Effect of Diet Supplemented with Quinoa Seeds on Oxidative Status in Plasma and Selected Tissues of High Fructose-Fed Rats

Pawel Pasko · Henryk Barton · Pawel Zagrodzki · Aleksandra Izewska · Miroslaw Krosniak · Malgorzata Gawlik · Maciej Gawlik · Shela Gorinstein

Abstract Oxidative stress plays an important role as a mediator of damage produced by fructose metabolism. This work was designed to investigate the effect of diet supplemented with quinoa seeds on oxidative stress in plasma, heart, kidney, liver, spleen, lung, testis and pancreas of fructose administered rats. Fructose administration (310 g/kg fodder for 5 weeks) caused oxidative stress that was manifested by the increase in plasma malondialdehyde (MDA) (p<0.05), and by the non-significant changes in the enzymatic antioxidant potential in plasma and most of tissues. Co-administration of quinoa seeds (310 g/kg fodder) maintained normal activities of some enzymes. It also influenced the oxidative stress as evidenced by decreasing MDA in plasma, and decreasing the activities of antioxidant enzymes (erythrocyte superoxide dismutase - eSOD, catalase -CAT, plasma glutathione peroxidase - pGPX). These findings demonstrate that quinoa seeds can act as a moderate protective agent against potential of fructose-induced changes in rats by reducing lipid peroxidation and by enhancing the antioxidant capacity of blood (plasma) and heart, kidney, testis, lung and pancreas.

Keywords Catalase · Glutathione peroxidase · Malondialdehyde · Oxidative status · Quinoa seeds · Superoxide dismutase

Abbreviations
CAT catalase
eSOD erythrocyte superoxide dismutase
GPX glutathione peroxidase
FRAP ferric reducing ability of plasma
MDA malondialdehyde
pGPX plasma glutathione peroxidase
SOD superoxide dismutase

Introduction

The consumption of fructose has increased considerably during the past years, but there is still little evidence about fructose influence on carbohydrate and lipid metabolism in humans and animals [1, 2].

It is known that the administration of fodders enriched with fructose to rats induced oxidative stress leading to insulin resistance, hypertriglyceridemia, heart disease and
obesity [3]. The experimental model adding 31% of fructose was applied on purpose to induce oxidative stress. A number of oxygenated compounds are produced during the attack of free radicals against membrane lipoproteins, proteins, and polyunsaturated fatty acids. Natural antioxidant enzymes manufactured in the body provide a primary defense against oxygen reactive species. SOD, GPX and CAT are the most important enzymes of the antioxidant system.

Quinoa, ancient seeds, contain significant amounts of antioxidant phytochemicals including: flavonoids, phenolic acids, fat soluble vitamins, fatty acids, trace elements, squalene, and other compounds, which could change antioxidant status in the organism [4–6]. These non-enzymatic antioxidants, compounds of quinoa seeds, can prevent oxidative stress. Until now, none of reports has dealt with the influence of quinoa seeds upon the antioxidative status in animals.

The aim of this study was to assess the influence of quinoa seeds under conditions of oxidative stress induced by dietary fructose (31%) on antioxidant status of selected rat tissues, erythrocytes and plasma. Studies using animal models are useful to investigate the actions of natural compounds.

Materials and Methods

Plant Material

Quinoa seeds (Chenopodium quinoa) were imported from Bolivia. The dry seeds were packaged in moisture-proof containers, and stored in a deep freezer (–20 °C). They were conditioned at room temperature before use. The detailed composition of the seeds was not studied in this work.

Preparation of Diet

Diets were formulated according to a following scheme: compounds in constant amounts (i.e. their amounts were the same in each diet) [g/kg fodder]: casein 200, rapeseed oil 50, chalk 28, calcium monophosphate 29, lecithin 10, sodium chloride 3, cellulose 50, mixture of vitamins and microelements 10 (Premix LPM, BASF, Poland). Two groups (C-control; CF-control with fructose) were given either standard fodder, which contained 620 g/kg corn starch or a fodder in which 310 g/kg of corn starch was substituted with 310 g/kg of fructose, respectively. In the fodder for the group “Q” 310 g/kg of corn starch was substituted with 310 g/kg of quinoa seeds, and in the fodder for the group “QF” next 310 g/kg of corn starch was substituted with 310 g/kg of fructose.

Animals

Male Wistar rats (mean weight 245.7±2.8 g) were purchased from the Animal House of Jagiellonian University. The rats were housed in metal—plastic cages (3 animal per cage) and kept in an air-conditioned animals 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.

Sample Collections

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 analyses was rat plasma for MDA, FRAP and GPX, and erythrocytes for SOD (eSOD). In all tissues, the activity of GPX and CAT was determined as well. The samples of organs (ca. 1 g) were homogenized in 5 mL phosphate buffered saline pH=7.4 before analysis.

Analytical Procedure

Parameters (FRAP, MDA, catalase, glutathione peroxidase, superoxide dismutase) and methods for their determination were essentially the same as in our previous paper [7].

Statistical Procedure

Values are given as mean±standard deviation (SD). Kruskal-Wallis test was applied to check for statistical evaluations using Statistica 5.1 (StatSoft, Inc., 1997), following by Dunn post hoc test. Differences at $p<0.05$ were considered significant.

Results

In comparison with control group (C), the fructose caused in CF group significant increase in MDA level ($p<0.05$) (Table 1). In CF group, we observed a decrease by 5.6% of GPX activity in plasma, 14.5% activity of SOD in red blood cells (eSOD) and insignificant increase (7.2%) of FRAP in plasma, in comparison with the C group. The administration of quinoa protected plasma against peroxidation (decrease of MDA $p<0.01$). We also observed significant increase of GPX activity (Q vs. QF), and not significant decrease in eSOD activity. We did not reveal any significant influence of
Quinoa seeds on FRAP in plasma. We did not observe any significant difference in FRAP in plasma between rats in control group, fructose group or groups with seeds, at any time. Quinoa seeds showed decreasing effect on the eSOD activity.

The effects of fructose and quinoa seeds administration on the GPX and CAT activity in tissues were summarized in Table 1.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Plasma</th>
<th>Organs</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C</td>
<td>CF</td>
</tr>
<tr>
<td>FRAP [umolFe2+/L]</td>
<td>759±45</td>
<td>814±55</td>
</tr>
<tr>
<td>GPX [U/L]</td>
<td>1463±132</td>
<td>1379±128</td>
</tr>
<tr>
<td>MDA [mmol/L]</td>
<td>122±24</td>
<td>157±9</td>
</tr>
<tr>
<td>eSOD [%]</td>
<td>81±4.8</td>
<td>69±13</td>
</tr>
</tbody>
</table>

Values are given as mean±SD of six animals. C: control group; CF: control group with 31% of fructose; Q: Chenopodium quinoa; QF: Chenopodium quinoa with 31% of fructose

a Values are statistically significant at p<0.05. eSOD [%] indicates internal unit of SOD activity estimated as a percent of inhibition of ephedrine autoxidation in the experimental conditions applied

Fructose in hearts caused only weak increase in CAT activity and slight decrease in GPX activity, as compared to C group. The administration of quinoa seeds with fructose increased only GPX activity (p<0.05 Q vs. QF). CAT activity in group QF was higher than in group C or CF, but these changes were not significant. Fructose did not influence significantly the antioxidant/oxidant balance of the liver but co-administration of quinoa seeds caused significant decrease in activity of liver CAT and GPX in comparison to the control group. Fructose caused nonsignificant decrease in GPX and CAT activity in kidneys. In rat testis, we observed only significant increase of activity of CAT after dosing fructose. Fructose did not influence significantly the activity of CAT and GPX in lungs. The GPX and CAT activity did not significantly decrease in pancreas of animals fodder with fructose. We observed an increased activity of these endogenous scavengers in pancreas after adding quinoa seeds to the diet. Fructose significantly increased only the level of GPX activity in spleen. We observed significant increase of GPX after addition of quinoa seeds to the diet too.

**Discussion**

*In vitro* studies indicate that dietary antioxidants can protect against oxidative damage in some tissues, however, the antioxidant potential of such compounds have not been fully investigated in animal model or in humans. As far as we know, no results of studies on antioxidant properties of quinoa seeds *in vivo* have yet been published.

The present study confirmed the disadvantageous effect of the administered dose of fructose upon the antioxidative system in rats plasma. In comparison with control group,
the fructose caused significant increase in MDA level testifying the intensified lipid peroxidation.

The administration of quinoa protected plasma against peroxidation. We also observed significant increase of GPX activity (Q vs. QF) and minor decrease in eSOD activity. We did not reveal any significant influence of quinoa seeds on FRAP in plasma.

Busserollos et al. [8] showed that feeding rats with diet containing 34% fructose induced oxidative stress. In our study, the fructose caused in CF group significant increase in MDA level, in comparison with control group, testifying the intensified lipid peroxidation. The administration of quinoa decreased MDA level. This observation, with Chenopodium quinoa seeds treatment, suggests lowered lipid peroxidation. Similar effects were observed in rats treated with black currant juice [9].

We did not observe any difference in FRAP in plasma between rats in control group, fructose group or groups with seeds. Possibly fructose, as being not very strong pro-oxidative agent, did not induce—in this dosage-changes of activities and concentrations of species account for this parameter. We also observed significant increase in the GPX activity (Q vs. QF). In another study, the activity of GPX in serum was increased by Guan-Xin-Er-Hao (Chinese herbal medicinal formula used for treating ischemic heart disease, containing: Salvia officinalis, Carthamus tinctorius, Paeonia lactiflora, Ligusticum chuanxiong, Dalbergia odorifera, in the proportion 2:1:1:1:1 of the dry weight) when the oxidative stress was induced by acute ischemic myocardial injury [10]. Since FRAP is considered to reflect the cumulative action of all antioxidants present in plasma, no apparent changes in FRAP could be connected with the intermediate antioxidant activity of quinoa seeds in comparison to another good antioxidant plant sources, which induced increase of total antioxidant capacity of serum [10].

Quinoa seeds showed decreasing effect on the eSOD activity, in contrary to vitamin E and another antioxidant compounds, which caused increase of SOD activity [11, 12]. Perhaps, significant decrease in SOD activity could be associated with high content of methionine in quinoa seeds compared to normal diet [13]. This could be tentatively deduced from other studies [11], as the administration of methionine reduced the concentration of Cu and Zn in heart. Possibly the same changes can play a similar role in red cells because superoxide dismutase is a zinc and copper dependent enzyme.

Fructose in hearts caused only weak increase in CAT activity and slight decrease in GPX activity, as compared to C group. The administration of quinoa seeds with fructose increased only GPX activity. Similar observations were shown by Saravanan and Pugalendi [14], where the oxidative stress was induced by chronically administration of alcohol, or by isoprenaline, which induced myocardial infarction [15]. Co-administration of quinoa seeds in our study, preserved changes in the activities of the examined enzymes. The other studies [14, 15] have also 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. [12] observed similar changes in activity of both aforementioned enzymes in hearts, after Ajuga iva treatment, which enhanced GPX activity in hypercholesterolemic rats in comparison with the untreated group, but this plant did not influence CAT activity in this organ. Increasing the activity of enzymatic antioxidants demonstrate that quinoa seeds added to rats fodder can protect heart against free radicals attack leading to oxidative damage. Heart tissue exhibited lower antioxidant enzymes activities in comparison to the liver, therefore it may be more sensitive to oxidative damage [14].

Fructose did not influence significantly the antioxidant status of the liver. These results suggest that this tissue has a stronger capacity than blood or another organs to reduce fructose induced lipid peroxidation. Similar effect was observed in hypercholesterolemic rats [12]. Co-administration of quinoa seeds caused significant decrease in activity of CAT and GPX in comparison to the control group. Bouderbala et al. [12] observed the same effect in CAT activity after Ajuga iva treatment and explained it as the stimulation of the liver CAT activity against oxidation. Mehmeteck et al. [16] observed strong influence exerted by artichoke extract on antioxidant status in liver after carbon tetrachloride induced oxidative stress, which can be associated with hepatoprotective effect of this plant. On the other hand, the decreased liver antioxidant enzymes activities observed in our study after quinoa seeds ingestion, may suggest the alleviation of oxidative stress and consequently—less stimulation of antioxidant defenses in this organ. However, the scarcity of reported results preclude any firm conclusion, and this issue needs further intensive studies to be clarified.

Fructose caused non-significant decrease in GPX and CAT activity in kidneys. Similar, but significant, observation was showed by Blaszczzyk et al. [11], where the oxidative stress was induced by sodium fluoride. The strong decrease of CAT and GPX activity in comparison to control group was also observed in streptozotocin induced diabetic rats [17]. Furthermore, our findings demonstrate significant increase in activity of CAT and non significant increase in activity of GPX in rats kidneys after addition of quinoa seeds as compared to control group. But, when we compared groups of animals foddered fructose we observed a decreasing activity of both enzymes. The protective role of antioxidant agents (i.e. vitamin E, vitamin C, N-acetylcystein, methionin, selenium, zinc)
against nephrotoxicity has been previously demonstrated [11, 18, 19], and there were also a lot of evidences demonstrating nephroprotective effect of phytochemicals (i.e. curcumin, quercetin, catechin, resveratrol) in models associated with oxidative stress [19]. Bouderbala et al. [12] showed that Ajuga iva decreased GPX activity and increased CAT activity. Future works on this aspect are warranted because the modification of dosage could change antioxidative status in kidneys.

We observed only significant increase of activity of CAT in rat testis after dosing fructose. It is known that CAT is necessary for decomposition of the toxic product—hydrogen peroxide, produced during the course of anaerobic metabolism of spermatozoa [20]. Significant increase of CAT activity in our study could be part of a defense system in this tissue against the oxidative stress induced by fructose. But, on the other hand, fructose is a part of semen [21] and its content in diet can change positively the antioxidative status of testis. Türk et al. [22] observed similar effect in sperm of rats, when only a high dose of pomegranate juice significantly increased sperm GPX activity in comparison to control group. But even low, middle and high doses of pomegranate juice increased CAT activity in sperm. In our experiment, quinoa seeds with or without fructose had a positive influence of antioxidant status of testis, as we observed significant increase in activity of CAT (C vs. Q) and not significant increase in activity of GPX.

Fructose did not influence significantly the activity of CAT and GPX in lungs. We observed only slight decrease in activity of both enzymes. In another study, 7,12-dimethylobenz[a]anthracene (a tumour factor) caused significant decrease in CAT and GPX activity, but after addition of the novel synthetic organoselenium compounds to the diet, the activity of both enzymes strongly increased, which was ascribed to strong nucleophilic (and hence—antioxidant) properties of such compounds [18]. On the other hand, previous data on grape seed extract, containing natural antioxidants, used to ameliorate the fibrogenic effect of silica, suggest that dietary factors may also play an important role in the prevention of lungs against oxidative stress [23]. Grape seeds, similarly to quinoa studied in the present work, are rich in antioxidant phytochemicals, mainly phenolic compounds, and flavanols. Since the exact and specific action for lungs mechanism has not yet been revealed, more studies are required to verify this hypothesis.

The GPX and CAT activity did not significantly decrease in pancreas of animals fodder with fructose. Streptozotocin is known as a good factor inducing oxidative stress and causing beta cell damage in rats. This factor decreased the antioxidant enzymes (CAT, GPX, SOD) activities significantly [24]. In another study with rabbits [25], sodium taurocholate was used to induce acute pancreatitis, and the level of enzymatic antioxidants was strongly decreased. In a recent study, it was observed that in diabetes mellitus oxygen free radicals are generated by stimulation of H2O2 production in vitro as well as in vivo in pancreatic beta cells [17]. For pancreatic cells is very important to have a proper level of antioxidants in order to ensure both survival of beta cells and insulin secretion capacity during periods of increased oxidative stress. We observed and increased activity of these endogenous scavengers in pancreas after adding quinoa seeds to the diet. The activities of these two enzymes were higher as compared to control group. Such results could be a sign that this pseudocereal seeds in the doses used could cause stabilization of activity of these enzymes and—to some degree—could prevent pancreas against free radical oxygen species.

Fructose significantly increased only the level of GPX activity in spleen. However, we observed significant increase of GPX after addition of quinoa seeds to the diet too. It could be advantageous for spleen and the whole immune system, as its cells are particularly sensitive to changes in the antioxidant status, owing to the generation of a high number of free radicals [26]. The cells of immune system need an appropriate protection against free radicals, because they contain a lot of polyunsaturated fatty acid in their membranes. The antioxidant/oxidant balance is especially important for function of immune cells. After intoxication by methanol as an inducer of oxidative stress, GPX and CAT levels in spleen were strongly decreased [26]. Jung et al. [27] presented interesting results concerning decreased levels of CAT and GPX in streptozotocin-induced diabetic rats, and the significant rise of GPX level after addition of wild ginseng leaves extract, while CAT activity in spleen remained unchanged.

**Conclusion**

Fructose feeding (31% w/w of diet) negatively affected antioxidant capacity in the plasma of rats but in organs significant changes were not observed. Our results are consistent with results of other studies, which indicated an increase in lipid peroxides and a decrease in antioxidant enzymes in similar conditions in spite of the matter that fructose in this dose is not a very strong pro-oxidant. The decreased activities of CAT and GPX in many tissues during fructose addition to the diet may be due to the production of reactive oxygen free radicals that can themselves reduce the activity of these enzymes. If compared the antioxidative parameters between control group and group treated with Chenopodium quinoa seeds it could be suggested that the antioxidative system of plasma and heart, kidney, testis, lung and pancreas is more effective.
when these seeds are present in the diet. These seeds are able to reduce the oxidative stress, which may help to alleviate the free radicals generation during some pathological states.

Acknowledgement This work was supported in part by the grant PBZ-KBN 094/P06/2003/08 from Polish Ministry of Science and Higher Education.

References