes at the lower amaranth dose. The protective role of antioxidant agents (i.e. vitamin E, vitamin C, *N*-acetylocystein, methionine, selenium, zinc) against nephrotoxicity has been previously demonstrated (Błaszczyk et al., 2008; Talas, Ozdemir, Yilmaz, & Gok, 2009; Wongmekiat, Leelarugrayub, & Thamprasert, 2008) and there were also numerous evidence demonstrating nephroprotective effect of phytochemicals (i.e. curcumin, quercetin, catechin, resveratrol) in models associated with oxidative stress (Wongmekiat et al., 2008). Bouderbala et al. (2008) showed that *Ajuga iva* decreased GPX1 activity and increased CAT activity. Previously, a positive correlation between antioxidant activity of amaranth seeds and total phenolic contents was demonstrated (Pasko et al., 2009) and it is known that these seeds have various phenolic acids and flavonoids (Pasko et al., 2010). Future works on this aspect are warranted because modification of dosing could change antioxidative status in kidneys.

It is well known that CAT is necessary for decomposition of the toxic product; hydrogen peroxide, produced during the course of anaerobic metabolism of spermatozoa (Medan et al., 2008). Significant increase of activity of CAT in our study could be a part of defence system in testis against the oxidative stress induced by fructose. On the other hand, fructose is a part of semen (Melis, 1999) and its content in diet can positively influence the antioxidative status of testis. Türk et al. (2007) observed a similar effect in sperm of rats, when only a high dose of pomegranate juice significantly increased sperm GPX activity in comparison to the control group. But even low, middle and high doses of pomegranate juice increased CAT activity in sperm. In our experiment, amaranth

seeds in lower dose with or without fructose had no positive influence of antioxidant status of testis, as we observed significant decrease in activity of CAT and GPX. Higher dose of amaranth seeds improved antioxidant status in comparison to the control group.

Fructose did not significantly influence the activity of both antioxidant enzymes in lungs. Strong carcinogen *n*-nitrosodiethylamine caused a decrease in CAT activity (Mittal, Brar, & Soni, 2006), and similarly 7,12-dimethylbenz[a]anthracene caused significant decrease in CAT and GPX1 activity in the lungs (Talas et al., 2009). After addition of organoselenium compounds to the diet, however, the activity of both enzymes strongly increased. Our data showed that amaranth seeds caused increase in GPX1 and CAT activity and these changes were observed for both doses of seeds. Our study and studies of Hemmati, Nazari, and Samei (2008) suggest that dietary factors may play an important role in the protection of lungs against oxidative stress. However, more studies are required to verify this hypothesis.

Streptozotocin is known as a useful agent inducing oxidative stress and causing beta-cell damage in rats. This factor decreased the antioxidant enzyme (CAT, GPX, SOD) activity significantly (Coskun, Ocakci, Bayraktaroglu, & Kanter, 2004). In another study with rabbits (Czakó et al., 2004) sodium taurocholate was used to induce acute pancreatitis, and level of enzymatic antioxidants again was strongly decreased. In recent study it was observed that in diabetes mellitus oxygen-free radicals are generated by stimulation of H_2O_2 production *in vitro* as well *in vivo* in pancreatic beta cells (Kaleem et al., 2006). We observed decrease in activity of both enzymes, i.e. CAT and GPX1 in pancreas after adding any dose of amaranth seeds to the diet. Such results may be interpreted as effect of additional antioxidant protection and may be caused by amaranth constituents, rendering a decrease of stimulation of the enzymatic system of the pancreas.

In the spleen, we observed a significant decrease of GPX1 in both doses and increase in CAT in the lower dose of amaranth seeds included in the diet. Spleen cells are particularly sensitive to changes in the antioxidant status, owing to the generation of a high number of free radicals (Parthasarathy, Kumar, Manikandan, & Devi, 2006). The cells of immune system need an appropriate protection against free radicals, because they contain numerous polyunsaturated fatty acids in their membranes. After intoxication by methanol as an inductor of oxidative stress, GPX1 and CAT levels in the spleen were strongly decreased (Parthasarathy et al., 2006). Jung, Seog, Choi, Choi, and Cho (2005) presented interesting results concerning decreased levels of CAT and GPX1 in streptozotocin-induced diabetic rats, as well as a significant rise of GPX1 level after addition of wild ginseng leaves extract, while CAT activity in the spleen remained unchanged.

In the light of these data, the factors of our experiment, i.e. fructose and amaranth seeds have been proved to exhibit a weak activity in the spleen as oxidant stressor and antioxidant preventive factor, respectively.

5. Conclusion

Fructose feeding (31% w/w of diet) negatively affected antioxidant system in the plasma of rats, but in organs, generally, significant changes were not observed.

The administration of amaranth seeds reduced peroxidation of lipids and improved the activity of antioxidative enzymes in plasma and selected organs. In groups treated with *A. cruentus* seeds, the antioxidative system of plasma as well as of some organs, especially the heart and lungs, is more effective.

In conclusion, these ancient pseudocereal seeds are able to reduce the oxidative stress, and improve antioxidative enzymatic protection system, which may help to alleviate the free radicals generation during several pathological states.

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