The Effect of *Silybum marianum* (L.) Gaertn. Seed Extract (Silymarin) on Galactose Induced Cataract Formation in Rats

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Abstract

Background: Increased oxygen free radical and reduced glutathione level in the eye lens are important risk factor for cataract formation. The antioxidative property and increasing cellular and extra cellular glutathione level have been reported by several herbal medicines including silymarin.

Objective: In present interventional study *Silybum marianum* L. seed extract (silymarin) was tested against galactose-induced cataract development in rats.

Methods: Thirty male 45 days old wistar rats (150 – 200 g), were divided in three groups of 10 rats each. Cataract was induced in two groups of rats following feeding them with 30% galactose diet for 40 days. One group kept as control and silymarin in the dose of 200 mg/kg/d was administered orally (mixed with galactose diet) to other group for 40 days. Cataract development in the rats lens was observed daily by ophthalmoscope and naked eye during the study. The glutathione (GSH) and lipid peroxides (LPO) levels were determined after 20 days in all rats left eye lens.

Results: The results indicated that, in silymarin treated group all stage of cataract development were significantly delayed as compared to control group. In rats treated with silymarin the lens GSH level was increased significantly ($p<0.01$) and LPO levels was decreased significantly as compared to control group ($p<0.05$).

Conclusion: Administration of silymarin to galactose fed rats showed beneficial effect on prevention of cataract development as well as antioxidative defence system such as increase in lens GSH and decrease LPO levels.

Keywords: Cataract, Silymarin, Antioxidant, Herbal medicine, Galactose

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Introduction

Cataract is the main cause of blindness in most of the nations [1]. In human the age of above 50 years, diabetes, ultraviolet radiation, smoking and chronic steroid therapy are among risk factor for accelerating cataract development [2 – 6]. The increased oxygen free radical levels are important mechanism underlying cataract development as seen in several chronic disease [7, 8].

The body and lens natural offensive against free radicals are glutathione peroxides and catalase, superoxid as well as nutritional derived compounds such as vitamin E, C and A [9-11]. In this connection administration of antioxidant like vitamin E have been reported to prevent galactose-induced cataract in experimental animals [12]. The antioxidative property and increasing body glutathione level has been reported by silymarin [13]. The antioxidative, anti-inflammatory, increasing blood and cellular glutathione level and cellular membrane stabilizing property of silymarin may affect the process of cataract development [13 - 16].

In present study the preventive effect of silymarin on galactose induced cataract formation in rat has been investigated.

Materials and Methods

Drugs and chemicals

The silymarin were kindly given by Institute of Medicinal Plants (ACECR), Tehran, Iran. Galactose, EDTA sodium salt, KCl, glutathione (GSH), glutathione reductase, 5',5'-dithio-bis (2-nitrobenzoic acid) and NADPH were obtained from Sigma, USA. The other chemicals and reagents were at analytical grade.

Experimental animals

Thirty male 45 days old wistar rats (150 – 200 g), bred in the central animal house of Shaheed Beheshti University were used. The animals were housed under standard conditions of light and dark cycle with free access to food (Behparvar products) and water. All the animals experimental protocols were approved by the Institutional Ethical Committee of Jahad Daneshgahee, Tehran Iran. The galactosemic food was contains 30% galactose and 70% prepared food according to methods used by Gupta et al [17].

Experimental groups

In present study, 30 rats were randomly divided in 3 groups of 10 animals each and caged in same environmental condition. One group fed in normal rat chow kept as healthy normal group. The rats in silymarin group received galactosemic diet and 200 mg/kg silymarin daily orally (mixed with foods). Control group fed on galactosemic diet only, for 40 days. The rats eyes lenses were observed by naked eye as well as ophthalmoscope every day and cataract formation were graded as follow: Grade I: vacuoles present at a part of the cortical equator of the lens; Grade II: a Y shape cavity at center of lens and vacuoles around it; Grade III: the lens center become milky color and cavity disappeared; Grade IV: the lens becomes opaque [17].

Determination of glutathione (GSH) and lipid peroxides (LPO)

At the end of 20 days all the left eye lens of animals in 3 groups were removed following anesthesia. The lenses were homogenized in 50mM PBS with pH=7.4, 1.0 mM EDTA, 0.15 M KCl. The concentrations of glutathione were assayed by the methods of Griffith [18]. Briefly, the glutathione levels were measured by the enzymatic recycling method using glutathione reductase and 5',5'-dithio-bis (2-nitrobenzoic acid) in which GSH

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is oxidized by 5',5'-dithio-bis (2-nitrobenzoic acid) and reduced by NADPH in the presence of glutathione reductase. 2-Nitro-5-thiobenzoic acid formation is monitored at 412 nm. The levels of GSH were calculated based on protein concentration. The amount of lens LPO determined is expressed as that of malonaldehydes (MDA). LPO assayed spectrophotometrically were diene and triene conjugates, and malonaldehydes (MDA) were determined as thiobarbituric acid-reactive material [19]. The protein concentration of samples were determined by using of dye binding method [20].

Statistical analysis

Values were represented as mean ± SD. All the results were analyzed by computerized statistical package (SPSS ver.11.5). Data were analyzed using one-way analysis of variance (ANOVA). P values <0.05 were considered significant.

Results

Cataract progression

As summarized in table 1 in control group rats fed galactose diet the grade I cataract formation observed after 8 days, grade II after 11, grade III after 19 and complete cataract formation after 31 days. In silymarin treated groups all grade of cataract progression was retarded significantly as compared to control group. The grade IV cataract formation was not observed in 40% of animals in silymarin treated group.

Lens glutathione (GSH) and lipid peroxides (LPO) content

As summarized in table 2 in rat fed on galactose diet and treated with silymarin for 20 days the lens GSH level was increased significantly and LPO level was decreased significantly as compared to control group.

Discussion

In present study administration of daily 200 mg/kg silymarin for 40 days to rats fed on galactose diet prevent all 4 stage of cataract progression in all the animals. In addition the stage IV or complete cataract formation did not observed in 40% of rats in silymarin treated group. Furthermore in silymarin treated group the rat lens glutathione and lipid peroxides level decreased significantly.

The mechanism underlying the preventive effect of silymarin on galactose induced cataract development is not known.

Table 1: The average (means ± SD) days of cataract progression in control and silymarin treated groups.

		Grade of cataract progression			
	Groups	**1**	**2**	**3**	**4**
Control	10	8.0 ± 0.8	11.0 ± 0.8	19.0 ± 1.4	31.0 ± 1.8
Silymarin 200 mg/kg/d	10	21.0 ± 1.4*	25.0 ± 0.8*	31.7 ± 1.3*	43.2 ± 5.1*

*p<0.01

Table 2: The average (means ± SD) lens GSH and LPO level after 20 days in control and silymarin treated as well as control group.

Groups N=10	GSH level (nmol/mg protein)	LPO (MDA) level (pmol/mg protein)
Normal (rat chow)	140.8 ± 12.3	30.4 ± 3.2
Control	60.4 ± 7.3	60.8 ± 5.3
Silymarin 200 mg/kg/d	75.3 ± 11.2*	45.6 ± 4.2*

(The results of control and silymarin treaeted groups were analyzed statistically)
*p<0.05

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The galactose - induced cataract in rats is a type of diabetic cataract in which oxidative damage; osmotic stress and other cellular metabolic abnormality could be associated with pacification of lenses [21]. The increase lipid peroxidation may be one of the mechanisms of cataractogenesis, initiated by enhanced production of oxygen free radicals in the eye fluids and tissues and impaired enzymatic and non-enzymatic antioxidant defenses of the lens in the favor of cataract development [9, 22]. The increase cellular glutathione and decrease lipid peroxidase level in silymarin treated galactosemic rat lens may reduce the oxidative damage induced by galactose on rat lenses leading to inhibition of cataract development. In support to present observation the anti cataractogenesis of vitamin E a powerful antioxidant and curcumin an herbal medicine with antioxidative property have been also reported previously [23-25].

In the process of cataract development oxygen free radicals react with and damage several lens enzyme and crystalline proteins as well as cellular membrane component. These effect render cellular membrane permeability defect, cellular metabolic derangement and thereby accumulation of a waste protein in the lens [26, 27]. The accumulation of waste proteins in the lens acts as the center of cataract formation [27]. Silymarin due to its cellular membrane stabilizing properties, increasing glutathione and decreasing lipid peroxides level as well as scavengering free radical may counteract the damage induced by free radical on lens cellular component and metabolic presses [13, 14, 15].

The osmotic stress due to accumulation of galactitol via aldose reductase within lens fibers link to cataractogenesis [21]. Silymarin is known to function as aldose reductase inhibitors may reduce accumulation of galactitol there by osmotic stress in the lens [29].

In conclusion the result obtained in present study, indicate that the administration of silymarin to galactose fed rat prevented cataract progression as well as induced favorable effect on antioxidative defence system such as increase in lens GSH and decrease LPO levels. It may suggest that antioxidative and increasing GSH level property of silymarin contribute to anticataractogenic effect observed in present study.

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