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Short communication/Kurzmitteilung

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A comparison of the impact of amaranth flour and squalene on plasma cholesterol in mice with diet-induced dyslipidemia

Vergleich des Einflusses von Amaranthmehl und Squalen auf Plasma-Cholesterol bei Mäusen mit diätetischer Dyslipidämie

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Summary

Amaranth was identified as a possible component of an anti-sclerotic diet. To date, particular substances responsible for this effect have not been exactly specified. Squalene, which is contained in amaranth, could be responsible for this effect. However, there are also other potential substances and the hypolipidemic effect of amaranth can be caused by a synergistic effect of several components. This study investigated and compared the impact of amaranth flour and squalene on the total cholesterol ($CHOL_{TOT}$) and LDL cholesterol ($CHOL_{LDL}$) levels in mice with dyslipidemia induced by a cholesterol- and sugar-rich diet.
The experiment included 40 inbred mice (C57Bl/6J SPF). After a 7-days acclimatization period, animals were divided into four groups by random. Individual groups were fed different diets for 49 days: control (group C), high energy diet (group HED), high energy diet with amaranth flour (group HED+A) and high energy diet with squalene (group HED+S). The sugar- and cholesterol-rich diet in HED resulted in the significant increase in the levels of $CHOL_{TOT}$ by 125% ($P < 0.05$) and $CHOL_{LDL}$ by 304% ($P < 0.05$), and at the same time in a decrease of HDL cholesterol ($CHOL_{HDL}$) levels by 58% ($P < 0.05$) compared to group C. To the contrary, amaranth flour enriched diet in group HED+A led to a decrease of $CHOL_{TOT}$ levels by 33% ($P < 0.05$) and $CHOL_{LDL}$ by 37% ($P < 0.05$), compared to HED. Both, amaranth flour and squalene, had a positive impact on $CHOL_{HDL}$ levels. Compared to group HED, there was a 47% increase in HED+A and a 60% increase in HED+S. Results proved the favorable impact of amaranth flour on the levels of total cholesterol $CHOL_{TOT}$ and also on $CHOL_{LDL}$. Furthermore, the results imply that amaranth flour contains besides squalene other substances, which can actively participate in its hypolipidemic effect.

Keywords: nutrition, mouse, hypercholesterolemia

Zusammenfassung

Amaranth wurde als mögliche Komponente einer antisklerotischen Diät identifiziert. Die für diesen Effekt verantwortlichen Inhaltsstoffe wurden bisher nicht genau spezifiziert. Squalen, das im Amaranth enthalten ist, könnte für diesen Effekt verantwortlich sein, es kommen jedoch auch andere Stoffe in Frage. Die hypolipidämische Wirkung kann auch Folge eines synergistischen Effekts mehrerer Komponenten sein. Im Rahmen dieser Studie wurde der Effekt von Amaranthmehl und Squalen auf den gesamten Cholesterinspiegel ($CHOL_{TOT}$) und LDL-Cholesterinspiegel ($CHOL_{LDL}$) bei Mäusen, bei denen mit Hilfe einer Cholesterin- und Zuckerreichen Diät eine Dyslipidämie induziert wurde, untersucht und verglichen.
Im Versuch wurden 40 durch Inzucht erzeugte Mäuse untersucht (Linie C57Bl/6J SPF). Nach einer 7-tägigen-Adaptation wurden die Mäuse zufällig in vier Gruppen eingeteilt. Den einzelnen Gruppen wurden im Zeitraum von 49 Tagen unterschiedliche Diäten verabreicht: Kontrollfutter (Gruppe C), hochenergetische Diät (HED), hochenergetische Diät mit Amaranthmehl (HED+A) und hochenergetische Diät mit Squalen (HED+S). Die Verfütterung der Zucker und Cholesterin enthaltenden Diät bei der HED-Gruppe hatte einen signifikanten Anstieg des $CHOL_{TOT}$-Spiegels um 125 % ($P < 0,05$) und des $CHOL_{LDL}$-Spiegels um 304 % ($P < 0,05$) bei gleichzeitiger Senkung des HDL-Cholesterinspiegels ($CHOL_{HDL}$) um 58 % ($P < 0,05$) zur Folge. Die Verfütte-

rung der mit Amaranthmehl angereicherten Diät bei der HED+A-Gruppe hingegen führte zu einer Senkung des $CHOL_{TOT}$-Spiegels um 33 % ($P < 0,05$) und des $CHOL_{LDL}$-Spiegels um 37 % ($P < 0,05$) im Vergleich mit der HED-Gruppe. Sowohl das Amaranthmehl als auch Squalen haben den $CHOL_{HDL}$-Spiegel positiv beeinflusst, wobei es im Vergleich mit der HED-Gruppe zu einem Anstieg um 47 % bei HED+A und um 60 % bei HED+SQ gekommen ist.
Die Ergebnisse zeigen einen positiven Einfluss des Amaranthmehls auf den Cholesterol Gesamtspiegel ($CHOL_{TOT}$) und auf das $CHOL_{LDL}$, und geben Hinweise darauf, dass Amaranthmehl neben Squalen auch weitere Inhaltsstoffe enthält, die eine hypolipidämische Wirkung aufweisen.

Schlüsserwörter: Ernährung, Maus, Hypercholesterolämie

Introduction, Material and Methods

Amaranth belongs to the family Amaranthaceae, which includes over 60 species (Kalač and Moudrý, 2000). It was grown on a large scale by Aztecs, Incas and Mayas in Latin America during the pre-Columbian era (Mendonca et al., 2009). It has been tested as a potential component of feeding mixtures for food animals due to its nutritious characteristics (Písařiková et al., 2005; Roučková et al, 2004). From the nutrition perspective, amaranth is rich in high quality proteins, whose content of amino acids (lysine, tryptophan) resembles animal proteins (Pond et al., 1991). Several studies highlighted, except for the high nutritional effect, the potential hypolipidemic effect of amaranth products (Berger et al., 2003; Czerwičsky et al., 2004; Ferreira et al., 2007; Kim et al., 2006; Shinn et al., 2004). Tests focused on grain, oil and flour, and examined the mechanism, by which amaranth products can influence the metabolism of lipids (Mendonca et al., 2009). Some studies imply that the amaranth oil fraction, especially in respect to a favourable fatty acid profile and high content of some unsaponifiable components, such as phytosterols, tocopherols and tocotrienols, can be responsible for this effect (Quereshi et al., 1996). It has been suggested that the mechanism is based on the inhibition of cholesterol absorption from intestines and its increased excretion (Berger et al., 2003; Kim et al., 2006). This theory is supported by the fact that phytosterols are in general able to influence the cholesterol absorption from intestines through a competition for available surface space with newly formed micelles (Zadák et al., 2001). Nevertheless, recent reports imply that amaranth free of the lipid moiety is also able to efficiently reduce blood cholesterol, and conclude that other components, possibly the protein fraction, are responsible for this effect (Mendonca et al., 2009; Plate and Areas, 2002). This is in accordance with the results of an experiment on Syrian hamsters (Mendoca et al., 2009). There has also been a discussion regarding the ability of squalene, which is ample in amaranth (Becker et al., 1981), to decrease pathologically elevated levels of $CHOL_{TOT}$ and $CHOL_{LDL}$. Squalene (2,6,10,15,19,23-hexamethyl-2,6,10,14,18,22-tetracosahexane) is a biologically and nutritionally important polyisoprenoid, which is a precursor in the biosynthesis of steroids, including cholesterol (Sulpice and Ferexou, 1984; Zadák et al., 2001). The hypolipidemic capacity of squalene was confirmed by a study in rats (Farvin et al., 2009), which investigated its synergic effect with simvastatin. Results imply that squalene supplementation increased the fecal excretion of cholesterol in the animals tested, and the combination of squalene and simvastatin was found to be more efficient than the individual drugs alone. Chaturvedi carried out an experiment in male Wistar rats, and also attributed the hypocholesterolemic effect of amaranth seed to squalene (Chaturvedi et al., 1993).

The present study was designed to compare the ability of amaranth flour and squalene to decrease the $CHOL_{TOT}$ and $CHOL_{LDL}$ levels in mice with experimentally induced dyslipidemia.

Animals

The experiment included 40 laboratory inbred male mice C57Bl/6J SPF (Anlab s.r.o., Prague, Czech Republic), all five weeks old and of similar weight: 19 ± 1 g. Following a week of adaptation, the animals were randomly divided into four groups of ten animals each, and kept in collective cages (five animals/cage). Their average weight at the onset of the experiment was 21.64 ± 0.72 g. All experiments were carried out in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC) and in agreement with the guidelines of the Animal Protection Law of the Czech Republic.

Environment

The experiment was carried out in the accredited laboratory of the Institute of Human Pharmacology and Toxicology at the University of Veterinary and Pharmaceutical Sciences Brno. The animals were kept in K3 cages (AnLab s.r.o. Praha, Czech Republic) sized 360 x 205 x 155 mm, provided food and water ad libitum, under standard condition, in an environment with controlled light and temperature (12:12 light dark cycle, temperature 20–24 °C, humidity 75–86%).

Feeding

During the adaptation period (7 days), mice were fed a standard feeding mixture Biostan MYPO (Biokron, Blučina, Czech Republic). During the experiment (49 days), mice were fed the following mixtures ad libitum: control feeding mixture (C) Biostan MYPO (Biokron, Blučina, Czech Republic), high energy diet (HED), high energy diet with amaranth (HED+A) and high energy diet with squalene (HED+S) (Tab. 1). The composition of feeding mixtures complied with the nutritional requirements of laboratory mice.

Used Substances

Squalene was purchased from Sigma-Aldrich (St. Louis, MO, USA). Amaranth flour was purchased from AMR Amarant a.s., (Hradec Králové, Czech Republic). The manufacturer declares the following content per 100 g of mixture: saccharides 58.2 g, fats 6 g, out of which saturated fatty acids 0.3 g and unsaturated fatty acids 1.2 g, cholesterol 0 g,

gliadin content max. 100 mg per 100 g of dry matter, fiber 15.2 g, sodium min. 21 mg, calcium min. 190 mg, magnesium min. 240 mg, phosphor min. 420 mg, iron min. 7.6 mg, copper min. 0.7 mg, zinc min. 2.8 mg, vitamin B6 min. 0.2 mg, vitamin C min. 3.7 mg, vitamin E 0.7–1 mg, squalene 300 mg.

TABLE 1: *Composition of individual diets used during the experiment*

Diet components g/100 g	Group			
	C	HED	HED+A	HED+S
Biostan MYPO*)	100	68.5	48.5	67.3
Cholesterol	0	1.5	1.5	1.5
Sugar	0	30	30	30
Amaranth flour	0	0	20	0
Squalene	0	0	0	1.2

*) Composition (wt/wt): 5.4% fat, 53.8% carbohydrate, 21.9% protein, 2.9% fibre, 6.6% minerals, added vitamins A, D, E, and 0.02% cholesterol (357kcal per 100g)

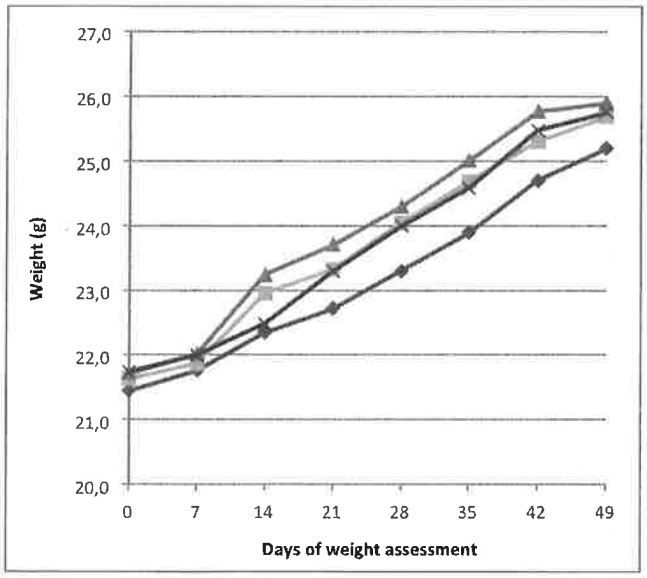

FIGURE 1: *Body weight of individual groups during the experiment ◆ = control, ■ = HED, ▲= HED+A, × = HED+S.*

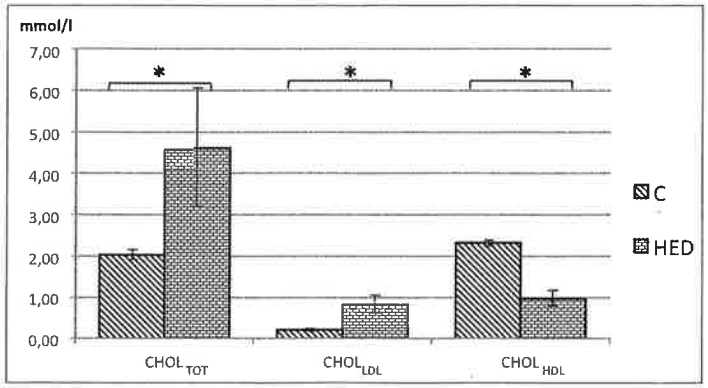

FIGURE 2: *Effects of control (C) and high energy diet (HED) administered for 49 days on levels of $CHOL_{TOT}$, $CHOL_{LDL}$ and $CHOL_{HDL}$. Values are means ± standard deviation, * $P < 0.05$, significantly different from C group.*

Laboratory Methods

The weight of the mice was monitored on day 0, 7, 14, 21, 28, 35, 42 and 49 of the experiment. The health condition was monitored by adspection during the experiment. On the 49th day of the experiment, after a 12 hours period of fasting animals were sacrificed under total anesthesia by exsanguination from heart. The animals were anesthetized (0.2 ml per 10 g of body weight) by a mixture containing 1% solution of ketamine (Narkamon® inj., Zentiva, Prague, Czech Republic) and 2% solution of xylazine (Rometar® inj., Zentiva Prague, Czech Republic) in the ratio 1:20. Blood was collected and serum was obtained by centrifugation at 1500 g for 15 min. Serum $CHOL_{TOT}$, $CHOL_{LDL}$ and $CHOL_{HDL}$ were determined by enzymatic colorimetric kits of the StarDust-MC 15 photometer (Bio-Vendor, Brno, Czech Republic).

Statistical Analysis

Statistical analysis of the data was performed using the R package software, version 2.10.1 (R Development Core Team, Vienna, Austria, 2009). Grubb's test was used to detect outliers. Statistical significance was tested using the two sample Welch's test. Statistical significance was determined at the levels of $P < 0.05$ and $P < 0.01$.

Results and Discussion

Body weight and feed intake

The body weight of all groups is illustrated in Figure 1. The mice gained weight throughout the study. Growth rates in all groups were similar. Mean baseline body weight of the tested mice was 21.63 g, and it reached 25.56 g at the end of the experiment. Mean feed intake for all groups was similar with 4.5 ± 0.2 g/mouse.

The Effect of High Energy Diet on Serum Lipids

Testing was carried out under conditions, which allowed an objective assessment of the effect of particular feed mixtures on the selected indicators of lipid metabolism. No death was observed in animals during the experiment; one mouse was killed because it had too large teeth and refused to eat.

A mixture containing cholesterol and sugar was applied to induce pathological changes in lipid levels in animals. The high energy feeding for 7 weeks (HED) led to a significant increase in $CHOL_{TOT}$ by 125%, ($P < 0.05$) and $CHOL_{LDL}$ by 304%, ($P < 0.05$), with a significant decrease in $CHOL_{HDL}$ levels by 58%, ($P < 0.05$) compared with C group (Fig. 2).

Effect of Amaranth Flour and Squalene on Experimentally Induced Dyslipidemia in Mice

To evaluate the effect of amaranth flour and squalene on dyslipidemia in mice, amaranth flour and squalene were added into the feed mixtures. As demonstrated in Figures 3 and 4, amaranth flour enriched feeding mixture (HED+A) significantly decreased the levels of $CHOL_{TOT}$ by 33%, ($P < 0.05$) and $CHOL_{LDL}$ by 37%, ($P < 0.05$). The decrease of $CHOL_{TOT}$ levels by 5% in the group fed squalene enriched diet (HED+S) was not statistically significant; to the contrary, the level of $CHOL_{LDL}$ slightly increased (by 10%). The use of amaranth as well as squalene had a positive impact on the levels of $CHOL_{HDL}$. The levels of $CHOL_{HDL}$ increased in the group HED+A by 47% ($P < 0.05$) and in the group HED+S by 60% ($P < 0.05$) (Fig. 3, 4 and 5).

Non-pharmacological treatment of dyslipidemia is still an integral part of the dyslipidemia therapy. Its advantages are inexpensiveness, safety and the possibility to combine it with a standard pharmacological treatment. Due to their potential hypolipidemic effect amaranth products seem to be well-suited for such a treatment. The hypolipidemic effect of amaranth and the possible mechanism, by which different amaranth products (usually seed, oil, grain) influence the metabolism of lipids, were subject to various studies. Literature screening showed that different products from amaranth can have a different impact on the metabolism of cholesterol. Amaranth flour can easily be applied to the preparation of feeding mixtures for animals or it could be used as an ingredient in the preparation of a hypocholesterolemic diet for humans. Amaranth flour however was not systematically used as a subject for the testing of its hypocholesterolemic potential.Therefore we wanted to study the effect of amaranth flour in hypercholesterolemic mice. Mixture containing cholesterol and sugar was applied to induce pathological changes in lipid levels in animals. As we expected, the high energy diet administered for seven weeks led to significant negative changes in serum lipids and led to dietary induced dyslipidemia. As shown in Figure 2, we found both $CHOL_{TOT}$ and $CHOL_{LDL}$ plasma levels significantly increased in HED group, with concurrent $CHOL_{HDL}$ levels significantly decreased when compared to control ($P < 0.05$). This is in accordance with data of others, who have found a nutritionally induced hypercholesterolemia in experiments on animal (Plate and Areas, 2002; Kishida et al., 2002).

Addition of amaranth flour into the feeding mixture was supposed to verify its hypocholesterolemic effect. After 49 days of investigation, we observed that the diet supplemented with amaranth flour was able to decrease the levels of $CHOL_{TOT}$ and $CHOL_{LDL}$, and at the same time increase the levels of $CHOL_{HDL}$ (Fig. 3, 4, 5). Many authors agreed on the ability of amaranth flour to positively influence the levels of $CHOL_{TOT}$ and $CHOL_{LDL}$. Similar results were obtained in a study in hamsters (Berger et al., 2003), which tested amaranth grain and oil, which had a positive impact on the levels of $CHOL_{TOT}$ as well as $CHOL_{LDL}$, and in an experiment carried out in rats (Grajeta, 1997) fed A. cruentus seed, which also resulted in a decrease of total cholesterol levels. Nevertheless, available studies have not achieved a general agreement on the ability of amaranth products to positively influence the levels of $CHOL_{HDL}$. Experiments in hamsters (Berger et al., 2003) and rats (Grajeta, 1997) did not confirm the ability of amaranth products to statistically significantly increase the pathologically low levels of $CHOL_{HDL}$. Even though the ability of amaranth to have a positive impact on the $CHOL_{HDL}$ fraction was indicated in an experiment in white rats (Chaturvedi et al., 1993), other authors (Czerwinsky et al., 2004) were less definite too and pointed out the necessity of further investigation into this effect.

Squalen was assumed to have a comparable impact on plasma lipids as amaranth flour. Squalene is an important inter-stage in the cholesterol biosynthesis. Studies confirmed that squalene is able to stimulate the activity of acyl-coenzyme A: cholesterol acyltransferase (ACAT), and at the same time is a feedback inhibitor of hydroxymethylglutaryl coenzyme A-reductase HMG-CoA (Strandberg et al., 1989). Therefore, squalene, due to its high content in amaranth (Lehman et al., 1994), can be considered one of the substances, which actively participate in the hypolipidemic effect of amaranth. A study in rats fed on cholesterol-rich diet (Shinn et al., 2004) tested the hypocholesterolemic effect of amaranth grain, oil and squalene. Both amaranth grain- and amaranth oil-enriched diet lowered serum and hepatic cholesterol and triglyceride levels in the tested animals. The experiment also demonstrated the hypolipidemic effect of injected squalene on both serum and hepatic cholesterol. Authors suggested that the cholesterol lowering effect of amaranth squalene can be caused by increased fecal elimination of steroids through the interference with cholesterol absorption. There was an interesting finding, that squalene from a shark liver did not have any of the above-described effects. This implies that different squalene sources can have different impacts on cholesterol metabolism, which complies with the data obtained during our experiment that did not reveal a statistically significant decrease of $CHOL_{TOT}$ (Fig. 3), whereas the increase in $CHOL_{LDL}$ and $CHOL_{HDL}$ levels did achieve statistical significance (Fig. 4, 5).

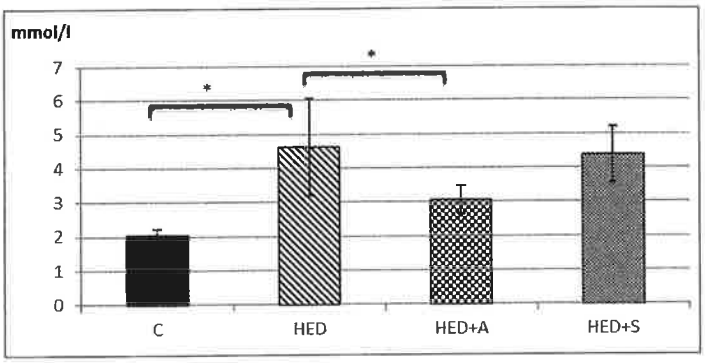

FIGURE 3: *Effects of experimental diets administered for 49 days on $CHOL_{TOT}$ levels. Values are means ± standard deviation, * $P < 0.05$, significantly different from C or HED group.*

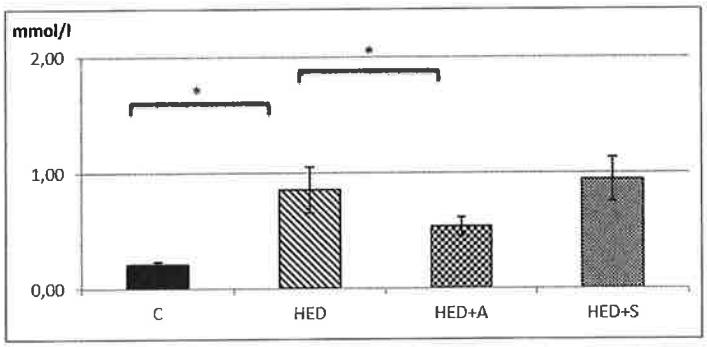

FIGURE 4: *Effects of experimental diets fed for 49 days on $CHOL_{LDL}$ levels. Values are means ± standard deviation, * $P < 0.05$, significantly different from C or HED group.*

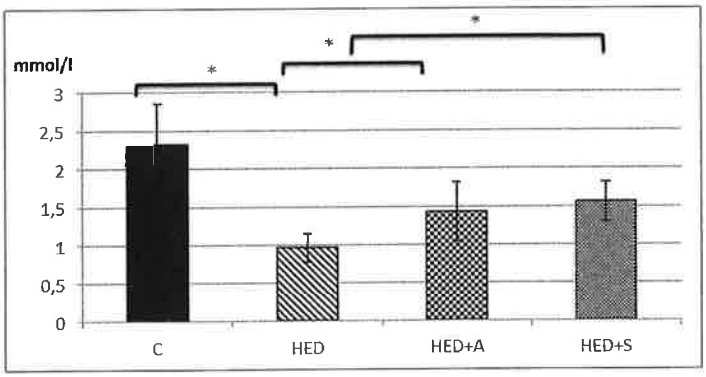

FIGURE 5: *Effects of experimental diets fed for 49 days on $CHOL_{HDL}$ levels. Values are means ± standard deviation, * $P < 0.05$, significantly different from C or HED group.*

Results obtained through statistical analysis of weight increments in individual groups did not indicate problems concerning nutritional value of amaranth flour. This finding is in accordance with literal sources that also showed that amaranth products can form an adequate component of feed mixtures (Acar et al., 1988)

Conclusion

This experiment confirms the impact of amaranth flour on pathologically increased levels of serum cholesterol in mice. It could be suggested, after the comparison of our results with data from available literature that amaranth contains several substances (e.g. squalen phytosterols, tocopherols, tocotrienols), which can actively participate in its hypocholesterolemic effect, and that different amaranth products (grain, oil,...) are not equally effective.

Despite discrepancies regarding the impact of amaranth flour on the levels of $CHOL_{HDL}$, amaranth flour should be further investigated as a potential active hypocholesterolemic component of diet for patients with disorders of lipid metabolism. It should be accentuated that the lipoprotein patterns of mice differ from those in humans, so we need to conduct further experiments in animals and humans to examine the ability of amaranth flour to positively affect the plasma cholesterol in animals and humans. As the experiment did not indicate problems with the nutritional value of amaranth flour, it can be recommended as a potential component of the feeding mixture for animals.

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Conflict of interest: The authors declare that no competing interests exist.

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THE EFFECT OF AMARANTH FLOUR ON PLASMA CHOLESTEROL PROFILE IN MICE WITH DIET-INDUCED DYSLIPIDAEMIA

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ABSTRACT: ***The present study was designated to investigate the effect of amaranth flour on plasma lipids profile in mice with dietary induced dyslipidemia. 50 inbred mice, males, were randomly assigned into 5 groups. Individual groups were on different diets during 49 days: Control diet (C group), high energy diet (HED), high energy diet with amaranth flour (HED+A), high energy diet with amaranth flour and simvastatin (HED+A+S), and high energy diet with simvastatin (HED+S). Addition of cholesterol and sugar (glucose) to the diet resulted in significant increase of total (TOT-C) and low-density lipoproteins cholesterol (LDL-C) with concomitant decrease of high density lipoproteins cholesterol (HDL-C) in HED group. Addition of amaranth into the feed mixture resulted in the decrease of TOT-C levels by 41% (P<0.05) and LDL-C levels by 50% (P<0.05) in HED+A group. Similar effect was observed in the HED+A+S group where TOT-C decreased by 37% (P<0.05) and LDL-C by 54% (P<0.05). Addition of amaranth into the feed mixture (HED+A group) also resulted in HDL-C level decrease by 27% (P<0.05). However, inclusion of simvastatin corrected this effect (HED+A+S group). The results showed the ability of the amaranth flour to influence positively the TOT-C and LDL-C levels. However with respect to the findings related to HDL-C the necessity of rational approach has to be emphasized.***

KEY WORDS: Amaranth, Hypercholesterolemia, Simvastatin

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RUNNING TITLE: *Amaranth flour intake and cholesterol profile*

INTRODUCTION

Increased blood cholesterol is usually associated with low density lipoproteins cholesterol (LDL-C), triglycerides (TG) and very low density lipoproteins cholesterol (VLDL-C) levels increase. Pathological LDL-C increase is a significant risk factor of arteriosclerotic vascular disease frequently manifesting with coronary heart disease. A series of studies pointed to the relationship between the incidence of coronary heart disease and cholesterol levels (Conroy et al., 2003, D´Agostino et al., 2008).

The therapy of pathologically changed levels of individual fractions of lipid metabolism is complex. It includes measures concerning lifestyle, primarily efforts to exclude risk factors such as high fat food, smoking, obesity, or de-compensated diabetes mellitus, and, in indicated cases, pharmacotherapy. Other treatment methods can be used in specialised centres, such as LDL apheresis or ileal bypass (Češka, 2005).

Decreased intake of saturated and increased intake of unsaturated fatty acids represents significant part of the prevention of cardiovascular diseases within the frame of non-pharmacological approach. Cereals and pseudo-cereals are important components of the diet of patients with hypercholesterolemia due to their high nutritional value and content of quality proteins, antioxidants, minerals and mainly tocotrienols and squalen (Gorinstein et al., 2002; Skibniewska et al., 2002).

Amaranth belongs to the family of Amaranthaceae that includes over 60 species (Kalač and Moudrý, 2000). Recently, it is investigated both due to its high nutritional value, and to its ability to affect positively pathologically changed plasma lipids levels.

Amaranth nutritional value is based upon its chemical composition characterised by relatively high protein content. Proteins are of very high quality from nutritional point of view and their amino-acid content (lysine, tryptophan) is

close to animal proteins (Pond et al., 1991). The seeds are primarily used for nutritional purposes, green parts of the plant are used to a lesser extent. Hypocholesterolemic effect of different amaranth products was studied both in animal models, and in humans. Some of the studies confirmed these effects (Plate and Areas 2002, Grajeta, 1997; Quereshi et al., 1996; Chatuverdi et al., 1993; Danz and Lupton, 1992) others not (Berger et al., 2000; Maier et al., 2000). At the same time it has not been clarified whether amaranth seed, oil, or flour are able to effect cholesterol levels by the same mechanism. Amaranth flour was chosen because it was not systematically used as a subject for the testing of its hypocholesterolemic potential and because it is easy to introduce it in a diet of both human and animal.

Simvastatin belongs to the group called 3-hydroxymethylglutaryl acetyl-coenzyme A (3-HMG-CoA) reductase inhibitors, commonly used for the treatment of dyslipidemia. Scandinavian Simvastatin Survival Study (Pedersen et al., 1994) showed that simvastatin was able to decrease TOT-C of -25%, LDL-C of -35% and HDL-C of +8% in patients with angina pectoris or previous myocardial infarction, on lipid lowering diet and suffering from moderate hypercholesterolemia between 5.5-8.0 mmol/l. This lead to a 30% reduction in the relative risk of death in the group treated with simvastatin. As the simvastin is an hypocholestrolemic agent with an influence on endogenous formation of cholesterol it would interesting to compare it with an effect of amaranth flour whose site of action could be according the study performed on rats (Takeda and Kiriyama, 1991) the intestine under the condition of dietetically induced hypercholesterolemia.

The aim of the study was to test, whether amaranth flour is capable to decrease the total cholesterol (TOT-C) and LDL-C levels in mice with experimental dyslipidemia, and at the same time to monitor the effect of individual diets on the weight of experimental animals.

We hypothesized that amaranth flour would be capable to decrease the TOT-C and LDL-C and simultaneously not decrease high density lipoproteins cholesterol (HDL-C).

MATERIAL AND METHODS

Animals

The experiment included 50 laboratory male inbred mice C57Bl/6J SPF (Anlab s.r.o. Praha, Czech Republic), of identical age of 6-7 weeks and of similar weight 19 ± 1g. The day of delivery was counted as postnatal day (PD) 0. The animals were randomized into five groups, 10 animals each, and stalled in groups of five after 1 week of acclimation. The average baseline weight at the day 0 (the beginning of the experiment) was 20,12 ± 0,42g.

Environment

The experiment took place in accredited laboratory of the Department of Human Pharmacology and Toxicology of the Veterinary and Pharmaceutical University, Brno. Animals were placed into special stall containers K/3 (AnLab s.r.o. Praha, Czech Republic) measuring 360 x 205 x 155 mm with the food access ad libitum, under standard condition, with controlled light and temperature regimen (12:12 light dark cycle, lights on 6 am, temperature 20 - 24°C, humidity 75%). SAWI Type HW 300/500 bedding was used (supplied by AnLab s.r.o. Praha, Czech Republic). All experiments were carried out in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC) and in agreement with the guidelines of the Animal Protection Law of the Czech Republic.

Diet

Mice were fed with standard feed mixture Biostan MYPO (Biokron, Blučina, Czech Republic) during the adaptation period. The mice were fed ad libitum with the following mixtures: control feed mixture (C) Biostan MYPO (Biokron, Blučina, Czech Republic), high energy diet (HED), high energy diet with amaranth flour (HED+A), high energy diet with amaranth flour and simvastatin (HED+A+S) and high energy diet with simvastatin (HED+S) during 49 days of the experiment (Table 1). Composition of the feed mixtures complied with nutritional requirements of the laboratory mice.

TABLE 1. Experiment design – composition of individual diets used during the experiment.*) Composition: cereals, side products of plant origin, milk and dairy products, minerals, L-lysine, monohydrochloride, D,L-methionine, and premix of supplementary substances.

Diet components g/100g	Arm				
	C	HED	HED+A	HED+A+S	HED+S
Biostan (MYPO*)	100	68.5	48.5	48.49	68.49
Cholesterol	0	1.5	1.5	1.5	1.5
Sugar (glucose)	0	30	30	30	30
Amaranth flour	0	0	20	20	0
Simvastatin	0	0	0	0.01	0.01

Materials

Simvastatin was purchased from Sigma-Aldrich (St. Louis, MO, USA). Cholesterol ≥ 95% (GC) was purchased from Sigma-Aldrich (St. Louis, MO, USA). Amaranth flour was purchased from AMR Amarant a.s., (Hradec Králové, Česká republika). The manufacturer declares the following composition per 100g of the mixture: carbohydrates 58.2 g, fats 6g containing 0.3g of saturated fatty acids, 1.2g of unsaturated fatty acids and 0g of cholesterol, gliadine

content maximum 100mg per 100g of dry matter, fibre 15.2 g, sodium at least 21mg, calcium at least 190mg, magnesium at least 240mg, phosphorus at least 420mg, iron at least 7.6mg, copper at least 0.7mg, zinc at least 2.8mg, vitamin B6 at least 0.2mg, vitamin C at least 3.7mg, vitamin E 0.7-1mg, squalen 300mg.

Laboratory procedures

The weight of mice was monitored on Day 0, 7, 14, 21, 28, 35, 42, and 49 of the duration of the experiment. Health status was monitored visually during the experiment. The animals were sacrificed after 12 hours of fasting in anaesthesia by heart exsanguinations on Day 49. The deep surgical anaesthesia was performed by the application of 0.2ml/10g body weight of a mixture of 1% ketamine solution (Narkamon® inj., Zentiva, Prague, Czech Republic) and 2% xylazine solution (Rometar® inj., Zentiva Prague, Czech Republic) in 1:20 ratio. Blood was collected and serum was obtained by centrifugation at 1500 g for 15 min. Samples were stored frozen. Serum TOT-C, LDL-C and HDL-C were determined by enzymatic colorimetric kits (BioVendor, Czech Republic) using an automatic photometer StarDust-MC 15 (BioVendor, Brno, Czech Republic). All samples were run in duplicate.

Statistical analysis

Statistical analysis of the data was performed by the use of the R package software, version 2.10.1 (R Development Core Team, Vienna, Austria, 2009). The design of the experiment was in favour of normal distribution of all measured parameters. Therefore it was possible to use Grubb's test for outliers to exclude potential outliers from tested data sets (α=0.05). The normality of the remaining data in each data set was confirmed by Shapiro-Wilk test (α=0.05). All used data sets were normally distributed after exclusion of few outliers. Statistical significance of differences between groups was tested using the one-tailed Student´s t- test with Welch correction for unequal variances (independent two sample t-test), and differences between two conditions were retained for P<0.05. P-values of t-tests were categorized in three intervals for better visualization of test results (* - 0.05>P>=0.01, ** - 0.01>P>=0.001 and *** - P<0.001).

RESULTS

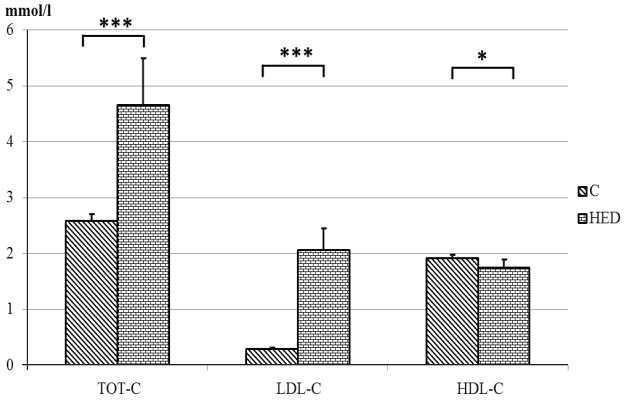
Effect of high energy diet on serum lipids

Testing was carried out under conditions that allowed an objective assessment of the effect of particular feed mixtures on selected lipid metabolism indicators. No death was observed in animals during the experiment.

Mixture containing cholesterol and sugar (glucose) was applied to induce pathological changes in lipid levels in animals. The high energy feeding for 7 weeks in C57Bl/6J mice (HED) led to a significant increase in TOT-C by 81 %, (P<0.001) and LDL-C by 612 %, (P<0.001), with significant decrease in HDL-C levels by 8,4 %, (P<0.05).

As shown in Fig. 1 cholesterol and sugar (glucose) addition to the control diet resulted in significant increase of TOT-C and LDL-C with concomitant decrease of HDL-C, which is a condition simulating dietary dyslipidemia.

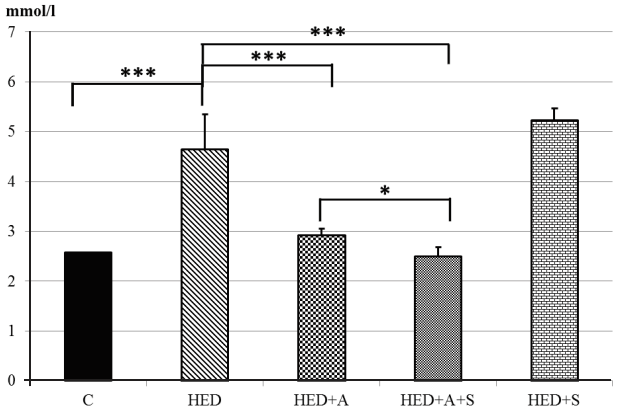
FIGURE 1. Effects of control (C) and high energy diet (HED) administered for 49 days on TOT-C, LDL-C and HDL-C levels. Values are means ± standard deviation, * - 0.05>P>=0.01, ** - 0.01>P>=0.001, *** - P<0.001; C=Control; HED=High Energy Diet



Effect of amaranth flour on experimentally induced dyslipidemia in mice

To evaluate the effect of amaranth flour on dyslipidemia in mice, amaranth flour was added into the feed mixture. As shown in Fig. 2 and 3, amaranth flour decreased significantly the TOT-C level by 37%, (P<0.001) and the LDL-C level by 46%, (P<0.001). HDL-C levels decrease was not noticed only when simvastatin was given (Fig. 4). Amaranth flour alone had not potential to influence this trend, moreover, HDL-C levels were significantly reduced by 27%when amaranth flour and simvastatin were applied simultaneously (P<0.001). Simvastatin in combination

FIGURE 2. Effects of experimental diets administered for 49 days on level of TOT-C. Values are means ± standard deviation, * - 0.05>P>=0.01, ** - 0.01>P>=0.001, *** - P<0.001; C=Control; HED=High Energy Diet; HED+A=High Energy Diet+Amaranth flour; HED+A+S= High Energy Diet+Amaranth flour+Simvastatin; HED+S= High Energy Diet+Simvastatin



with amaranth flour statistically significantly reduced both TOT-C levels by 46%; (P<0.001), and LDL-C levels by 53%; (P<0.001). On the other hand, administration of simvastatin alone did not result in positive effect on these parameters (Fig. 2 and 3). Statistical evaluation did not show additive effect of simvastatin in this experimental design (Fig. 2, 3, and 4).

Fig. 2 shows statistically significant increase of TOT-C level (HED group) after the administration of high energy diet and concomitantly its statistically significant decrease after the administration of amaranth flour (HED+A, HED+A+S).

FIGURE 3. Effects of experimental diets administered for 49 days on level of LDL-C. Values are means ± standard deviation, * - 0.05>P>=0.01, ** - 0.01>P>=0.001, *** - P<0.001; C=Control; HED=High Energy Diet; HED+A=High Energy Diet+Amaranth flour; HED+A+S= High Energy Diet+Amaranth flour+Simvastatin; HED+S= High Energy Diet+Simvastatin

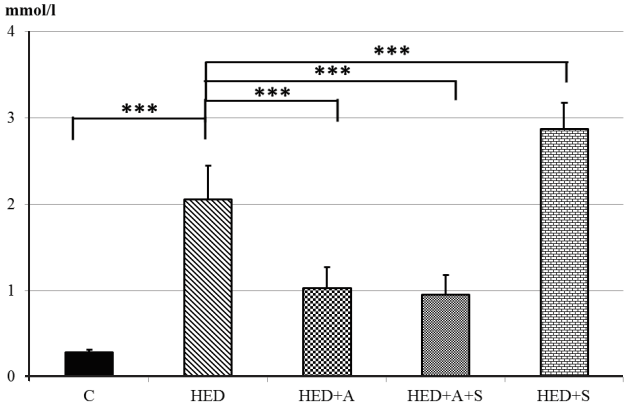


Fig. 3 shows statistically significant increase of LDL-C level after the administration of high energy diet (HED) and concomitantly its statistically significant decrease after the administration of amaranth flour (HED+A, HED+A+S).

Figure 4. Effects of experimental diets administered for 49 days on level of HDL-C. Values are means ± standard deviation, * - 0.05>P>=0.01, ** - 0.01>P>=0.001, *** - P<0.001; C=Control; HED=High Energy Diet; HED+A=High Energy Diet+Amaranth flour; HED+A+S= High Energy Diet+Amaranth flour+Simvastatin; HED+S= High Energy Diet+Simvastatin

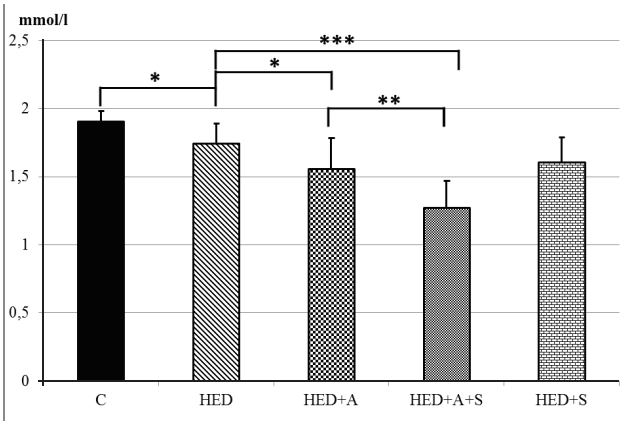
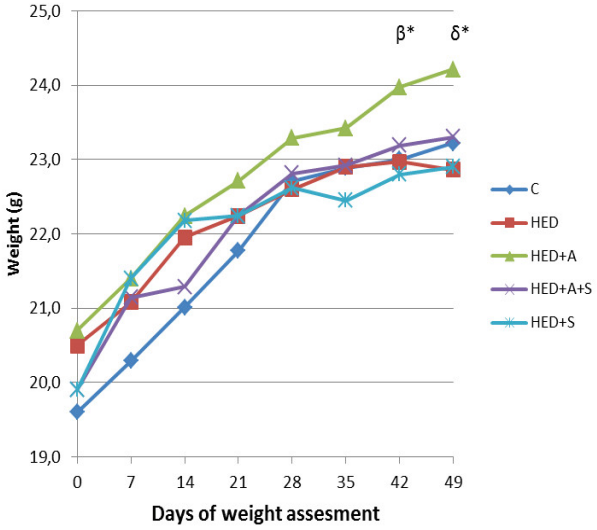


Fig. 4 shows statistically significant decrease of HDL-C levels after the administration of both high energy diet (HED), and high energy diet with amaranth flour (HED+A).

Effect of individual diets on the weight of experimental animals

Animals from individual groups were weighed to determine their body weight during the experiment on indicated days (Fig. 5).

FIGURE 5. body weight of individual groups during the experiment. * - 0.05>P>=0.01, ** - 0.01>P>=0.001, *** - P<0.001; C=Control; HED=High Energy Diet; HED+A=High Energy Diet+Amaranth flour; HED+A+S= High Energy Diet+Amaranth flour+Simvastatin; HED+S= High Energy Diet+Simvastatin; α=HED+A significantly different from C; β=HED+A significantly different from HED ; γ=HED+A significantly different from HED+A+S; δ=HED+A significantly different from HED+S



Mean baseline body weight of the tested mice was 20.12g, and it reached 23.66g at the end of the experiment. There were no statistically significant differences in the mean body weight at the beginning of the experiment. Mean body weights of individual tested groups were comparable, except of HED+A group, which showed statistically significant increase of bodyweight from HED and HED+S group between Days 42 and 49. This group showed higher mean weight from Day 21.

DISCUSSION

Dyslipidemias represent one of the most important risk factors for cardiovascular diseases. TOT-C levels, and first of all LDL-C levels are both a significant risk factor, both primary endpoint in the treatment and prevention of cardiovascular diseases. The results of non-pharmacological aggressive

treatment (i.e. lipid modification accomplished by Partial Ileal Bypass surgery) support the core significance of LDL-C decrease (Cintra and Sposito 2010; Buchwald et al., 1998).

Non-pharmacological treatment forms fundamental part of the treatment of dyslipidemias due to a series of reasons (economic efficiency, safety, usability for the whole family in the case of hereditary disorder, possibility of achieving target values without pharmacological intervention in asymptomatic persons, suitable adjuvant even for the most effective hypolipidemics). Since unsuitable high cholesterol, carbohydrates and saturated fatty acids diet belongs among significant risk factors for development of dyslipidemias (AHA, 2006), this condition was induced through diet for the purposes of the experiment, not through the use of genetically modified animals.

As we have expected the high energy diet administered for 7 weeks led to significant negative changes in serum lipids and led to dietary induced dyslipidemia. As shown in Fig. 1, we found plasma levels of both TOT-C, and LDL-C significantly increased in HED group, with concurrent HDL-C levels significantly decreased when compared to control This is in accordance with data of others, who have found a nutritionally induced hypercholesterolemia in animal experiments (Plate and Areas 2002; Kishida et al., 2002).

After 49 days of the experiment we observed that diets supplemented with amaranth flour and administered to mice were able to decrease the levels of TOT-C a LDL-C. Interestingly, amaranth flour was more effective than simvastatin to inhibit diet-induced elevation of both negative factors of atherosclerosis TOT-C and LDL-C (Fig. 2 and 3). These findings are in compliance with the study on hamsters (Berger et al.., 2003b) where the ability of amaranth products (seed and oil) to influence the levels of both TOT C, and LDL-C was recorded. Study on rats (Shinn, 2004) testing amaranth seeds and amaranth oil showed the ability of amaranth products to decrease the levels of TOT -C as well.

Administration of simvastatin alone did not result in the decrease of TOT-C and LDL-C serum levels (Fig. 2 and 3). Also, the additive effects of the combination of amaranth flour with simvastatin had not been shown in the experiment. It might be primarily due to the fact that simvastatin as 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitor influences mainly endogenous cholesterol formation, but not its exogenous intake (Daskalopoulou and Mikhailidis, 2006). These results, together with the results of the experiment performed on rats (Takeda and Kiriyama, 1991) support the hypothesis stipulating that the site of amaranth flour action is the intestine. Hypothesis concerning the ability of amaranth flour to block the cholesterol absorption in the intestine is in compliance with the study on streptozotocin induced diabetic rats where statistically significantly increased excretion of faecal cholesterol and bile acid was found (Kim et al., 2006). Study on rats also described the ability of orally administered amaranth seeds and oil to interfere with enterohepatic bile acids cycle (Shinn et al., 2004). Amaranth ability to influence cholesterol and bile acids absorption and the content of liver cholesterol was described also in the experiment on hamsters (Berger et al., 2003b). The above indicated hypothesis is supported also by data from other literal resources presenting that phytosterols generally are able to influence the absorption of cholesterol from intestine through the competition for newly formed micellae (Zadák et al., 2001). Linoleic acid, one of the four fatty acids significantly presented in amaranth, could also support recovery of faecal bile acid and thus reduce cholesterol content (Punita and Chaturvedi, 2000).

Although some studies found also positive effect of amaranth grain and oil on HDL cholesterol levels (Berger et al., 2003b), in study performed with amaranth flour in rats fed with cholesterol containing diets (Czerwińsky et al., 2004) no statistically significant increase was found. Similarly, no effect of amaranth on HDL-C levels was observed in a study performed on 6 week old female chickens (Quereshi et al., 1996). Study on hamsters also did not show any ability of amaranth to influence the HDL cholesterol levels (Berger et al., 2003a). No positive effect of amaranth flour on HDL-C levels was observed in our experiment, but statistically significant decrease of HDL-C levels was recorded (Fig. 4). These findings are in compliance with some literal data (Češka, 2005) that point out to the necessity of rational approach to the so called antisclerotic diet (e.g. addition of oleic acid). However, the ability of simvastatin to correct this development is a positive finding that is in accordance with some literature data describing the ability of statins to slightly increase the level of HDL-C (Češka, 2012). This finding further emphasizes the importance of the combination of both approaches to the treatment of dyslipidemias, both pharmacological, and non-pharmacological.

All these data suggest, as well as results obtained from a study performed on rats fed with cholesterol containing diet (Czerwińsky et al., 2004), that amaranth can form valuable part of the diet of hypercholesterolemic patients, since it is in the form of a flour (i.e. it is easy to introduce it in the diet of human) capable of positive effects on plasma lipids profile, and thus to enable also a better control of, primarily, LDL-C, and thus to contribute to complex therapy of these patients.

Results obtained through statistical analysis of weight increments in individual groups of animals did not indicate problems concerning nutritional value of amaranth flour. This finding is in compliance with literal sources that also showed that amaranth products can form adequate component of feed mixtures (Acar et al., 1988).

In the conclusion this study confirmed the potential of amaranth flour to influence pathologically increased levels of serum lipids in mice. It could be suggested, after the comparison of our results with data from available literature, that the primary site of action of amaranth flour is probably the intestine where it could inhibit the absorption of exogenous cholesterol. Based on these results, amaranth flour can be recommended for further investigations as a suitable component of dietary regimens in patients with dyslipidemias,

including those allergic to cereals. Nevertheless taking into account the finding related to HDL-C and the fact that lipid metabolism in mice differs from that in humans further experiments in animals and humans to examine the ability of amaranth flour to positively affect the plasma cholesterol in animals and humans have to be conducted.

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CONFLICT OF INTEREST DISCLOSURE

The authors stated that there are no conflicts of interest regarding the publication of this article.

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Review Article

Amaranth as a potential dietary adjunct of lifestyle modification to improve cardiovascular risk profile

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ABSTRACT

The aim of this review was to summarize data regarding amaranth as a potential component of lifestyle modification to improve cardiovascular risk profiles by modifying cardiovascular risk factors such as cholesterol, diabetes, and hypertension. PubMed was searched for appropriate articles. The main inclusion criteria for articles were as follows: interventions with amaranth; conducted in humans or animals or in vitro; and reported serum lipids and lipoprotein levels, and antidiabetic, antihypertensive, and antioxidant abilities. The outcome measures were changes in serum lipids and the presence of antidiabetic, antihypertensive, and antioxidant activity. A total of 33 articles were included herein. Regarding hypolipidemic activity, most studies investigated the effect of intervention with amaranth in animals, and fewer studies were performed in humans. Most studies in animal models demonstrated the ability of amaranth to decrease total cholesterol and low-density lipoprotein cholesterol. Pilot studies in humans were not convincing regarding amaranth's lipid-lowering activity. Based on this search, it is not clear which constituents are potentially responsible for the hypocholesterolemic effect of amaranth. Some authors tend to think that squalene can play a role in this effect, whereas others suggest that different components of amaranth are of greater importance (eg, sterols, oil fractions rich in fatty acids, proteins, amino acids, or fiber) for its hypocholesterolemic effect. It is possible that several constituents are jointly responsible for this action. Regarding the antidiabetic, antihypertensive, and antioxidant activities, most studies were performed in vitro and showed good potential for all three biological effects. Future research should focus on clarifying the effect of amaranth on high-density lipoprotein cholesterol, identifying the constituents responsible for these beneficial effects, and providing more data regarding its use in humans, ideally using randomized controlled trials. The antidiabetic, antihypertensive, and antioxidant activities found in vitro should be confirmed further in animal or human models.

Abbreviations: ACE, angiotensin-converting enzyme; apo, apolipoprotein; CVD, cardiovascular disease; HMG-CoA, hydroxymethylglutaryl coenzyme A; LDL, low-density lipoprotein; TC, total cholesterol; TGs, triglycerides; VLDL, very low density lipoprotein.

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Article Outline

1. Introduction

Total cholesterol (TC) and low-density lipoprotein (LDL) cholesterol have been identified, in both interventional and epidemiological studies [1,2], as important risk factors for cardiovascular diseases (CVDs) because cholesterol delivery is a crucial step in atherosclerosis pathogenesis. Thus, correction of dyslipidemia remains a cornerstone of CVD prevention strategies [3]. Vast data have been collected to support this notion. Meta-analysis of 170 000 patients showed that a 1-mmol/L decrease in the level of LDL cholesterol resulted in a 20% decrease in cardiovascular risk [4].

Many authors have also reviewed the role of nutrition in the prevention of CVD [5-7]. Currently, the role of dietary interventions in the prevention of CVD has been studied. One the one hand, meta-analyses performed to obtain an overall estimate of the impact of dietary modifications have not resulted in consistent outcomes. On the other hand, a healthy diet can still be recommended for all patients because it is universal, applicable to most patients, does not have any "typical" adverse drug reactions, and can be considered a component of a healthy lifestyle.

In the newest recommendation of the European Society of Cardiology and European Atherosclerosis Society for the management of dyslipidemia, lifestyle modifications are mentioned as a baseline for any treatment approach [8]. Patients should be motivated to reduce the amount of some high-risk components in their diet, especially dietary trans fatty acids [9] and saturated fatty acids [10,11]; conversely, patients should be encouraged to consume more dietary fiber (particularly the soluble type), which has a direct hypocholesterolemic effect [12], and to consume functional foods enriched with phytosterols [13,14].

The potential of amaranth to positively influence pathologically modified lipid levels, in particular, TC and LDL cholesterol, has been tested [15,16]. Generally, a consensus on the potential of amaranth products to reduce TC and LDL cholesterol was reached for animal models; however, the effect on high-density lipoprotein (HDL) cholesterol remains unclear as well as the components that are potentially responsible for its hypocholesterolemic effect. Additionally, in vitro experiments showed the potential of amaranth to influence other important CVD risk factors, namely, hypertension and diabetes. Antioxidant activity was also observed in some studies, which can provide further benefits [17-19].

1.1. *Botanical aspect and human use of amaranth*

Amaranth belongs to the pseudocereal group. Pseudocereals are plant not belonging to the grass family and produce seeds that are used to make flour. Amaranth is an annual plant, 0.9-1.8 m high. Amaranth is cultivated mostly for its seeds, which are a source of flour and oil. Amaranth is fast growing and can be used in grain crop rotation systems. Amaranth was popular in the Inca, Maya, and Aztec civilizations where it served as staple food. It has become popular among consumers because of its potential medicinal properties. Grain can be used for making breads and cakes, and the plant components can be used in salads or soups.

1.2. *Chemical composition*

The chemical composition of the amaranth grain is dependent on the species and variety as well as on climatic conditions. Amaranth seeds and leaves have higher nutritional value than other cereals. Amaranth grain also has a higher protein content compared to other cereal grains (15%) and has a significantly higher lysine content (3 times more than maize) [20]; an even higher protein content was

recorded in amaranth leaves. Amaranth grain consists of 6% to 9% oil, which is also higher than in most other cereals, with a predominance of unsaturated fatty acids (approximately 75%). Another component, which is found in greater amounts in amaranth grain than in other cereals, is fiber.

It is not clear which constituents are potentially responsible for amaranth's hypocholesterolemic effect. We shall discuss some of the concepts and their specific roles in modification of lipid metabolism.

1.2.1. *Squalene*

Amaranth is ranked among the most important sources of squalene in the plant kingdom [21]. Amaranth oil contains 6 to 8% squalene, whereas amaranth seeds contain approximately 7% of this oil [22-24]. Squalene plays important roles in many biological processes, such as the biosynthesis of steroids or coenzyme Q10, and is also an important part of cell membranes. The antioxidant properties of squalene have been demonstrated in a model of lipid peroxidation of liposomes, whereas the antidiabetic properties of squalene were tested by measurement of the inhibition of α-amylase [25]. Some suggest that squalene can also act as a scavenger of hydroxyl radicals. In a study performed in vitro in a bone marrow model, the antioxidant effect of squalene was even better than that of glutathione [26]. Interestingly, we found reduced levels of squalene in individuals hospitalized long term due to multiple traumas (ie, being in a stressful situation) and who needed parenteral nutrition [27]. Squalene has also been attributed to the ability to reduce the risk of certain cancers, such as bronchogenic carcinoma [28]. In this context, an interesting finding was observed in a study of amaranth lunasin-like peptide. This peptide can internalize into the cell nucleus faster than soybean lunasin and inhibits the chemical carcinogen-induced transformation of mouse fibroblast cell line cells [29]. Among the remaining doubts is that, according to some literature, squalene may be potentially responsible for the ability of amaranth to reduce cholesterol in blood serum via hydroxymethylglutaryl coenzyme A (HMG-CoA) inhibition, but according to others, there must be an inhibitor in amaranth other than squalene [30,31].

1.2.2. *Sterols*

Sterols are another group of substances that may contribute to the hypolipidemic effect of amaranth. The most common sterol contained in amaranth is spinasterol (50%), omega-7-stigmasterol (15%), omega-7-ergosterol (14%), and 24-methylene cycloartenol (10%) [32]. Phytosterols are normally absorbed from food in a minimal amount; they are chemical homologs of cholesterol, and the mechanism of their lipid-lowering effect is based on competition between phytosterols and cholesterol in the newly forming micelles, resulting in a reduced absorption of dietary cholesterol [27].

1.2.3. *Proteins*

It has also been observed that amaranth without its fat component is still able to positively influence the lipid spectrum. It has been inferred from this observation that the other components, such as the protein fraction, may be responsible for this effect [16,33,34]. Proteins from *Amaranthus cruentus* were further tested by Soares [35]. An enzymatic hydrolysis simulating in vivo digestion was used to prepare a protein isolate. Certain peptides were detected, and their inhibitory activity on HMG-CoA reductase was determined. The authors of this work suggested that these peptides showed enough inhibition capacity to be considered potentially bioactive substances responsible for the hypocholesterolemic effect of amaranth. Generally, this particular higher proportion of the protein component in comparison with other cereals and its amino acid composition is considered one of the greatest advantages of amaranth. The proteins in amaranth are of high quality [27]. It is alleged that the protein content may be up to twice that of other cereals [36]. The amino acid composition with a higher proportion of essential amino acids (lysine, tryptophan), approaching the quality of proteins of animal origin, is also highly regarded [37]. Because of the low content of prolamins and the absence of α-gliadin in amaranth grains, amaranth flour is suitable for patients with celiac disease. Amaranth allergies are also very rare because of the low antigenic structure of the amaranth protein.

1.2.4. *Fatty acids*

The total amount of fat in amaranth seeds can vary greatly. Generally, however, the lipid component in the seeds of amaranth is higher than that in other cereals [38]. Amaranth seed lipid components contain unsaturated and saturated fatty acids with a significant predominance of unsaturated fatty acids, including linoleic, oleic, and α-linoleic acid. The ratio of saturated to unsaturated fatty acids is approximately 1:3. The most abundant fatty acid in amaranth oil is the essential linoleic acid, which comprises approximately 40% of all fatty acids. The second highest in the oil is oleic acid (approximately 20%). Saturated fatty acids, including palmitic and stearic acid, occur in amaranth oil in low levels. [24,38-41]. Concerning the lipid component, it should be mentioned that a significantly high proportion of vitamin E (especially tocopherols) is reported [38]; however, the content is variable depending on the species of amaranth.

1.2.5. *Fiber*

Fiber is an important component of the diet that helps with proper functioning of the digestive tract. Because fiber can impair the absorption of cholesterol from the digestive tract, it is considered another possible component contributing to the hypolipidemic effect of amaranth. The fiber content in amaranth is comparable to the content of fiber in wheat and maize [42]. Amaranth fiber is of high quality (characterization of soluble and insoluble content and physicochemical properties) and is of very low gliadin content (less than 0.01% in some varieties) [43].

2. Approach

2.1. *Search strategy*

PubMed was searched for relevant studies through August 2019 using the following keywords: *amaranth, cholesterol, cardiovascular disease, risk, studies, human,* and *animal.* Articles were evaluated manually based on their abstracts. For articles in which the abstract was not sufficient, the full texts were used for making decisions.

2.2. *Inclusion and exclusion criteria*

Articles describing the effect of amaranth on cholesterol, hypertension, and diabetes were included as well as articles regarding its antioxidant activity. In vitro and in vivo studies in humans and animals were included. The included articles addressed amaranth and its potential to influence CVD risk factors. This was defined as an influence on cholesterol, hypertension, diabetes, and antioxidant activity. Articles describing only the chemical composition of amaranth and mentioning the potential of amaranth to influence CVD risk factors as a reference only were excluded as well as articles not available in English.

3. Findings

In total, 33 articles were included, 21 articles dealt with potential cholesterol effects, 8 dealt with potential antidiabetic effects, and 4 discussed antihypertensive effects. Studies testing the effect on lipids were mostly performed in animal models, and studies exploring antidiabetic, antihypertensive, and antioxidant effects were mostly performed in vitro.

3.1. *Hypolipidemic effects reported in animal and human studies*

The summarized findings for hypolipidemic effects are presented in Table 1 for animal models and Table 2 for humans. As stated previously, most published studies in animal models exceed investigations in the human, and clinical trials are lacking for amaranth in CVD.

The positive effect of extruded amaranth on TC, LDL, and very low density lipoprotein (VLDL) cholesterol and triglycerides (TG) was found in a study of hypercholesterolemic rabbits [16]. In an experiment, Kabiri et al obtained similar results [44] that in rabbits fed a high-cholesterol diet and an alcoholic extract from amaranth, TC, LDL cholesterol, TGs, oxidized LDL, apolipoprotein (apo)-B, C-reactive protein, and the atherogenic index decreased and HDL cholesterol and apo-A increased compared to the same parameters in the group fed a high-cholesterol diet only.

Similar findings regarding TC and LDL cholesterol were obtained in 2 small pivotal studies testing amaranth flour in mice [45,46]. In both experiments, a pathological state was induced by dietetic manipulation. The introduction of amaranth flour to a high-cholesterol diet led to a decrease in the levels of TC and LDL cholesterol. The effect on HDL cholesterol was not consistent and, thus, remains unclear.

In a study conducted on hamsters fed a hypercholesterolemic diet, the introduction of amaranth grain and oil led to a decrease in TC and non-HDL cholesterol and an increase in HDL cholesterol. Amaranth grain and oil also reduced VLDL cholesterol. Interestingly, amaranth oil increased the synthesis of cholesterol, possibly due to a compensatory mechanism [47]. Reduction of plasma cholesterol (mostly non-HDL) was also observed in hamsters fed a diet containing protein isolate from amaranth. The authors suggested that amaranth protein

Table 1 – Summary of amaranth studies conducted in animal models

Source	Animal	Product	TC	LDL	HDL	Non-HDL	VLDL	TG	Notes
Berger et al, 2003 [47]	Hamster	Grain and oil	↓		↑	↓	↓		↑ of cholesterol synthesis rate, ↓ of hepatic cholesterol ester
Mendonca et al, 2009 [33]	Hamster	Protein	↓			↓			
de Castro et al, 2013 [59]	Hamster	Oil							No hypocholesterolemic effect
Quereshi et al, 1996 [48]	Chicken	Seed and oil	↓	↓	0				↓ of activity of 7œ hydroxylase and 3 HMG-CoA reductase
Chmelik et al, 2013 [45]	Mouse	Flour	↓	↓	↑				
Chmelík et al, 2013 [46]	Mouse	Flour	↓	↓	↓				
Andrea, Plate, Arêas 2002 [16]	Rabbit	Extruded amaranth and oil	↓	↓	0		↓	↓	
Kabiri et al, 2010 [44]	Rabbit	Extract (alcoholic)	↓	↓	↑				↓ of apo-B, oxidized LDL, CRP, athero. Index, ↑ of apo-A
Danz and Lupton, 1992 [55]	Rat	Fiber	↓						
Chatuverdi et al, 1993 [49]	Rat	Seed	↓		0			↓	
Grajeta, 1999 [15]	Rat	Seed	↓					↓	↓ of TC in blood and liver, ↓ of TG in liver
Czerwiński et al, 2004 [50]	Rat	Meal							Positive effect on plasma lipid profile
Shinn et al, 2004 [30]	Rat	Squalene and grain	↓					↓	↓ of HMG-CoA reductase
Escudero et al, 2005 [54]	Rat	Protein concentrate	0	↓	↑				
Kim et al, 2006a [63]	Rat	Grain and oil	↓				↓	↓	
Montero-Quintero et al, 2014 [52]	Rat	Bread	↓	↓					
Lado et al, 2015 [53]	Rat	Protein	↓						

↓↑: significant effect observed by authors, 0: no effect observed by authors.

Table 2 – Summary of amaranth studies conducted in humans

Source	Product	TC	LDL	HDL	non-HDL	VLDL	TG	Other
Maier et al, 2000 [55]	Muffins	↓						
Berger et al, 2000 [57]	Seed							No hypocholesterolemic effect
Martirosyan et al, 2007 [31]	Oil	↓	↓			↓	↓	Effect dependent on concentration of squalene
Chávez-Járegui et al, 2010 [58]	Defatted snacks	0	0	↓			0	

↓↑: significant effect observed by authors, 0: no effect observed by authors.

may have an effect on endogenous cholesterol metabolism [33]. Interestingly, in an experiment on chickens, the authors reported a decrease in the activity of 3-HMG-CoA reductase. In this study, serum TC and LDL cholesterol were lowered by 10%-30% and by 7%-70% ($P < .01$), respectively, and HDL cholesterol was not affected [48].

Several studies performed in rats [15,30,49-55] showed a response that supports consensus on the hypocholesterolemic effect of different amaranth products (grain, flour, oil, and seed). In a study of amaranth squalene [30], a slight inhibition of 3-HMG-CoA reductase was observed, similar to the results of the study in chickens (Fig. 1). Amaranth grain and oil supplementation in streptozotocin-induced diabetic rats improved glucose and lipid metabolism together with lowering serum and liver lipids, such as TC, TGs, and VLDL cholesterol [51]. In a study of a protein concentrate, a positive effect was also observed on HDL with a concomitant decrease in LDL cholesterol. The authors also found a hypotriacylglycerolemic effect and concluded that the tested substance had an effect on lipid metabolism in the liver [54].

A total of 180 hypercholesterolemic human subjects were included in the clinical trials performed by Meier et al [56]. The authors observed that 28 days of dietary intervention including the consumption of 50 g of amaranth per day led to a 45% decrease in TC levels [56]. Very interesting results were obtained in a randomized placebo-controlled clinical trial of 125 patients suffering from coronary heart disease and arterial hypertension. The patients were randomized to use amaranth oil at different doses (3-18 mL amaranth oil daily). After 3 weeks of amaranth oil consumption, a significant decrease in the levels of TC, LDL, VLDL cholesterol, and TGs was reported. This effect was dependent on the concentration of squalene. HDL cholesterol was not affected [31].

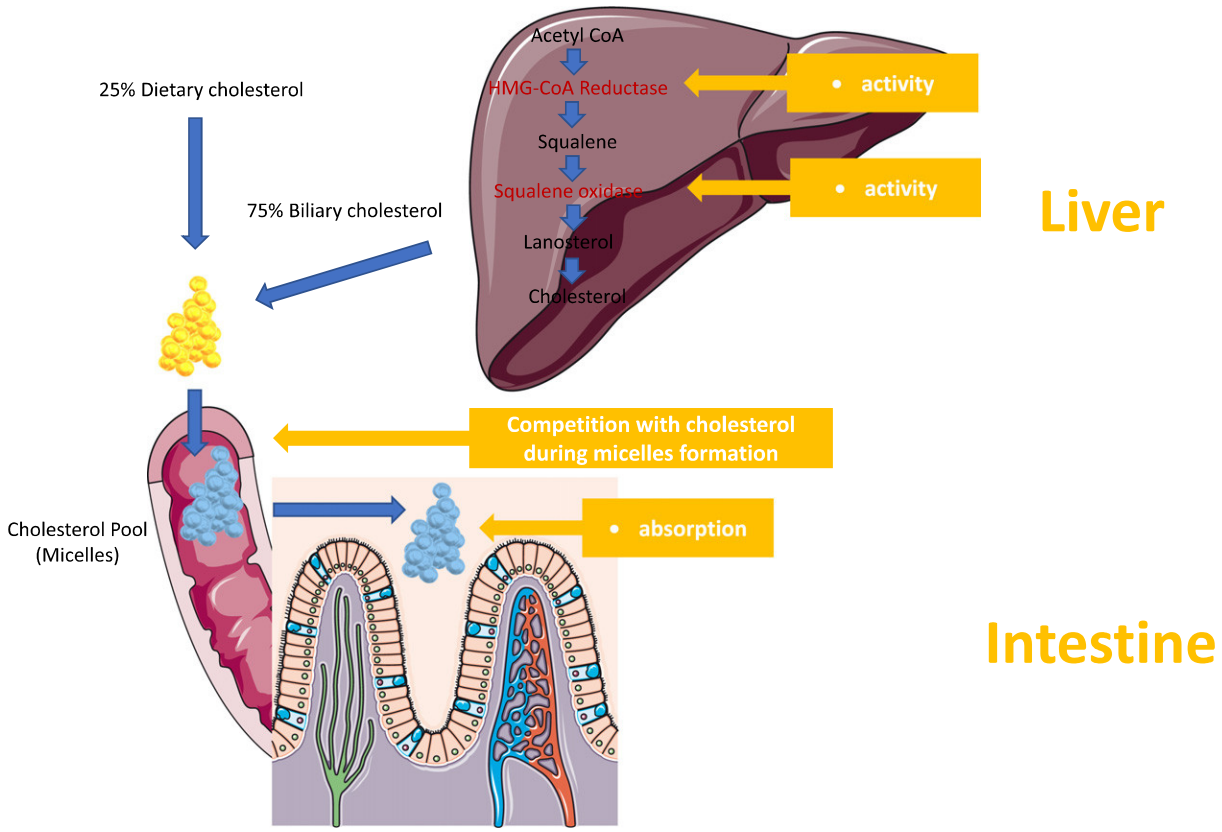

Fig. 1 – Hypocholesterolemic effect.Potential mechanisms of action for amaranth and its components are highlighted by yellow arrows. The following actions include inhibition of 3-HMG-CoA reductase activity [30,48], interference with the absorption of cholesterol [30], and inhibition of the squalene oxygenase enzyme [62].

It is important to mention a small pilot study in humans [57] in which the hypolipidemic effect of amaranth was not observed. When the effect of defatted amaranth was studied in patients with moderate hypercholesterolemia, a significant reduction in HDL cholesterol without a positive effect on LDL cholesterol was recorded [58].

Hypolipidemic effects were not evident in the experiment carried out on hamsters [59]. The findings demonstrated that amaranth oil, and its component squalene, increased the excretion of bile acids but did not have a hypocholesterolemic effect in hamsters fed a diet containing high amounts of saturated fatty acids and cholesterol.

3.2. *Active substances responsible for the hypocholesterolemic effect*

As already indicated in the section describing the chemical composition of amaranth, there are several active substances that are potentially responsible for the hypocholesterolemic effect. Taking into consideration the favorable composition of fatty acids together with the presence of other substances (phytosterols, tocopherols, tocotrienols), the authors of a study performed on 6-week-old broilers [48] suggested that the oil fraction from amaranth played an important role in the hypocholesterolemic effect. Similarly, the authors of experiments performed on rats hypothesized that the unsaturated fatty acids in amaranth can be responsible for the hypocholesterolemic effect [49]. In a study conducted by Danz and Lupton, the authors observed that rats fed amaranth showed similar hypocholesterolemic effects as those fed soluble fiber, indicating another mechanism of action [55].

Authors of experiments conducted with defatted amaranth proposed a hypothesis that other components, such as protein, are responsible for the hypocholesterolemic effect of amaranth [16,33,34,54]. Additionally, the ability of squalene, which amaranth contains in a large quantity [60], to decrease pathologically elevated levels of TC, especially LDL cholesterol, was tested. This is consistent with the results obtained in an experiment performed in rats [30] in which the observed hypocholesterolemic effect was attributed to squalene. The hypolipidemic effects of amaranth squalene were evident in both the serum and liver. Amaranth squalene also increased the fecal excretion of cholesterol and bile acid. Interestingly, a slight inhibition of 3- HMG-CoA reductase activity was observed. The authors of the study suggested that amaranth squalene lowers cholesterol via increased fecal elimination of steroids through interference with the absorption of cholesterol. The authors also noted a potential difference between sources of squalene (plant versus animal) and their influence on cholesterol metabolism. In a placebo-controlled trial performed with patients suffering from cardiovascular disease [31], a positive result after the application of amaranth oil containing various doses of squalene was reported. Amaranth oil containing 100, 200, 400, and 600 mg of squalene reduced TC by 14%, 17%, 20%, and 12%, respectively, and LDL cholesterol by 19%, 23%, 25%, and 12%, respectively. The authors noted that the role of squalene (especially the dose of squalene) in cholesterol reduction has not yet been completely clarified. Dietary supplementation with 1 g of squalene daily for 9 weeks in 18 male subjects caused increases in VLDL and LDL cholesterol concentrations by 34% and 12%, respectively, and the subsequent 6-week period on lower doses of squalene supplementation (0.5 g daily) normalized serum sterols [61].

According to some authors, the potential mechanism of action might be connected to the effect on the inhibition of squalene biosynthesis, particularly the inhibition of the squalene oxygenase enzyme, which was identified as a potential target for hypocholesterolemic therapy. There can be 1 or more active substances with this effect in amaranth [62]. This can explain the results from a study performed with amaranth squalene in rats in which the authors concluded that the effect of amaranth from squalene was different from that of squalene from sharks [30]. The potential mechanisms of action are illustrated in Fig. 1.

3.3. *Antidiabetic and antioxidant effects*

Antidiabetic activity was tested in a study performed by Kim et al in streptozocin-induced diabetic rats [63]. The inclusion of amaranth grain resulted in decreased blood glucose and increased serum insulin levels. The antidiabetic and antioxidant effects of amaranth products (2 varieties of *Amaranthus caudatus* seeds: var. Oscar Blanco and var. Victor red) were tested on a model of α-amylase inhibition and on a model of lipid peroxidation of liposomes. The inhibition of α-amylase blocks the hydrolysis of glycosidic bonds in digestible carbohydrates, decreases the absorption of glucose from the intestinal tract, and reduces postprandial blood glucose peaks [25]. Authors of the study found that methanol and ethyl acetate extracts from amaranth seed showed appreciable antidiabetic activity. As the squalene itself was less effective, the authors came to conclusion that other components may also contribute to this effect. Kasozi et al [64] in their work on rats attributed antidiabetic properties to improved calcium homeostasis in blood, kidney, and liver, while Velarde-Salcedo et al [65] attributed them to with inhibitory activity upon dipeptidyl peptidase IV. The ethyl acetate extract together with squalene showed good antioxidant activity in a model of lipid peroxidation of liposomes. Fructose-induced changes in rats by reducing lipid peroxidation and enhancing antioxidant activity were shown in the work of Pasko et al [18]. The same authors further concluded in another in vitro study that amaranth seeds and sprouts possess relatively high antioxidant activity [19]. Similarly, Klimczak et al [17] showed appreciable antioxidant activity in β-carotene and linoleate models. Inhibition of lipid peroxidation of *Amaranthus caudatus* hydrolyzed protein was shown in experiment of Vilcacundo et al [66] on zebrafish larvae and embryos. Interesting results were obtained in study of Lee and Joo [67] where antioxidative properties and rectal cancer cell (CT 26 cell) inhibition effect of amaranth (*Amaranthus cruentus* L.) cauline leaves were observed.

It is important to note that Chatuverdi et al [68] tested the effect of differently processed amaranth grain products on postprandial glycemia in non–insulin-dependent human subjects, with results suggesting not to recommend isolated ingestion of amaranth grain due to the high digestibility of its starch. Whether extrusion affected the digestibility and the high glycemic index of the starch of amaranth seed was tested by Guerra-Matias and Areas [69], who produced a

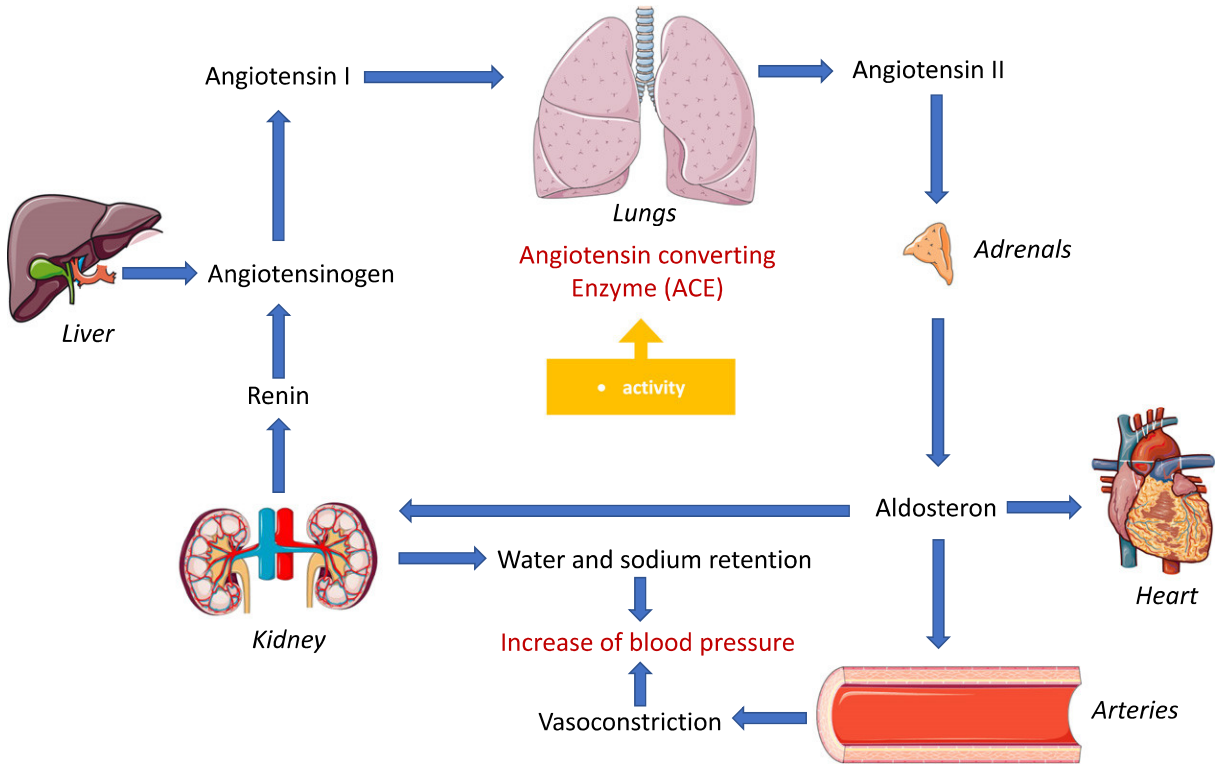

Fig. 2 – Antihypertensive effect.Potential mechanisms of action for amaranth are highlighted by yellow arrow (inhibition of the function of the ACE). The actions are supported by the articles cited in the reference list [71–74].

negative result. In vitro experiments performed by Capriles et al [70] led to similar conclusions.

3.4. *Antihypertensive effect*

An important pathway in blood pressure regulation is the renin-angiotensin-aldosterone pathway (Fig. 2). According to some authors, proteins in amaranth can be capable of generating antihypertensive effects via inhibition of the function of the angiotensin-converting enzyme (ACE). Silva-Sanchez et al [71] reported that amaranth can contain peptides with such effects. Tiengo et al [72] tested the inhibitory activity of defatted amaranth flour and its in vitro products with positive results. Tovar-Perez et al [73] studied peptides from amaranth grain by alcalase hydrolysis and also obtained positive results. The presence of antihypertensive peptides was also observed in the work of Vecchi and Anon [74]. The aforementioned potential mechanisms of action are presented in Fig. 2.

4. Discussion

This review showed the potential of amaranth to influence some important CVD risk factors: cholesterol, blood glucose, and blood pressure. The antioxidant activity discussed in the text may further contribute to the above-mentioned effects.

Discrepancies found in the literature suggest the complexity of the issue. Not only the substances within amaranth but also the actual pathology and product selection (oil, seed, flour) can play a significant role in the final hypolipidemic effect of amaranth. This indicates the need for other studies to identify the active hypolipidemic substances in amaranth, the optimal dose, and the conditions under which a product from amaranth would be able to achieve the best effect, that is, determination of a suitable form and identification of the target population.

To date, the presented data regarding the effect of amaranth on blood glucose seem to be inconsistent. Positive results represented by a decrease in blood glucose in some experiments are in contrast to results showing a high glycemic index of amaranth that can lead to an excess in food intake with potential negative consequences (weight gain, changes in lipid profile, and insulin secretion).

The present results are consistent regarding the presence of bioactive proteins capable of inhibiting ACE function. This suggests that amaranth may be a part of a diet recommended for patients suffering from hypertension. However, human experiments are needed to confirm this clinical benefit.

It is still not known whether the effect of amaranth is consistent in humans, as there are few studies, and the conclusion regarding the effect of amaranth on HDL cholesterol has not yet been drawn. It should also be emphasized that metabolic pathways in animal models differ from those in humans, so it is not easy to extrapolate result from animal models to humans. Additionally, we did not find sufficient information from randomized studies on how the inclusion of amaranth in the diet impacts the risk of atherosclerotic vascular disease.

Future research should include double-blind, randomized, placebo-controlled trials should in human subjects. Well-

defined active substance needs to be used to avoid variability between different products from amaranth. Studies need to address the effect of amaranth along with other nutraceuticals on the prevention and treatment of dyslipidemia, atherosclerosis, and CVD. Ideally, mortality should be used as a main efficacy criterion in these studies. Additionally, future research needs to test the role of amaranth in the context of modern lipid-lowering therapies, antihypertensive therapy, and antidiabetic therapy. Finally, the potential of amaranth as a part of an interventional strategy for statin intolerance is another area in which future research should focus.

5. Conclusions

The presented data show the potential of amaranth to be integrated into lifestyle interventions and thus help to form a solid background for the success of pharmacological therapy. Most published studies in animal models have demonstrated the ability of amaranth to decrease TC and LDL cholesterol. Interestingly, the data in this work also showed that amaranth products impacted TGs in the same positive way. A high level of TGs is considered an independent risk factor; the importance of lowering plasma levels of TGs has been called into question many times, but it is currently considered an integral part of residual cardiovascular risk reduction strategies [75,76]. This, together with some positive findings related to the antidiabetic and antioxidant effects, may suggest positive complex effects of amaranth products.

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Hypolipidemický účinek obsahových látek amarantu

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SOUHRN

Hypolipidemický účinek obsahových látek amarantu
V současné době rozlišujeme více než 60 druhů rostlin rodu *Amaranthus* rozšířených od tropů až po mírné pásmo. Amarant (český název laskavec – *Amaranthus sp.*) je již řadu let považován za plodinu s vysokým nutričním indexem. Díky této vlastnosti se stal významnou součástí stravy nejen u lidí, ale i vhodnou komponentou krmných směsí hospodářských zvířat. Potenciální biologické účinky amarantu, které by bylo možné terapeuticky využít v prevenci či terapii metabolických poruch, byly studovány v celé řadě preklinických studií. Cílem zkoumání těchto prací byla nutriční hodnota amarantových produktů, ale především hypolipidemický efekt. Obě tyto výhodné vlastnosti jsou společně s poměrně nízkými nároky na pěstování považovány za jedny z největších pozitiv, které použití amarantu nabízí. Přestože amarant má velmi dobré předpoklady v možnosti nefarmakologického působení v terapii či profylaxi dyslipidémií, je potřeba dalších experimentů pro detailnější analýzu mechanismu účinku jeho obsahových látek.
Tato práce si klade za cíl shrnout poznatky o hypolipidemických vlastnostech amarantu a jeho obsahových látek.
Klíčová slova: amarant – skvalen – hypolipidemikum – nutriční výživa

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SUMMARY

Hypolipidemic effect of amaranth constituents
More than 60 plant species of the genus *Amaranthus* spreading from the tropical to moderate zone have been classified. For a number of years, the amaranth (*Amaranthus sp.*) has been considered to be a crop-plant with a high nutritional index. Thanks to this property it has become an important component of food not only for people but also a suitable component of fodder mixtures for farm animals. Potential biological effects of the amaranth which could be employed therapeutically in the prevention or therapy of metabolic disorders have been studied in a number of preclinical studies. The papers have focused on the nutritional value of amaranth products, in particular on the hypolipidemic effect. Both of these useful properties are considered, together with relatively small demands for cultivation, to be one of the greatest positive properties offered by the use of the amaranth. Though the amaranth has a very good potency for a possible non-pharmacological action in the therapy or prophylaxis of dyslipidemias, further experiments are needed for a more detailed analysis of the mechanism of effect of its constituents. The present paper aims to summarize the knowledge concerning the hypolipidemic properties of the amaranth and its constituents.
Key words amaranth – squalene – hypolipidemic agent – nutrition

Čes. a slov. Farm., 2009; 58, 200–202 *Má*

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Úvod

Amarant (český název laskavec – *Amaranthus sp.*) byl již od předkolumbovské éry považován za plodinu s vysokým nutričním indexem. Proto se stal významnou součástí stravy nejen u lidí, ale i vhodnou komponentou krmných směsí hospodářských zvířat. Až mnohem později výzkum odhalil výhodné chemické složení této rostliny, představující látky schopné aktivně snižovat hladinu cholesterolu v krvi. Tohoto hypocholesterolemického efektu amarantu by mohlo být využito například k produkci potravin se sníženým obsahem cholesterolu (např. vajec) nebo pro možnost nefarmakologického ovlivňování dyslipidémií.

Botanická charakteristika

V současné době rozlišujeme více než 60 druhů rostlin rodu *Amaranthus* rozšířených od tropů až po mírné pásmo. Zejména pro potravinářské, ale i pro další účely jsou využívány druhy *Amaranthus hypochondriacus*, *Amaranthus cruentus*, *Amaranthus caudatus*, popřípadě jejich kříženci s *Amaranthus hybridus* [1)].

Amarant je jednoletá bylina řadící se k C4 rostlinám s hluboce kořenícím hlavním kůlovým kořenem s četným postranním větvením. Lodyhy kulturních forem jsou jednoduché nevětvené 0,9–1,8 m vysoké. Řapíkaté listy mají velké lysé čepele nejčastěji vejčitého tvaru s výrazným špičatým zakončením. Různá barevná kombinace fialové a zelené, případně i kresba listů nebo jen jejich jednotlivých částí (řapík, čepel), je charakteristická pro jednotlivé odrůdy rodu *Amaranthus*. Jednopohlavní květy jsou seskupené v klubíčkách uložených v podlouhlých latnatě rozvětvených vzpřímených lichoklasech. Plodem je nejčastěji vejčitá tobolka, v níž jsou drobná okrouhle elipsovitá lesklá semena čočkovitého tvaru [2)].

Chemické složení

V porovnání s běžnými obilovinami mají semena amarantu poměrně vysoký obsah bílkovin s téměř optimálním zastoupením esenciálních aminokyselin zejména množstvím lysinu a histidinu [1)]. Toto příznivé bílkovinné respektive aminokyselinové složení je uváděno jako velmi významná výživová hodnota semen amarantu se značnou biologickou kvalitou, která však kolísá nejen mezi druhy, ale i uvnitř druhů, což se přisuzuje především genetickým faktorům. Na celkovém množství těchto látek se nejvíce podílí zárodečná vrstva semena [3)].

Ve všech částech této rostliny převyšuje zastoupení škrobů s velmi nízkým obsahem amylózy. Toto složení není vhodné pro výrobu pícnin, lze je však využít pro úpravu konzistence řady potravin nebo například jako pomocnou látku ve farmaceutickém průmyslu [4)].

Vysoká energetická hodnota semen amarantu je dána značným obsahem nenasycených mastných kyselin, zejména kyseliny palmitové, olejové a linolové [5)].

Nejčastěji se pro extrakci zmíněných obsahových látek využívají semena. Základní informace o chemickém složení amarantu zaznamenává tabulka 1.

Tab. 1. Chemické složení semen a listů pěstovaných druhů amarantu – upraveno podle [1) 3)]

Složka	Semena	Listy
Voda (%)	6–11	70–94
Minerální látky (% sušiny)	3–4	8–22
Bílkoviny (% sušiny)	13–18	17–38
Tuky (% sušiny)	5–10	1–11
Sacharidy (% sušiny)	50–65	38–47
Vláknina (% sušiny)	2–8	5–25

Skvalen – důležitá složka Amarantu

Skvalen je biosyntetickým prekurzorem steroidů nebo antioxidačních látek, jakým je například koenzym Q10. Je také součástí buněčných membrán, v nichž určuje jejich kvalitu a odolnost proti tepelnému a chemickému poškození [6)]. Představuje jednu z nejdůležitějších lipidových složek kůže ovlivňujících její metabolismus a zachování příznivých mechanických vlastností. Díky svým fotoprotektivním vlastnostem je významnou součástí pleťové kosmetiky [7)].

Skvalen má celou řadu pozitivních biologických účinků. Předpokládá se, že snížení rizika vzniku různých typů nádorového bujení spojovaného s vysokou spotřebou olivového oleje je dáno právě přítomností skvalenu [8)]. V některých pracích se uvádí i možný chemoprotektivní efekt u rakoviny tlustého střeva [9)]. Mezi další pozitivní vlastnosti skvalenu patří také jeho hypocholesterolemické působení, obzvláště pak v kombinaci s tokotrienoly [10)]. Tato kombinace však není nezbytně nutná, jak ostatně prokázala práce provedená Meittinenem [11)], která demonstrovala schopnost samotného skvalenu efektivně snižovat cholesterol v krevním séru. Skvalen vykazuje také poměrně silné antioxidační vlastnosti a zpomaluje proces stárnutí kůže, reguluje látkovou přeměnu tuků a kladně ovlivňuje obranyschopnost organismu [12)]. Tradičním zdrojem skvalenu jsou v současné době mastné kyseliny některých druhů mořských ryb (*Centrophorus squamosus*, *Physeter macrocephalus*) [13)]. Stále stoupající tlak na ochranu mořské fauny a flóry však způsobuje nutnost hledání nových zdrojů skvalenu. Jedním z alternativních zdrojů může být právě amarant.

Biologické účinky amarantu

Potenciální biologické účinky amarantu, které by bylo možné terapeuticky využít v prevenci či terapii metabolických poruch, byly studovány v celé řadě preklinických studií. Cílem zkoumání těchto prací byla nutriční hodnota amarantových produktů, ale především hypocholesterolemický efekt. Obě tyto užitečné vlastnosti jsou společně s poměrně nízkými nároky na pěstování považovány za jedny z největších pozitiv, které použití amarantu nabízí.

Vliv amarantu na hladinu cholesterolu

Podrobnější studium hypocholesterolemického účinku amarantu vedlo ke zjištění několika možností jeho vy-

užití k redukci hladiny cholesterolu. Kromě přímého využití amarantu v dietetických režimových opatřeních při hypercholesterolémii lze amarantovými produkty začleněnými do běžné stravy působit profylakticky.

Dosud byla provedena celá řada studií vlivu amarantu na lipoproteinové a cholesterolové spektrum na různých živočišných druzích i na lidech. V mnoha případech však nebylo dosaženo stejných závěrů. To deklarují klinické studie provedené na lidech Maierem [14] nebo Bergerem přímo na hypercholesterolemických mužích [15]. V obou případech nebyl výsledek práce významný v porovnání s experimenty provedenými na zvířecích modelech, které naopak vykazovaly signifikantní změny výsledných hodnot cholesterolu. V podrobnějším pokusu s kuřaty, kde se sledovaly rozdíly v hladinách cholesterolu pod vlivem amarantu, bylo zaznamenáno nezměněné množství HDL na rozdíl od celkového cholesterolu, včetně LDL frakce [16]. Navíc byla prokázána vyšší aktivita jaterního enzymu podílejícího se na transformaci cholesterolu na žlučové kyseliny. K podobným závěrům dospěl i Grajeta [17] při pokusech na laboratorních potkanech. U zvířat, kterým byla podávána semena *A. cruentus*, výrazně poklesl obsah celkového cholesterolu v krevním séru i v játrech, kde bylo eliminováno i množství triacylglycerolu, zatímco jeho hladina, stejně jako hladina HDL a volných mastných kyselin, ovlivněny v krevním séru nebyly. Biologický účinek se odlišoval i u různých forem amarantu, které byly experimentálním zvířatům podány. V případě odtučněných i neodtučněných semen byl efekt velmi obdobný s účinkem popsaným výše. Ale při současném podávání olejů s vysokým podílem nenasycených mastných kyselin klesl obsah triacylglycerolu v játrech ještě výrazněji než při podávání samotného amarantu [17]. K podobnému závěru došel i Berger [18] při pokusu s křečky krmenými hypercholesterolemickou dietou po dobu 4 týdnů. Výsledky práce ukázaly, že podání amarantového oleje mělo za následek pokles jak celkového, tak non-HDL cholesterolu o 15 %, respektive 22 %. V další práci provedené tentokrát na laboratorních potkanech zjistil Chatuverdi [19], že hladina sérových lipidů a triglyceridů poklesla, zatímco frakce HDL cholesterolu stoupla, což se opět shoduje s předchozími pracemi. Autor na základě provedeného experimentu konstatuje, že hypocholesterolemický efekt amarantu byl jednoznačně prokázán.

Ačkoliv se uvedené práce shodují v tom, že na hypocholesterolemickém efektu amarantu se nepodílí zřejmě jen jedna látka (skvalen), nýbrž i další dosud neznámé složky; právě skvalen zaujal výzkumný tým vedený Shinem, který realizoval experiment [20], jehož cílem bylo prostudovat zmíněný efekt u amarantového zrna a oleje. Bylo zjištěno, že jak zrno, tak olej snižují celkové sérové hladiny cholesterolu i triglyceridů. Dále bylo prokázáno, že skvalen z amarantu podaný injekčně hypercholesterolemickým potkanům snižuje nejen již zmíněné sérové parametry, ale dochází také k inhibici 3-HMG-CoA-reduktasy.

Uplatnění amarantu může mít ještě jeden zajímavý aspekt, kterým je výroba potravin se sníženou hladinou cholesterolu. Ty by mohly být využity jako součást diety pro pacienty s dyslipidémií. Zajímavou z tohoto pohledu je práce, zabývající se schopností amarantu snížit obsah cholesterolu ve vejcích [21]. Autoři studie zkoumali, zda by bylo možné přídavkem amarantu a RPO (red palm oil – olej získaný z oplodí palmy) do diety nosnic snížit hladinu cholesterolu ve vejcích. Na základě získaných výsledků pak bylo zjištěno, že jak RPO, tak amarant (obzvláště byly-li zkombinovány) tento efekt vykazovaly.

Přestože amarant má velmi dobré předpoklady v možnosti nefarmakologického působení v terapii či profylaxi hyperlipidémie, hypercholesterolémie a hyperlipoproteinémie, je potřeba dalších detailnějších experimentů pro konkrétnější analýzu mechanismů účinků. Teprve dlouhodobá epidemiologická studie by mohla prokázat skutečnou terapeutickou účinnost diety nebo doplňkové léčby výtažky z amarantu.

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