Soy milk and Sesame seeds (Phytoestrogens) Ameliorate cardiotoxcity induced by adriamycin in experimental animals

Amal H. Hamza, Rasha H. Mahmoud

Biochemistry Department, King Abdulaziz University, Faculty of Science, Jeddah, KSA
Corresponding Author: Rasha H Mahmoud
E-mail address and telephone number of the corresponding author:
Dr.rasha.asu@gmail.com telephone: 00966505149183

Abstract

Soy milk and sesame seeds consumption has been linked to a lower incidence of chronic diseases such as cardiovascular diseases and atherosclerosis. In this study, fifty male adult albino rats (120-125 g) were divided into five experimental groups, healthy control group, positive control group injected with single dose of adriamycin ADR (10 mg/kg b.w) for induction of cardiotoxicity, group fed on diets supplemented with 20% soy milk powder after ADR injection, group fed on diets supplemented with 10% sesame seeds powder after ADR injection and group fed with diets supplemented with soy/sesame combination after injection. Results showed that, ADR injection induced cardiovascular disorders manifested by increased plasma amylase activity, troponin-T concentration, acid phosphatase activity, anticardiolipin IgM, C-reactive protein and apolipoprotein- β with highly elevated levels of triacylglycerols, total cholesterol, LDL-C and reduction of HDL-C. Observation of this study indicated that supplementation of animal diet with either soy protein or sesame seeds powder or combination of them has significant beneficial effects on ADR injected rats , through the improvement of lipid profile and reduction of elevated cardiac disease biomarkers levels. It should be noticed here that the efficiency of soy ⁄ sesame combination in reduction of adverse effects of ADR is greater than either supplement alone.

Key words: Soy protein-Sesame seeds- Adriamycin- Cardiotoxicity- Cardiac Biomarkers-Lipids profile

{**Citation:** Amal H. Hamza, Rasha H. Mahmoud. Soy milk and sesame seeds (phytoestrogens) ameliorate cardiotoxcity induced by adriamycin in experimental animals. American Journal of Research Communication, 2013, 1(10): 1-15} www.usa-journals.com, ISSN: 2325-4076.

Introduction

Cardiotoxicity occurs during therapy with several cytotoxic drugs and may be the dose limiting factor in cancer treatment and hence tumor response. Furthermore, cardiotoxicity can also be responsible for long term side effects and may cause severe morbidity in surviving cancer patients. Cardiotoxicity includes a wide range of cardiac effects from small changes in blood pressure and arrhythmias to cardiomyopathy. In literature , different mechanisms of chemotherapy induced cardiotoxicity are postulated including cellular damage due to the formation of free oxygen radicals and the induction of immunogenic reactions with the presence of antigen presenting cells in the heart. Moreover, the influence of the cytotoxic agent on certain phospholipids, especially cardiolipin, may also explain the development of cardiotoxicity [1].

Adriamycin (ADR) is an anthracycline chemotherapeutic agent that has been commonly used in treatment of a wide range of cancers. Unfortunately, the clinical use of ADR is associated with sever cytotoxic side effects including cardiotoxicity and nephrotoxicity [2].

There is great interest in the potential of soy and soy foods to prevent or treat chronic diseases, including cardiovascular disorders, osteoporosis and cancer. These potential benefits are mostly attributed to dietary isoflavones, a subclass of flavonoids that possess numerous biological properties and are most commonly found in legumes; with the highest amounts found in soybeans. The predominant isoflavones found in soybeans are the β-glycoside forms of genistein, daidzein and glycitein, which are not bioavailable. Upon ingestion, small intestinal brushborder membrane enzymes and bacterial β- glycosidases remove the glycoside group, after which the isoflavones are readily absorbed and become bioactive [3].

Soy consumption has been linked to a lower incidence of chronic diseases such as cardiovascular diseases, atherosclerosis, type II diabetes, and certain types of cancers [4]. These beneficial effects are attributed mainly to the abilities of soy components in improving blood lipid profiles, such as lowering total cholesterol, LDL cholesterol, and triglyceride levels, and decreasing the ratio of LDL to HDL cholesterol. However, the bioactive components in soybean responsible for the hypocholesterolemic and hypotriglyceridemic properties have not been consistently identified [5]. The interest in the potential health effects of soy and soy isoflavones is growing as epidemiological studies have associated with a diet rich in isoflavones with a lower risk of certain diseases [6].

It is well known that, each 200 gm of soy milk consists of (carbohydrates 1g, fat 3.5 g, saturated fats 0.5 g, monosaturated fat 0.5 g , fiber 0.25g , genistein 9.96 mg, daidzein 6.68 mg, glycetein 0.94mg) [7].

Sesamum *indicum Linn*. (Pedaliaceae) has long been used extensively as a traditional food in the orient for various purposes and commonly known as sesame. Sesame is an important oilseed crop of the world, India being a major producer. Sesame oil is widely used in cooking and as an ingredient of confectionery and for making margarine, and provides highly stable oil and nutritious protein and meals; also it has varieties of medicinal properties. The seeds are used as a demulcent in respiratory affections, infantile cholera, diarrhea, dysentery and other bowel infections and bladder diseases. The seed powder is useful in amenorrhea, dysmenorrhea, ulcers and bleeding piles. Unsaponifiable matter (sterols), fibers as well as lignan-type compounds such as sesamin, sesamolin, sesamol and sesaminol are recognized to be potent therapeutic agents [8].

Therefore, this study aimed to investigate the protective effect of soy milk powder and sesame seeds on ADR-induced cardiotoxicity in rat model.

Materials and Methods

Drug and plant materials: Adriamycnie (ADR) was purchased from NOVARITS Co. Egypt. Soy milk powder, sesame seeds were purchased from local markets, sesame finely powdered in electric miller for use in animal diet. Adriamycin was dissolved in saline immediately before use and injected to rats with a single dose of 10mg/Kg according to Itoh et al., [2]. To induce cardiotoxcity ADR was intraperitoneally injected with a single dose of 10 mg/kg, which is well documented to induce cardiotoxicity in rats [9].

Experimantal Design: Fifty male adult albino rats (120-125) g were obtained from Animal House of National Research Center, Egypt are used in the present study. Rats were acclimatized for 7 days, the animals were housed one per cage in wire bottomed stainless steel cages in a temperature controlled room (25 ± 5 °C) , with relative humidity (50± 10 %) , and with 12hour light / dark cycle. The animals were randomly assigned into five experimental groups (10 rats /group) which were classified as follows:

Group (1) rats were fed standard control diet and set as healthy control group.

Group (2) rats were fed standard control diet and injected ADR, and set as positive control.

Rats of groups (3, 4, 5) were intraperitoneally injected with a single dose of ADR 10 mg/kg. At the second day of the experiment, rats were fed on different experimental diets:

Groups (3) rats were fed on diet supplemented with 20% soy milk powder [10] **.**

Group (4) rats were fed on diet supplemented with 10% sesame seeds powder [11] **.**

Group (5) rats were fed on diet supplemented with combination of 20% soy milk and 10% sesame seeds powder.

The experiment lasts for 6 weeks. At the end of the experimental period, fasting blood samples were collected from retro-orbital venous plexus under diethyl ether anesthesia. Blood samples were collected in dry clean centrifuge tubes and then centrifuged at 3000 rpm for 15 minutes at 4°C. Serum samples were collected, stored at -80°C in clean plastic eppindorff tubes till analysis.

Biochemical analysis: Serum amylase, acid phosphatase and C-reactive protein were measured [12, 13,14] . Troponin T immunoassay was measured [15] **.** While, immunometric enzyme immunoassay for the quantitative determination of anti-cardiolipin (IgM) was performed [16] **.** Apo-lipoprotein-β was also measured in serum [17] **.** Serum cholesterol , triacylglycerols (TAG), High density lipoprotein cholesterol (HDL-C) and low density lipoprotein cholesterol (LDL-C) were measured colorimetrically [18,19, 20,21].

Statistical Analysis:

The data were statistically analyzed using SPSS computer Program. The results were presented as mean ± SE. The differences between mean values were determined by analysis of variance (ANOVA test), where $P \leq 0.05$ was considered significant.

Results

Table (1): Effect of Soy milk and Sesame on Troponine-T, Acid phosphatase and Amylase levels in all experimental animals

Groups	Tropopine-T (µg/dL)	Acid phosphatase (U/L)	Amylase (U/L)
Healthy control	0.008 ± 0.00029	8.66 ± 0.561	1827.4 ± 7.5
Positive control	0.123 ± 0.00017	14.66± 0.38	2608.5 ±10.6
Adriamycin+Soy	0.004 ± 0.00029	6.759 ± 0.164	2080.7 ± 5.11
Adriamycin + Sesame	0.008 ± 0.0003	7.50 ± 0.512	2066.1 ± 17.01
Adriamycin + Soy +Sesame	0.0037± 0.0003	5.04 ± 0.338	1928.3 ± 12.4

Data are represented as Mean ± SE

Table (1) shows the activity of amylase, acid phosphatase and troponine-t in healthy, ADR injected and treated groups. It is clear from our results that ADR injection showed a significant increase in amylase activity comparing to healthy control group ($p \leq 0.05$). While addition of soy or sesame decreased amylase levels significantly as compared to the positive control group. The obtained results showed that, animals fed both soy and sesame showed significant lower levels of amylase comparing to groups fed soy or sesame only.

It is clear that the activity of acid phosphatase and troponine-t level increased significantly in ADR injected group as compared to healthy control animals ($p \leq 0.05$). On the other hand, the addition of soy or sesame plus soy showed a significant improvement in acid phosphatase and trpopnine-t levels as compared to positive control and group fed on sesame only. Noteworthy, group fed on sesame only showed the highest level of acid phosphatase when compared to either group fed on soy or the group fed on soy and sesame combination.

Table (2): Effect of Soy milk and Sesame on Anticardiolipin , C-reactive protein and Apo-lipoprotein β levels in all experimental animals

Group	Anticardiolipin-IgM (GPL/ml)	C-reactive protein (mg/dL)	Apo-lipoprotein β (mg/dL)
Healthy control	1.359 ± 0.055	2.158 ± 0.086	10.48 ± 0.276
Positive control	1.873± 0. 068	5.150 ± 0.087	31.2 ± 1.142
Adriamycin+Soy	1.528 ± 0.053	4.350 ± 0.029	15.24 ± 0.127
Adriamycin + Sesame	1.500 ± 0.055	2.620 ± 0.066	13.14 ± 0.396
Adriamycin + Soy+Sesame	1.482 ± 0.073	3.080 ± 0.076	12.03 ± 0.630

Data are represented as Mean ± SE

Data in table (2) showed that levels of anticardiolipin IgM ,CRP increased significantly in positive control group as compared to healthy control group which indicates inflammation as a result of adriamycin injection. Animals fed on soy, sesame and both of them showed a significant decrease ($P \leq 0.05$) in anticardiolipin IgM levels as compared to the positive control. It could be also concluded from table (2) that groups fed sesame or (soy+ sesame) showed a significant decrease in CRP and apolipoprotein β levels comparing to group fed o soy only. On the other hand, group fed on sesame showed the lowest levels of CRP as compared to all other treatments. While there was no significant change between these groups in Anticardiolipin IgM levels.

Table (3): Effect of Soy milk and Sesame on Total cholesterol, TAG , LDL-C and HDL-C, in all experimental animals

Group	Total cholesterol (TC) (mg/dl)	Triacylglycerols (TAG) (mg/dl)	LDL-C (mg/dl)	HDL-C (mg/dl)
Healthy control	160.17±3.29	128.45± 2.34	66.9±1.49	61.67 ± 1.42
Positive control	200.30±4.58	272.86± 2.33	83.0± 1.15	40.01±1.29
Adriamycin+Soy	158.55±2.65	188.24±1.34	66.8 ± 2.1	59.7 ± 1.92
Adriamycin + Sesame	145.02±1.83	179.2± 2.818	68.1 ± 1.5	60.25 ± 1.15
Adriamycin + Soy+Sesame	167.6 ± 2.03	184.1 ± 1.81	55.8±2.45	77 ± 1.49

Data are represented as Mean ± SE

Levels of cholesterol, triglycerides, HDL-C and LDL-C are shown in table (3). Data represented in this table showed significant increase in cholesterol, triglycerides and LDL-C levels in ADR injected group (positive control) as compared to healthy control group, while HDL decreased significantly ($p \leq 0.05$) comparing also to the healthy control group. Groups of animals fed on soy, sesame or both of them showed a significant decrease in cholesterol, triglycerides and LDL-C levels and significant increase in HDL-C level as compared to positive control ($p \leq 0.05$). It is also clear that group fed soy + sesame combination showed a significant increase in HDL concentration as compared to groups fed soy or sesame only.

Discussion

The present study revealed that levels of amylase, acid phosphatase and troponine-T in healthy, ADR injected and treated groups. In this study it was found that, a single ADR dose (10 mg/kg) induced marked acute cardiotoxicity. ADR-induced cardiotoxicity was manifested by increased serum amylase activity troponin-T concentration and acid phosphatase in adriamycin injected group as compared to healthy control animals. Amylase is derived from the Greek word "amylone," which means starch. The main sources of amylase in humans are the pancreas and salivary glands, but it can be found in other tissues in small quantities. The main function of amylase is to cleave starch into smaller polysaccharides at the internal 1 to 4 alpha linkage in the process of digestion. Among healthy individuals, the pancreas and the salivary glands account for almost all serum amylase, 40-45% from the pancreas and 55-60% from the salivary glands [22] **.** Addition of soy or sesame decreased amylase levels significantly as compared to the positive control group. On the other hand, feeding animals with soy and sesame combination showed significant lower levels of amylase comparing to groups fed either soy or sesame only. A study by [23] showed that, isoflavones positively affect serum amylase level and was able to normalize its level in the blood. Amylase was previously used in assessment of cardiotoxicity [24].There was a clear positive correlation between the yield of free phenolics and amylase activity.

Troponine-T is a complex of three regulatory proteins that is integral to muscle contraction, skeletal and cardiac muscle. It is the biomarkers of myocardial damage [25] **.** Our results indicated that supplementing diets with either sesame or soy protein or combination of them remarkably reduce serum levels of troponine T. Cardiac troponin T is currently the preferred biomarker for the detection of myocardial infarction, due to superior sensitivity and specificity. Furthermore, troponin concentrations provide powerful prognostic information across a spectrum of disease states, even at the lower limit of detection. Troponin testing is primarily used as a tool in diagnosing heart attacks - where damage to the heart is caused by blocked blood flow to the heart [26].

ADR administration increases the acid phosphatase levels. This was in agreement with study which showed that ADR injection causes alterations in acid phosphatase levels [27]. Significant activity of acid phosphatase is found in the spleen, erythrocytes, platelets and prostate gland [28]. Enhanced reduction in the acid phosphatase level observed with increased supplementation of soy and sesame in diet suggests that increased soy consumption may reduce or suppress the release of the enzyme into the blood stream. This reduction may be ascribed to the protein content of either soy or sesame. A study [29] concluded that, supplementing diets with phytoestrogens normalize acid phosphatase level in human model.

Levels of anticardiolipin IgM, CRP and apolipoprotein-β increased significantly in positive control group as compared to healthy control group, thus indicating inflammation as a result of adriamycin injection. Animals fed on soy, sesame and both of them showed a significant decrease in anticardiolipin IgM levels as compared to the positive control, thus reflects the role of soy and sesame, either alone or in combination, in reduction of hazardous effects on ADR. Anticardiolipin antibodies ACA is a member of antibody family, they are autoantibodies that target one or more phospholipids or phospholipid binding proteins present on cellular membranes, bind cardiolipin in the presence of their cofactor b2-glycoprotein [30]**.** It could be also concluded from this study that groups fed on sesame or soy/sesame combination showed a significant decrease in CRP, while animal group fed on sesame showed the lowest levels of CRP as compared to all other treatments, similar results were previously reported [31] **.** C-reactive protein (CRP) is a hepatically-derived acute-phase reactant protein and its serum levels are associated with clinical inflammation. Elevated serum levels of CRP can predict the risk of first myocardial infarction and ischemic stroke in humans**.** Studies have been published proposing a role for CRP in determining lipid response to various dietary interventions [32]. In addition to dyslipidaemia, cardiotoxicity is typified by low grade chronic inflammation. Elevated levels of inflammatory markers, that is, C-reactive protein (CRP), have been shown to predict all-cause and cardiovascular mortality [33]. Results obtained from this study were in agreement with previous studies [34, 35, 36].

Results of this study revealed that, supplementing diets with soy, sesame either alone or in combination reduced the elevated level of apolipoprotein-β. Apolipoprotein-β (apo-β) is the main structural proteins of atherogenic lipoproteins and HDL particles. Apolipoprotein -β levels reflect the entire spectrum of pro-atherogenic particles, including very-low-density, intermediate-density, and low-density lipoproteins, whereas LDL cholesterol levels do not. Apolipoprotein -β levels also provide a good measure of the number of LDL particles, which reflects the atherogenicity of LDL. This association and the ability to measure apolipoprotein in non fasting blood samples have led to recommendations that the apo-β be used in routine clinical care [37].

It is now evident that an increased serum apo-β concentration is an important coronary heart disease (CHD) risk factor. Even in hypertriglyceridemic models, however, most of the total plasma apo-β is associated with LDL, making apo-β a good surrogate for LDL particle concentration. The larger apo-β carrying particles may be less atherogenic than the smaller LDL particles, suggesting that specific measurement of apo-β might be a better predictor of heart diseases [38].

The protective effect of soy and sesame against elevation of cardiac disease biomarkers may due to their content of linolenic acid .Soy bean is rich in linolenic acid and is similar to sesam in its linolenic acid content. Five fatty acids make up nearly the entire oil portion of soybean seed. Soybean oil averages 12% palmitic acid (16:0), 4% stearic acid (18:0), 23% oleic acid (18:1), 53% linolenic acid [39]. Sesame seeds powder contains about 45% linolenic acid [40] **.**Alpha-linolenic acid (ALA) is one of the two essential fatty acids in humans, it may prevent cardiac arrhythmias and sudden cardiac death [41]. Epidemiological studies as well as dietary trials including moderate amounts of ALA in the experimental diet suggest that this essential fatty acid, despite its low concentrations in blood and tissues, may be important in relation with the pathogenesis (and prevention) of heart diseases. Soy-based foods are an important source of dietary protein in Asian countries. A study with Chinese women found that soy food consumption was associated with a lower risk of coronary disease [42]. Sesame seeds supplementation appeared to reduce cardiac disease biomarkers levels to a greater extent than soy protein this effect may due to its content of

Sesamol (3,4-methylenedioxyphenol), the lignan of sesame oil, is a potent antioxidant and anti-inflammatory agent in various oxidative systems, including endotoxin and iron intoxication [43] **.**

A noteworthy result is significant increase in cholesterol, triglycerides and LDL-C levels with a reduction HDL-C level in adriamycin injected group as compared to healthy control.ADR injection is associated with marked hyperlipidemia , ADR treatment also resulted in high levelof plasma triglycerides and phospholipids [44]. Our results corroborated with previous studies [45] and[46]. A previous work demonstrated myocardial toxicity of ADR paralleled by an increase in serum lipids especially cholesterol and triglycerides. ADR administration may lower the level of cytochrome P 450 which may in turn depress cholesterol 7 -hydroxylase activities, the key enzyme in the conversion of cholesterol to bile acids [47].

The results of this study demonstrated that animals fed on soy, sesame or both of them showed a significant decrease in cholesterol ,triglycerides and LDL-C levels , with higher HDL-C level, when compared with positive group. Recent research has focused primarily on efforts to identify the components of soy protein responsible for the beneficial lipoprotein changes. Amino acid composition of soy was investigated for its effect on plasma lipid and lipoprotein metabolism, and its role in atherosclerosis inhibition [48]. Studies investigated that, there were components (such as isoflavones, saponins, and fibers) of the intact soy protein other than amino acids that independently lowered plasma cholesterol concentrations interacted with the protein moiety to affect lipoprotein metabolism favorably [49]. Clarkson noted a reduction in LDL cholesterol concentrations with increasing isoflavone content in soy protein [50].

Several studies [51, 52] had examined the effect of soy and supported the role of soy protein in the reduction of elevated cholesterol. Most of the previous studies used a relatively large quantity of soy protein (36-50 g). A study [53] used composite soy products containing 8-17 g soy milk, and one study by Cicero *et al* [54] used 20 g soy protein have found favourable effects of soy on lipids. Several mechanisms were suggested to contribute to the cholesterol-lowering effect of soy protein, including enhancement of bile acid excretion, increased tissue LDL receptor activity, and reduced absorption of dietary cholesterol. Interest in the potential role of soy isoflavones as a hypocholesterolemic agent was raised because of their estrogenic activity. In addition to soy isoflavones, a number of other components were shown to have cholesterol-lowering effect, such as saponins [55]. The observations that soy isoflavones including genistein and daidzein lower serum lipid levels in rodents raise the possibility that the isoflavone, not protein, component of a soy milk preparation is responsible for the hypolipidemic propensity. In this context, studies have indicated that soy milk low in isoflavone had a weaker lowering effect on serum and liver lipid levels in rats and mice [56].

Many attempts have been made to elucidate the mechanisms involved in the multiple beneficial effects when following soy diets. A study [57] stated that**,** an increased excretion of cholesterol from the liver into the intestine via bile acids is unlikely to be the reason for the diminished cholesterol concentration in the liver because the concentration of mRNA coding for CYP7A1, the initial and rate-limiting enzyme in the conversion of cholesterol to 7a-hydroxylated bile acids, was decreased by 40% in rats fed soy protein isolate compared to rats fed casein. This confirms the findings from a recent study in which the soy-protein-induced reduction of hepatic cholesterol**.** Torres *et al.,* [58] demonstrated that soy milk protein compared to animal proteins lowers serum lipid levels in experimental animals and humans. With regard to the mechanism underlying this hypolipidemic effect, the study shows that soy protein decreases cholesterol absorption.

On the other hand, Hyson *et al.,* [59] proved the role of Soy proteins, plant sterols, and almonds reduce LDL-C .Well-documented experimental studies have shown beneficial effects of soy in serum lipids, including reduction of low-density lipoprotein (LDL), cholesterol and triglycerides **.** In general, the present results were consistent with the finding of Anthony *et al.,* [60] who reported that isoflavones were effective in decreasing serum total cholesterol concentration. However, further investigations are required to assess an independent and favorable effect of isoflavones on blood lipids.

Administration of sesame reduced the elevated levels of cholesterol and triglycerides as compared to ADR injected animals. Studies on isolated or extracted physiologically active components such as sesamin, sesaminol, globulin and defatted sesame seed fraction have been demonstrated to possess beneficial effects on cholesterol metabolism and oxidative stress. A study [61] reported that, lipid-lowering effects of sesame seed in hypercholesteraemic rats is related to an increased excretion of cholesterol, neutral sterols, bile acid and an increase in hepatic bile acid content. The increased intake of sesame seeds or purified sesamin, has hypocholesterolemic effects in rats, and can reduce blood pressure. Sesame may also have antioxidant and anti-inflammatory effects. Oral administration of sesame seeds has been shown to reduce iron-induced lipid peroxidation and lipopolysaccharide-stimulated proinflammatory cytokine production in rats. These properties may be important in diseases such as atherosclerosis where inflammation and oxidative damage have been suggested to be key pathological mechanisms. Apart from its direct effect on pathological mechanisms in cardiovascular diseases, sesame could also increase nutrients which may protect against cardiovascular diseases [8].

In this study, soy administration reduces lipid profile when compared to ADR injected rats. Many attempts have been made to elucidate the mechanisms involved in the multiple beneficial effects following soy diets. These studies have suggested an inhibition of cholesterol absorption at the small intestine by soy protein, a reduced rate of saponin-mediated bile salt absorption, and an antioxidant effect on lipids associated with soy protein with or without isoflavones. The higher increase of high-density lipoprotein (HDL) cholesterol serum levels appears to be associated with the presence of isoflavones in the soy protein. In addition, an activation of peroxisome proliferator-activated receptors (PPARs) was recently reported, which may explain these interesting effects of soy protein on lipid metabolism [62]**.**

The phytosterol (1.275%) and fiber (17.166%) content of sesame seed in this context could be important in cholesterol elimination and an increase in hepatic bile acid content [11]. Although globulin fractions of sesame seed has been shown to behypocholesteraemic and induce HMG-CoA reductase activity in hypercholesteraemic rats [63] **.**The present study shows that whole seed preparation could effectively bring down the cholesterol levels.

It is also clear that group fed a soy/sesame combination (G5) showed a significant increase in HDL concentration as compared to groups fed soy or sesame only. It is well documented that while low-level HDL-cholesterol is positively correlated with a high risk for CAD, an increase in HDL-C level is found to be beneficial. Epidemiological studies have shown that high HDL-cholesterol concentration could potentially contribute to its anti-atherogenic properties, including its capacity to inhibit LDL-oxidation and protect endothelial cells from the cytotoxic effects of oxidized LDL [64] **.**Presently, sesame seed powder administration raised HDL-C levels in hypercholesteraemic animals. While dietary fibers are not known to elevate HDL-cholesterol levels, flavonoids are reported to increase the HDL-cholesterol concentrations The sesame seed flavonoids (0.0446%) could thus have contributed to an increase in HDL-cholesterol concentrations [65,66].

In conclusion: Observation of this study indicated that supplementation of animal diet with either soy milk or sesame seeds powder or combination of them has significant beneficial effects on adriamycin injected rats , through the improvement of lipid profile and reduction of elevated cardiac disease biomarkers levels.

References

1.Singh B, Bhat T and Singh B Potential therapeutic applications of some antinutritional plant secondary metabolites. Journal of Agriculture and Food Chemistry 2003; 51:5579–5597.

2.Itoh M, Iwai K, Katone-Miyahara Y, Yamada H, Ohno H, Yamamoto K,Tashima M, Inoko M, Nohara R and Uchiyama T . Successful allogeneic bone marrow transplantation for acute myelogenous leukemia after druginduced cardiomyopathy. Tohoku J Exp Med 2004;204:85–91.

3.Cassidy A, Brown J, Hawdon A, Faughnan M, King L and Millward J. Factors affecting the bioavailability of soy isoflavones in humans after ingestion of physiologically relevant levels from different soy foods. J Nutr 2006;136:45–51.

4.Frank A , Charles P , Ann M, Stephen A , Amy D, Margaret T and Ronald L. Soy protein diet alters expression of hepatic genes regulating fatty acid and thyroid hormone metabolism in the male rat. Journal of Nutritional Biochemistry 2010;21: 1106–1113.

5.Navarro-Núnez L, Rivera J, Guerrero J, Martínez C, Vicente V and Lozano M .Differential effects of quercetin, apigenin and genistein on signalling pathways of protease-activated receptors PAR(1) and PAR(4) in platelets. Br J Pharmacol 2009;158:1548–56.

6.Hermansen K, Sondergaard M, Hoie L, Carstensen M and Brock B .Beneficial effects of a soy-based dietary supplement on lipid levels and cardiovascular risk markers in type 2 diabetic subjects. Diabetes Care 2001;24: 228–233.

7.Liliana P, Bricarello ND, Nelson K, Marcelo C, Bertolami M . Comparison between the eEffects of soy milk and non-fat cow milk on lipid profile and lipid peroxidation in patients with primary hypercholesterolemia. Nutrition 2004;20:200 –204.

8.Namiki M. Nutraceutical functions of sesame: a review. Crit Rev Food Sci Nutr 2007;47:651e73.

9.Mohamed H, Asker M, Ali S, el-Fattah T. Protection against doxorubicin cardiomyopathy in rats: role of phosphodiesterase inhibitors type 4. J. Pharm. Pharmacol 2004; 56: 757–768.

10.Sam J , Ali A, Ali I, Mohamedc C and Hansend M . Differential effects of dietary flaxseed protein and soy protein on plasma triglyceride and uric acid levels in animal models. Journal of Nutritional Biochemistry 2002;13 : 684–689.

11.Nishant P ,Visavadiya A and Narasimhacharya K . Sesame as a hypocholesteraemic and antioxidant dietary component. Food and Chemical Toxicology 2008; 46 :1889–1895

12.Rinderknecht H and Marbach E . A new automated method for the determination of serum α-amylase. Clinica Chimica Acta 1970; 29: 107-110.

13.Graham E, Alan B and David M . Colorimetric determination of serum acid phosphatase activity using adenosine 3'-monophosphate as substrate. J clin Path 1971; 24: 493-500.

14.Rifai N, Tracy R, Ridker P. Clinical efficacy of an automated high-sensitivity C-reactive protein assay. Clin Chem 1999; 45:2136-2141

15.Bialk P, Vogel D, Mayr S, Richter S and Franken N . Electro-chemiluminescent immunoassay for troponin T using the random-access analyzer Elecsys . Clin Chem 1995; 41:S60.

16.Miyakis S, Lockshin M ,Atsumi T, Branch D, Brey R, Cervera R and Derksen R. International consensus statement on an update of the classification criteria for definite antiphospholipid syndrome (APS). J Thromb Haemost 2006;4: 295-306.

17.Marcovina SM . International Federation of Clinical Chemistry Standardization Project for Measurements of Apolipoproteins A1 and B Clin.Chem 1991;37: 1676-1682.

18.Richmond W. Preparation and properties of a cholesterol oxidase from Nocardia sp and its application to the enzymatic assay of total cholesterol in serum. Clin Chem 1973; 19 (12), 1350–1356.

19.Pinter JA, Havashi J and Watson, J.A. Enzymatic assay of glycerol, dihydroxiacetone and glyceraldehyde, Arch. B&hem. Biophys., 1967;121: 404-412.

20.Warnick G, Benderson J, Albers J. Dextran sulfate-Mg++ precipitationprocedure for quantitation of high density lipoprotein cholesterol. Clin Chem 1982 ;28:1379–88.

21.Friedwald W, Levy R and Fredrichson D . Estimation of the concentration of low density lipoprotein cholesterol in plasma without use of the preparative ultracentrifuge. Clin Chem 1972; 226: 499-502.

22.Jianbo X, Guoyin K, Xiaoling N, Fan Y and Xiaoqing C. Interaction of natural polyphenols with α-amylase *in vitro*: molecular property–affinity relationship aspect. Mol Bio Syst 2011; **7**: 1883-1890

23.Hamden K, Jaouadi B, Carreau S, Aouidet A and Elfeki A . Therapeutic effects of soy isoflavones on α-amylase activity, insulin deficiency, liver-kidney function and metabolic disorders in diabetic rats. Nat Prod Res 2011; 25: 244-55.

24.Era A, Altan E, Cetin G and Dik B . Assessment of the cardiotoxicity of tulathromycin in rabbits. Acta Vet Hung 2011;59:327-335.

25.Wu A. and Lane P. Metaanalysis in clinical chemistry: validation of cardiac troponin T as a marker for ischemic heart diseases. Clin. Chem. 1995; 41:1228–1233.

26.Omland T, Lemos J and Sabatine M . A sensitive cardiac troponin T assay in stable coronary artery disease. N Engl J Med 2009; 361:2538 – 2547.

27.Nicola G, Gaetaro L and Mauro G . Lysosomal alterations in heart and liver of mice treated with doxorubicin. Cancer chemotherapy and pharmacology 1985; 15:26-36.

28.Anosike C, Ezeanyika L and Obidoa O . Effect of roasted soy bean diet on the histology of selected rat tissues. Biores 2007;5:237-240.

29.Lee Y, Lee H, Kim K, Lee J , Nam S, Cheon S and Sohn H.Evaluation of the preventive effect of isoflavone extract on bone loss in ovariectomized rats. Biosci Biotechnol Biochem 2004; 68:1040-1045.

30.Lhan T, Abdullah H, Pınar T, Berrin C and Zeynep C . Anticardiolipin and anti-b2 glycoprotein I antibody concentrations in patients with type 2 diabetes mellitus. Diabetes Research and Clinical Practice 2005;68 : 181–187.

31.Chan Y, Lau K and Yiu K . Reduction of C-reactive protein with isoflavone supplement reverses endothelial dysfunction in patients with ischaemic stroke. Eur Heart 2008; 29:2800-2807.

32.Teede H, Dalais F and McGrath B. Dietary soy containing phytoestrogens does not have detectable estrogenic effects on hepatic protein synthesis in postmenopausal women. Am J Clin Nutr 2004; 79:396 – 401.

33.Zoppini G, Targher G, Negri C, Stoico V, Perrone F and Muggeo M. Elevated serum uric acid concentrations independently predict cardiovascular mortality in type 2 diabetic patients. Diabetes Care 2009;32:1716-1720

34.Javier C, Jorge T, Arturo L , Luis C and Tamara L. Anti-Cardiolipin, Anti-Cardiolipin Plus Bovine, or Human Glycoprotein-I and Anti-Human Glycoprotein-I Antibodies in a Healthy Infant Population. Archives of Medical Research 2002; 33 : 175–179.

35.Antonio C, Miguel Á, García P and Juan J . Isoflavones and cardiovascular disease. Maturitas 2010; 67: 219-226.

36.Placido L, Celestino G, José F, Ana A, Fernando D, Ignacio A and Javier F. Soy isoflavones diet and physical exercise modify serum cytokines in healthy obese postmenopausal women Phytomedicine 2011;18: 245-250.

37.Barter PJ, Ballantyne CM, Carmena R, Castro C M, Chapman M and Couture P. Apo B versus cholesterol in estimating cardiovascular risk and in guiding therapy: report of the thirty-person/ten-country panel. J Intern Med 2006 259:247-58.

38.Rizzo M, Pernice V, Frasheri A and Berneis K .Atherogenic lipoprotein phenotype and LDL size and subclasses in patients with peripheral arterial disease. Atherosclerosis 2008;197:237-241

39.Masayuki S , Kiyohiko T , Aya U , Tetsuya Y, Jun A and Keisuke K. Genetic relationship between lipid content and linolenic acid concentration in soybean seeds. Breedin Science 2008; 58 : 361-366.

40.Wilson RF.Seed composition, In HR Boerma, JE Specht ,eds, Soyebans: Improvement, Production and Uses, Ed 3 ,American Society of Agronomy, Madison, 2004 pp 621-677

41.Salen P .Alpha-linolenic acid and coronary heart disease. Nutr Metab Cardiovasc Dis 2004;14:162-169.

42.Zhang X, Shu XO, Gao YT, Yang G, Li Q and Li H . Soy food consumption is associated with lower risk of coronary heart disease in Chinese women. J Nutr 2003;133:2874–8.

43.Hsu D, Chien S, Chen K T and Liu M .The effect of sesamol on systemic oxidative stress and hepatic dysfunction in acutely iron intoxicated mice. Shock 2007;28, 596–60.

44.Geetha A, Catherine J, Shankar R, Shyamala Devi CS. Lipids and lipoprotein profile in doxorubicin treated rats: influence of -tocopherol administration. Indian J Exp Biol 1990;28:1071–1074.

45.Manabe N, Kinoshita A, Yamaguchi M, Faruya Y, Nagano N, Yamada-uchio K, Akashi N, Miyamoto K, Miyamoto H. Changes in quantitative profile of extracellular matrix components in the kidneys of rats with adriamycin induced nephropathy. J Vet Med Sci 2001; 63:125–33

46.Saadatmandi J, Dagmar F, Uwe W, Hannelore D and Wendy L . Dietary isoflavones in the prevention of cardiovascular disease – A molecular perspective Food and Chemical Toxicology 2008;46: 1308-1319.

47.Koutinos G, Stathopoulos G, Dontas I, Perrea-Kotsarelis D, Couris E, Karayannacos P and Deliconstantinos G .The effect of doxorubicin and its analogue mitoxantrone on cardiac muscle and on serum lipids: an experimental study. Anticancer Res 2002;22 : 815–820.

48.Teixeira S, Potter S, Weigel R, Hannum S , Erdman J and Hasler C . Effects of feeding 4 levels of soy protein for 3 and 6 wk on blood lipids and apolipoproteins in moderately hypercholesterolemic men. Am J Clin Nutr 2000;71 : 1077–1084.

49.Potter S ,Baum J, Teng H, Stillman R, Shay N and Erdman J.Soy protein and isoflavones: their effects on blood lipids and bone density in postmenopausal women. Am J Clin Nutr 1998;68 : 1375–1379.

50.Clarkson TB .Soy, soy phytoestrogens and cardiocvascular disease.J Nutr 2002;132:566-569.

51.Zhan S. Meta-analysis of the effects of soy protein containing isoflavones on the lipid profile. Am J Clin Nutr 2005;81:397- 408.

52.Reynolds K, Chin A, Lees KA, Nguyen A, Bujnowski D and He J .A meta-analysis of the effect of soy protein supplementation on serum lipids. Am J Cardiol 2006;98:633-640.

53.Chiechi L, Secreto G, Vimercati A, Greco P, Venturelli E and Pansini F. The effects of a soy rich diet on serum lipids: the Menfis randomized trial. Maturitas 2002;41: 97- 104.

54.Cicero A, Minardi M, Mirembe S, Pedro E and Gaddi A . Effects of a new low dose soy protein/beta-sitosterol association on plasma lipid levels and oxidation. Eur J Nutr 2004; .43:319-322.

55.Song T, Lee S O, Murphy P A and Hendrich S .Soy protein with or without isoflavones, soy germ and soy germ extract, and daidzein lessenplasma cholesterol levels in golden Syrian hamsters. Exp Biol Med 2003;228: 1063–1068

56.Mezei O, Li Y, Mullen E, Ross-Viola J and Shay N .Dietary isoflavone supplementation modulates lipid metabolism via PPARα-dependent and -independent mechanisms. Physiol Genomics 2006;26:8–14.

57.Anjali S, Corinna B, Anja B, Frank H, Gabriele I and Klaus E. Isoflavone-poor soy protein alters the lipid metabolism of rats by SREBP-mediated down-regulation of hepatic genes. J Nutr Biochem 2007;18: 313–321.

58.Torres N, Torre-Villalvazo I and Tovar AR . Regulation of lipid metabolism by soy protein and its implication in diseases mediated by lipid disorders. J Nutr Biochem 2006;17:365–73.

59.Hyson D, Schneeman B and Davis P .Almonds and almond oil have similar effects on plasma lipids and LDL oxidation in healthy men and women. J Nutr 2002;132:703-707.

60.Anthony M, Clarkson T, Bullock B and Wanger J .Soy protein versus soy phytoesterogens in the prevention of diet-induced coronary artery atherosclerosis of male cynomolgus monkeys. Arterioscler Thromb Vasc Biol 1997;17:2524–2531

61.Grace P, Taylor J, Botting N, Fryatt T, Oldfield M and Bingham S .Quantification of isoflavones and lignans in urine using gas chromatography/mass spectrometry. Anal Biochem 2003;315:114-121.

62.Damasceno D, Goto H, Rodrigues F. Soy protein isolate reduces the oxidizability of LDL and the generation of oxidized LDL autoantibodies in rabbits with diet-induced atherosclerosis. J Nutr 2000;130:2641-2648.

63.Romero AL, West KL, Zern T and Fernandez ML .The seeds from plantago ovata lower plasma lipids by altering hepatic and bile acid metabolism in guinea pigs. J Nutr 2002;132:1194–1198.

64.Assmann G and Nofer J .Atheroprotective effects of high-density lipoproteins. Annual Review of Medicine 2003;54: 321–341.

65.Daniel R, Devi K and Augusti K .Mechanism of action of antiatherogenic and related effects of Ficus bengalensis Linn. Flavonoids in experimental animals. Indian Journal of Experimental Biology 2003;41:296–303.

66.Munro I, Harwood M, Hlywka J, Stephen A, Doull J and Flamm W. Soy isoflavones: a safety review. Nutr Rev 2006; .61:1–33

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