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Original Research Article

Antiatherogenic activity of silybin in Wistar rats: an experimental study

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ABSTRACT

Background: Dyslipidemia is the major contributor to an increased risk of atherosclerosis. Furthermore, Atherosclerosis presently comprises one of the essential contributors to a global epidemic of cardiovascular disease and turn out to be the leading cause of death and disability worldwide. Natural antioxidants have been shown to be effective in reducing lipid profiles and mitigate peroxidative modification of lipoproteins and atherosclerosis. The aim of the study was to explore the antiatherogenic effect of silybin through its antioxidant mechanism in Wistar rats fed on hypercholesterolemic diet.

Methods: Male Wistar rats of 150-200 g were used for this study. Hypercholesterolemia in rats was induced by administration of high cholesterol diet. The Wistar rats were divided into four groups, each with eight rats. After 60 days blood samples were drawn by retro-orbital puncture for biochemical analysis. The animals were sacrificed by cervical dislocation and liver and aorta were dissected out and processed for histopathological study and biochemical analyses.

Results: In the histopathological study high cholesterol fed Wistar rats showed fatty degeneration of hepatocytes with leucocytic infiltration of sinusoids. The level of TBARS was significantly increased in high cholesterol diet fed rats ($p < 0.05$). Silybin at both doses [300 mg/kg (1593.00 ± 81.08) and 600 mg/kg (1596.00 ± 28.81)] reduced the plasma TBARS significantly ($p < 0.05$). The antioxidant enzyme levels were also reduced significantly in high cholesterol diet fed rats ($p < 0.05$).

Conclusions: The study suggests a conclusive evidence of silybin has antiatherogenic action. Its safety profile, availability and low cost are an added advantage to the presently available pharmacological therapy. Hence, silybin can be considered in conjunction with other available dyslipidemic medication in the market.

Keywords: Dyslipidemia, Atherosclerosis, Coronary heart disease, Silybin

INTRODUCTION

Dyslipidemia is a primary contributor to an increased risk of atherosclerosis. Furthermore, atherosclerosis presently comprises one of the essential contributors to a global epidemic of cardiovascular disease and turn out to be the leading cause of death and disability worldwide.¹

Obesity and associated diabetes have aggravated the risk of Atherosclerosis both in low and high socio-economic

regions and, alarmingly, in the developing world. Atherosclerosis is not only the main underlying cause in coronary heart disease but also causes frequent strokes and affects peripheral arteries.² An integrated view of experimental results in animals and study of human atherosclerosis suggest that low-density lipoproteins accumulate in the intima of arteries, undergo oxidative modifications, and form foam cells, which are the key constituents of fatty streaks and the earliest phase of atherosclerotic plaque. In addition to lifestyle

modification, all patients with risk factors require medication as a part of primary and secondary prevention. Presently minimal medication is available to reduce oxidative stress associated with hyperlipidemia.

In recent years, antioxidants have been subjected to epidemiological studies³ that have related their consumption to a reduction in the incidence of oxidative damage associated with diseases like ageing, cardiovascular diseases, diabetes, inflammation and neurodegenerative disorders. Many antioxidants such as tocotrienol, probucol, vitamin C, have been reported to protect against induced hypercholesterolemia.⁴⁻⁶ Natural antioxidants have been shown to be effective in reducing lipid profiles and also mitigate peroxidative modification of lipoproteins and atherosclerosis.⁷ These antioxidants reduce LDL oxidation and preserve vasoreactivity by increasing endothelial nitric oxide release and reducing thrombogenicity.

They also reduce the risk of plaque progression and rupture. Silymarin a flavonolignans obtained from an edible plant 'milk thistle' has been used as an herbal medicine for the treatment of liver related disorders for more than 2000 years. Silymarin consists of four flavonolignan isomers, namely silybin, isosilybin, silydianin and silychristin.⁸ Silymarin, a powerful antioxidant, is said to protect liver cells and other cells in the body and brain. Various animal studies have reported that Silybin's antioxidant effect helps protect skin against photocarcinogenesis and renal tissue against cisplatin induced nephrotoxicity. The aim of the study was to explore the antiatherogenic effect of Silybin through its antioxidant mechanism in Wistar rats fed on hypercholesterolemic diet.

METHODS

The study was undertaken at Central animal house, Rajah Muthiah Medical College and Hospital, Annamalai University, Annamalai Nagar, Tamil Nadu. Male Wistar rats of 150-200 g were used for the experiment and a regular pellet diet was used to feed the animals and water ad libitum. The Institutional Animal Ethical Committee approved the study. The total duration of the study period was 60 days. Silybin (siliphos) was procured from Swanson Health Products (Indena S.p.A., Italy) U.S.A.

Each 300 mg capsule of siliphos contained 89-109 mg of silybin. The other ingredients were microcrystalline cellulose (plant fiber), gelatin, magnesium stearate and silica. Biochemical and enzymatic kits for measuring lipid peroxides and antioxidant enzymes were obtained from Sigma Aldrich chemicals. Cholesterol and sodium cholate in powder form were obtained from Hi Media chemicals. Hypercholesterolemia in Wistar rats was induced by administration of high cholesterol diet (1% cholesterol, 0.5% sodium cholate, 1% coconut oil) for 60 days in standard rat chow diet.

The rats were divided into four groups, each with eight rats: (a) group-I (n=8): normal control, treated with normal diet only; (b) group-II (n=8): experimental control, high cholesterol diet (1% cholesterol, 0.5% sodium cholate, 1% coconut oil); (c) group-III (n=8): high cholesterol diet+silybin 300 mg/kg orally once daily; and (d) group-IV (n=8): high cholesterol diet+silybin 600 mg/kg orally once daily. The animals in all four groups were fed with a standard pellet diet and water ad libitum during the experimental period. Silybin was mixed with normal saline and administered through an oral intragastric tube to groups III and IV. At the end of 60 days, the animals in all the groups were subjected to overnight fasting. Blood samples were drawn by retro-orbital puncture under intramuscular ketamine for biochemical analysis. The animals were sacrificed by cervical dislocation under intramuscular ketamine and liver and aorta were dissected out and processed for histopathological study and biochemical analyses.

Statistical analysis

Values of biochemical analyses were expressed as means±SD for eight rats in each group. The data were analyzed by Duncan's multi-range test. Statistical analyses were performed using a software package SYSTAT 12. A value of $p \leq 0.05$ was used as the criterion for statistical significance.

RESULTS

Histopathological evaluation

In the histopathological study, high cholesterol-fed rats showed fatty degeneration of hepatocytes with leucocytic infiltration of sinusoids (Figure 2). The tunica media of aortic tissue showed a large number of foam cells, necrotic core containing lipid debris, thrombi formation, neovascularization and inflammatory cells (Figure 6). Rats treated with 300 mg/kg of silybin showed only mild fatty degeneration of liver with only few microvesicular and macrovesicular droplets of fat (Figure 3). Liver tissue of silybin treated rats at 600 mg/kg showed almost normal hepatocytes with only few microvesicular droplets of fat (Figure 4). Aortic tissue of rats treated with silybin 300 mg/kg showed only intimal thickening with few foam cells and inflammatory cells (Figure 7). At the higher dose (600 mg/kg), silybin treated rats showed only streaks of foam cells in the intimal wall of aorta (Figure 8).

Plasma lipid peroxides and antioxidants level

From Table 1, it was observed that the level of TBARS was significantly increased in high cholesterol diet-fed rats ($p < 0.05$). silybin at both doses (300 and 600 mg/kg) reduced the plasma TBARS significantly ($p < 0.05$). The antioxidant enzyme levels were also reduced significantly in high cholesterol diet-fed rats ($p < 0.05$). The activities of all antioxidant enzymes (GPx, SOD and CAT) and non-enzymatic antioxidant (GSH) were increased even much

higher ($p < 0.05$) than the normal control group in silybin treated groups.

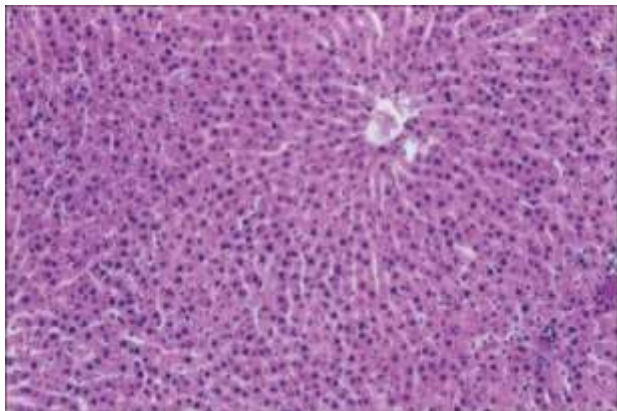


Figure 1: Lobules of liver showing normal hepatocytes.

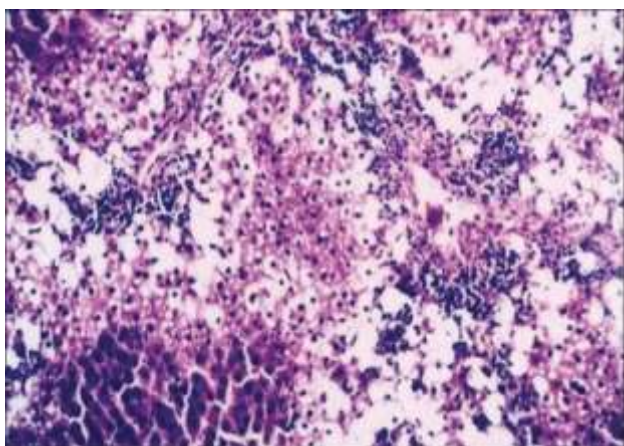


Figure 2: Liver tissue shows severe fatty degeneration of hepatocytes and infiltration of leucocytes in hepatic sinusoids and shows abundant macrovesicular and microvesicular droplets of fat within the hepatocytes.

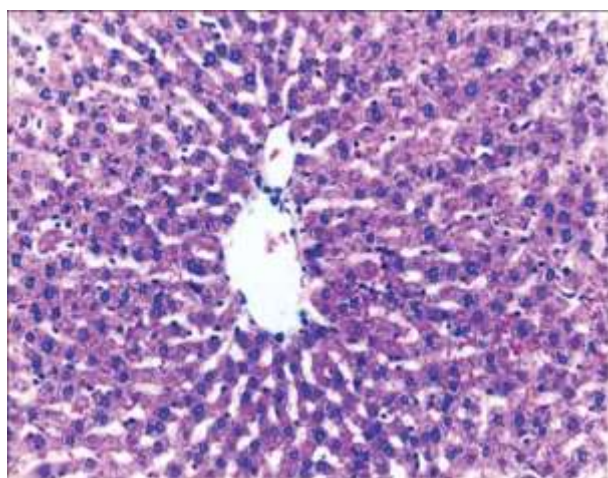


Figure 3: Mild fatty degeneration of hepatocytes with microvesicular and few macrovesicular droplets of fat.

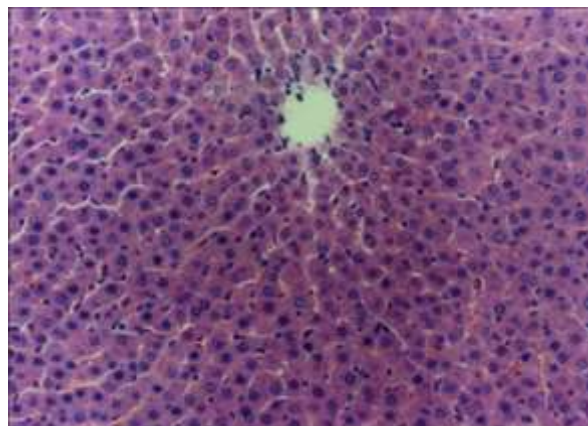


Figure 4: Hepatocytes shows few microvesicular droplets of fat.

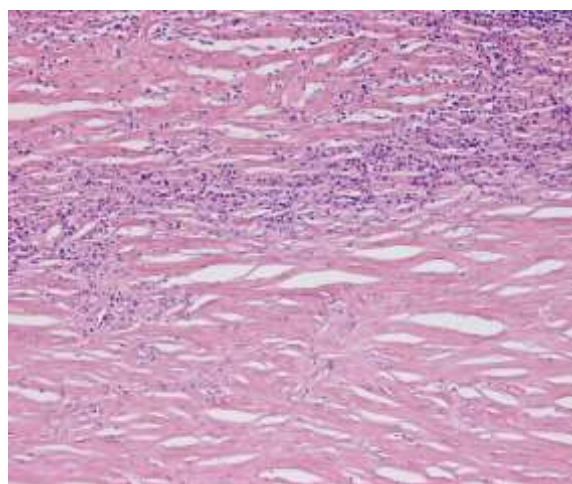


Figure 5: Few macrophage derived foam cells are seen in the intima of aorta.

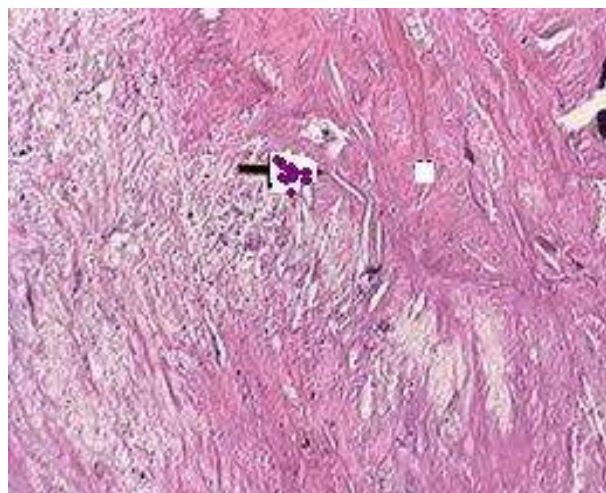


Figure 6: The internal and external elastic membranes are attenuated, the media of artery is thinned and necrotic core containing lipid debris from dead cells, foam cells fibrin organized thrombi. Scattered inflammatory cells and neovascularization were noticed.

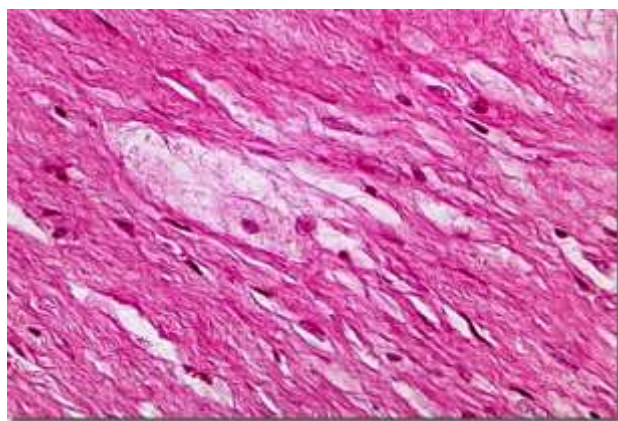


Figure 7: The wall of aorta shows intima thickening and lipid accumulation with few scattered inflammatory cells and foam cells.

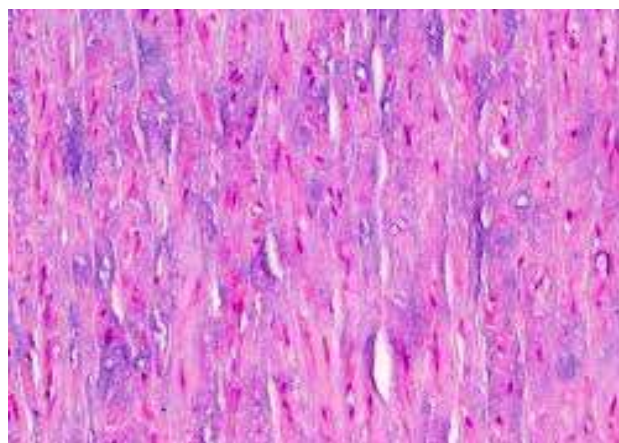


Figure 8: The intimal wall of aorta shows streaks of foam cells.

Table 1: Effect of silybin on plasma lipid peroxides and antioxidants in hypercholesterolemic rats.

Groups	TBARs $\mu\text{g/dl}$	GSH $\mu\text{g/dl}$	GPx $\mu\text{mol}^{\#}/\text{min}$	SOD NBT [@] / min	CAT $\mu\text{mol}^*/\text{min}$
Control	1768.66 \pm 93.75 ^b	855.33 \pm 45.10 ^b	54.00 \pm 3.97 ^b	616.66 \pm 26.72 ^b	55426 \pm 879.60 ^b
High cholesterol	2051.33 \pm 28.33 ^c	821.33 \pm 42.74 ^a	47.91 \pm 2.86 ^a	610.00 \pm 11.26 ^a	54996 \pm 407.23 ^a
High cholesterol+silybin 300 mg/kg	1593.00 \pm 81.08 ^a	909.00 \pm 29.11 ^c	61.11 \pm 1.83 ^c	623.33 \pm 29.02 ^c	60328 \pm 905.97 ^c
High cholesterol+silybin 600 mg/kg	1596.00 \pm 28.81 ^a	1026.00 \pm 21.85 ^d	62.58 \pm 1.34 ^c	628.00 \pm 13.78 ^d	62903 \pm 2146.82 ^d

Note: Values are expressed as means \pm SD for eight rats in each group. Values not sharing a common superscript differ significantly at $p \leq 0.05$ (Duncan's test). #= μmol of GSH utilized/minute/dl of plasma. @=One unit of enzyme activity is defined as the amount of enzyme that gave 50% inhibition of NBT reduction in one minute. *= μmol of hydrogen peroxide decomposed/minute/dl of plasma.

DISCUSSION

Dyslipidemia, including hypercholesterolemia and low levels of HDL-C are the major causes of atherosclerosis and atherosclerosis induced conditions, such as coronary heart disease (CHD), ischaemic cerebrovascular disease and peripheral vascular disease. Atherosclerosis remains the major cause of death and premature disability in developed societies. In India, atherosclerotic CHD is expected to be the single most important cause of death by the year 2015. Genetic factors and unhealthy dietary practices contribute to the dyslipidemias seen in countries around the world. The number of human epidemiological studies and animal studies shows that consumption of saturated fats, refined carbohydrates, a low diet of vegetables and fruits also tends to raise plasma cholesterol levels. Large epidemiological studies such as seven countries study and the Framingham heart study confirmed the strong relationship between the elevated serum cholesterol and subsequent atherosclerosis and atherosclerotic related CHD.⁹

Since hypercholesterolemia constitutes one of the major risk factors for the development of CHD, a large number of animal experiments are carried out to understand the better relationship between disorders in cholesterol metabolism and atherogenesis and to evaluate possible treatments. A great number of animal models, such as

rabbits, mice, rats, chickens, swine, cats and dogs have been tested.¹⁰ The most commonly used method to evaluate antihyperlipidemic drugs and antiatherosclerotic drugs is 'Cholesterol diet induced hyperlipidemia in rats'. Rats fed on high cholesterol diet for thirty days show a significant increase in serum and total hepatic cholesterol, LDL-C, VLDL-C, Triglyceride levels and a decrease in HDL-C level.¹¹

In the present study also male Wistar rats were used, and hypercholesterolemia was induced by giving 1% cholesterol, 0.5% sodium cholate, 1% coconut oil for 60 days in a standard rat chow diet.

Several epidemiological studies, clinical trials and animal experiments showed that hyperlipidemia induces oxidative stress and the oxidative modification of lipoproteins in the vessel wall might play a key role in atherogenesis.¹² Hence in the present study also, to assess the oxidative stress associated with hyperlipidemia, lipid peroxides and antioxidant levels in the plasma were analyzed. Elevated levels of TBARS in plasma of high cholesterol diet-fed rats was a clear manifestation of excessive formation of free radicals and activation of lipid peroxidation system by hyperlipidemia. Reduction in antioxidant enzymes (GSH, GPx, SOD, and CAT) in hypercholesterolemic rats was due to increased utilization of the above enzymes to counteract the increased formation of lipid peroxides.

Recent experimental and epidemiological evidence suggest that antioxidants like vitamin C, vitamin E, and β -carotene, ubiquinone, bioflavonoids and selenium reduce LDL oxidation and reduce the risk of plaque progression and rupture.¹³ When WHHL rabbits were treated with probucol (the only hypolipidemic drug with antioxidant properties) the amount of plaque in the arterial walls was significantly reduced compared with control animals.¹⁴ Silymarin a complex mixture of four flavonolignan isomers, namely silybin, isosilybin, silydianin and silychristin (with silybin being the most active) has been widely used from ancient times to treat human liver disorders.¹⁵ The antioxidant and free radical scavenging properties attribute to one of the several mechanisms for silymarin's hepatoprotective effect. Various animal studies have also demonstrated that silybin was shown to have anticancer and neuroprotective activities through its antioxidant defense mechanism.¹⁶ In the present study silybin treated rats showed a marked reduction in TBARS. Thus, silybin administration in hypercholesterolemic rats ameliorated the increased level of lipid peroxidation. Silybin also markedly increased antioxidants. In fact, the antioxidant levels (GSH, GPx, SOD and CAT) were much higher than the normal group of rats. This could be the compensatory response of silybin to oxidative stress due to LDL-C peroxidation. This result revealed the antioxidant and free radical scavenging properties of silybin.

The antioxidant role of silybin in preventing the progression of atherosclerosis was studied by histopathological analyses of aortic and hepatic tissues of rats. Scientists have strongly proved that antioxidants control LDL oxidation in vivo and potentially slow atherosclerotic process.¹⁷ Cell culture experiments showed that probucol prevented LDL oxidation and subsequent uptake and degradation in macrophages.¹⁸ In the present study also, cholesterol diet fed rats showed increased fatty degeneration and foam cells in liver. The aortic tissue showed a large number of foam cells, necrotic core containing lipid debris and inflammatory cells. Silybin treated rat showed only a few microvesicular droplets of fat in hepatocytes at the higher dose. The intimal wall of aortic tissue showed only few streaks of foam cells.

CONCLUSION

The study suggests a conclusive evidence of silybin has antiatherogenic action. Its safety profile, availability and low cost are an added advantage to the presently available pharmacological therapy. Hence Silybin can be considered in conjunction with other available dyslipidemic medication in the market. However, further studies are needed for the role of silybin on cytokines like IL-1, TNF α , growth factors and others that contribute to atheromatous plaque evolution and its complications.

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Conflict of interest: None declared

Ethical approval: The study was approved by the Institutional Ethics Committee

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