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Effects of Silymarin on Lipid Metabolism in Rats

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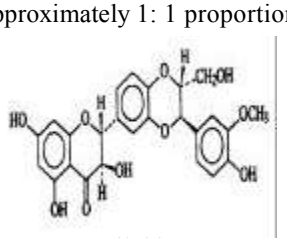
Abstract: The efficacy of silymarin on lipid metabolism as evaluate by the changes of total lipids, cholesterol, lipoproteins and triglycerides in rats was studied. Fifty rats were divided into five groups. The group1 was fed on standard diet during all the period of experiment; group 2, 3, 4 and 5 were fed on egg yolk for 20days to induce hypercholesterolemia. Group2 received egg yolk and injected with saline solution (0.9% NaCl) during the experimental time (7 days). Groups 3, 4 and 5 received egg yolk and injected with silymarin (25, 50 and 100 mg/kg during the 7days, respectively). Animals were sacrificed after the end of treatments and blood samples were collected. The results showed significantly increase in serum total lipids, triglycerides, cholesterol, LDL (low density lipoproteins), HDL (high density lipoproteins), VLDL (very low density lipoproteins) in non treated animals compared to control, but these parameters showed highly significant decrease in animal treated with silymarin. In conclusion, silymarin reduced blood total lipids, triglycerides, total cholesterol and Lipoprotein.

Key words: Silymarin · Lipid Metabolism · Lipoprotein · Cholesterol · Total lipids

INTRODUCTION

Silymarin is a flavonoid found in the herb milk thistle *Silybum marianum* (L.). A standardized extract obtained from the seeds of *S. marianum* was found to contain approximately 70-80% of the silymarin flavonolignans and 20-30 % chemically undefined fraction, comprising mostly polymeric and oxidized polyphenolic compounds. The main component of the silymarin complex is silybin (CAS No. 22888-70-6), synonymous and silibinin, sometimes incorrectly called silybinin. Besides silybin, which is a mixture of two diastereomers A and B in approximately 1: 1 proportion [1].



Silybin

Isosilybin

Silymarin is a powerful antioxidant said to protect liver cells (and other cells in the body and brain) from toxins. It promotes liver cell protein synthesis and decreases the oxidation of glutathione. Silymarin has metabolic and cell-regulating effects, namely carrier-mediated regulation of cell membrane permeability, inhibition of the 5-lipoxygenase pathway, scavenging of reactive oxygen species (ROS) of the R-OH type with an action on DNA-expression via suppression of nuclear factor (NF)-κB[2].

Cholesterol constitutes a component of extra and intra cellular membranes and is needed to maintain normal cell growth and multiplication. It is the precursor of steroid hormones and bile acids and it is a structural component of lipoprotein particles which involved in the transport of lipid energy to tissues [3].

Certain hormones such as male and female hormone have a powerful influence on lipoprotein receptor expression [4]. Adrenal steroid hormones are largely derived from lipoprotein cholesterol [5].

Flavonoids led to an inhibition of cholesterol synthesis, cellular cholesterol esterification, triglycerides and phospholipids synthesis and it inhibits the activity of HMG-CoA reductase [6].

Considerable interest was given to the benefit of diet rich in flavonoid, such as fruits, vegetables, wine and tea, with respect to cardiovascular disease and certain cancer [7].

The protective effects of flavonoids against the chronic disease have been attributed to their free radical scavenging property. In case of cardiovascular disease

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flavonoids reduced low density lipoprotein (LDL) oxidation which is an important step in atherogenesis [8]. Other study provided evidence about these flavonoids in reducing blood lipid Cholesterol levels in hyperlipidemic rats [9].

In humans the intake of soy protein rich in isoflavonoids was shown to significantly reduce serum cholesterol levels [1]. The citrus Flavonoids (naringenin and hesperetin decreased cholesterol synthesis by inhibiting acyl CoA cholesterol acyltransferase (ASAT) activity in HepG2 cells [10]. In contrast, silybin, the major flavolignan from fruit of *Silybum marianum* reduced cholesterol synthesis by suppressing 3- hydroxyl 3- methyl glutaryl coenzyme A reductase (HMGR) activity, the rate limiting enzyme in cholesterol synthesis[11]. In addition to silybin or flavolignans, Silymarin has hypocholesterolemic effects in rats [12]. Silymarin treatment decrease total lipids and total cholesterol following its stimulation by CCl4 toxicity which increased serum total lipid and total cholesterol levels in albino rats [13]. Rats fed standard diet did not respond to oral administration of silymarin , with mild increase in HDL cholesterol. Parenterally injected silymarin failed to reduce serum cholesterol both in rats fed on high cholesterol diet and standard diet. Silymarin could act by either fat mediating bioavailability and / or by inhibiting of resorption of dietary cholesterol [14].

The liver plays an important role in regulation of plasma lipoprotein metabolism[15]. The liver injury of different etiologies is often accompanied by secondary lipoproteinemia, which may lead to the development of atherosclerosis, particularly when associated with hyper cholesterolemia characterized by an increase in low density lipoprotein (LDL) cholesterol [16] and decrease in high density lipoprotein (HDL) cholesterol [17]. In hyperlipemic rats, silymarin as well as silybin administered orally decreased serum LDL cholesterol [18], while in normal rats silybin bishemisuccinate administered parenterally did not affect serum cholesterol level [19]. Other studies showed that silymarin is able to inhibit the development of diet induced hyper cholesterolemia in rats [12].

The aim of the present work was to evaluate the influence of silymarin administration on serum total lipid, total cholesterol, triglycerides, low density lipoprotein, high density lipoprotein, very low density lipoprotein levels in normal and in rats with high cholesterol diet following induced hypercholesterolemia.

MATERIALS AND METHODS

Material

Drug: The standard silymarin powder was received from Sediko company, Egypt.

Animals: Fifty male albino rats weighing 100-120 g were obtained from the college of EL-Tahady University Libya. They were maintained at standard housing conditions and fed with commercial standard diet (Hindustan Lever Ltd., Bangalore) and provided with water *ad libitum* during the experiment. Hypercholesterolemia was induced in rats by fed the rats on egg yolk for 20 days before the bingeing the experiment and during the experiment (7 days).

Five groups of rats were used for the study. The animals of group I served as the control and received slandered diet only and injected by saline solution 0.9% at a dose of 1 ml/kg/day i.p. for 7 days. Groups II received egg yolk and injected with saline solution (0.9% NaCl) at a dose of 1 ml/kg/day i.p., Groups III received egg yolk and injected with 25 mg silymarin /kg/day i.p., Groups IV received egg yolk and injected with 50 mg silymarin /kg/day i.p., Groups V received egg yolk and injected with 100 mg silymarin /kg/day i.p. for 7 days.

Samples: All the animals were scarified on day 7 under light ether anesthesia. The blood samples were collected separately by carotid bleeding into sterilized dry centrifuge tubes and allowed to coagulate for 30 min at 37°C. The clear serum was separated at 2500 rpm for 10 minutes and biochemical investigations were carried out.

Biochemical Analyses: Total lipids was estimated according to method described by Knight *et al*. [20], serum cholesterol was estimated according to method described by Stein [21], serum triglycerides was estimated according to method described by Chawla [22]. The low density lipoprotein (LDL), high density lipoprotein (HDL) and Very low density lipoprotein (VLDL) were estimated according to method described by Kostner *et al*. [23].

Statistical Analyses: The results were expressed as mean ± SD of ten animals from each group. The data were evaluated by one-way ANOVA followed by Tukey's multiple comparison test. P values ≤ 0.01 were considered statistically significant[24].

Table 1: Effects of silymarin on lipidogram in serum of hyperlipemic rats (Mean±SD)

Parameters	Control	Group 1 with high cholesterol diet	Group 2 silymarin 25 mg/Kg	Group 3 silymarin 50 mg/Kg	Group 4 silymarin 100 mg/Kg
Cholesterol (mg/dl)	98.54±0.82 e	189.56±0.91 a	153.82±1.25b	138.24±0.74 c	120.66±1.56 d
LDL (mg/dl)	15.92±0.91d	35.42±0.56 a	24.19±0.89 b	20.25±0.53 c	19.23±0.34 c
VLDL (mg/dl)	50.74±0.55 e	78.35±0.74 a	56.68±0.92 c	67.91±0.85 b	58.64±0.44 d
HDL (mg/dl)	78.63±0.54 d	97.36±0.81 a	86.42±0.63 b	87.43±0.38 b	79.86±0.72 c
Triglycerides (mg/dl)	289.47±1.12 c	321.54±0.86 a	320.66±0.77 a	295.34±1.42 b	290.32±1.78 c
Total lipids	884.3±0.86 e	976. 77±0.75 a	939.24±1.24 c	946.73±1.38 b	919.27±1.76 d

Mean with different letters for each parameter are significantly different($P<0.01$).

RESULTS

A significant ($P \leq 0.01$) increases in the serum total lipids, serum total cholesterol, serum triglycerides were observed in animal groups fed on high cholesterol diet as compared to the control group. However, following treatment with silymarin, these values were improved as shown in Table 1. LDL, HDLand VLDL were significantly($P<0.01$) increased in blood serum of rat groups fed on hypercholesterolimic diet as compared to the control group fed on free cholesterol diet and also these results showed improvement after treatment with silymarin Table 1.

DISCUSSION

The present results demonstrated significant increases in the serum total lipids, total cholesterol and triglycerides in animal groups fed on high cholesterol diet as compared to the control group. But these results showed improvement after treatment with silymarin. These results were in agreement with the view that silymarin has increased stimulation of protein synthesis and has a contributory hepatoprotective mechanism, which accelerate the regeneration process and the production of liver cells[25]. Silymarin also exerts antioxidant and membrane stabilizing activity and attributes important for liver secretion and uptake of plasma lipoproteins and so alter in lipid metabolism[26].

The LDL, HDL and VLDL values obviously increased in blood serum of rats groups fed on hypercholesterolimic diet and were improved after treatment with silymarin. It was reported that silymarin normalize the binding of low density LDL in paracetamol damaged hepatocyes in rats [27]. Previously the inhibition of 3 hydroxy 3- methyl glutaryl coenzyme A reductase, a key enzyme in cholesterol synthesis, by silybin has been demonstrated *in vitro*[11], implying its possible direct influence on liver cholesterol metabolism. Silymarin or silybin induced hypocholesterolemic effect [18]. Silymarin administrated concurrently with high cholesterol diet caused acceleration of LDL removal by the perfused rat liver. Silymarin is associated with the decrease in liver cholesterol [12]. This is probably the main base of silymarin induced improvement of removal of LDL by the liver, since is known that the liver LDL uptake is regulated by LDL receptor activity, which increased with the fall of cholesterol content in the liver[28].

In conclusion, levels of total lipid, triglycerides, cholesterol, lipoproteins decreased in the blood of rats fed on high cholesterol diet after treatment with silymarin.

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