

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

Fallah Huseini H (Ph.D.)<sup>1</sup>, Zaree Mahmoodabady A (Ph.D.)<sup>2\*</sup>, Heshmat R (Ph.D.)<sup>3</sup>, Raza M (M.D., Ph.D.)<sup>4</sup>

1- Department of Pharmacology, Institute of Medicinal Plants ACECR, Tehran, IR IRAN

2- Department of Biochemistry, Faculty of Medicine, Baqiyatallah (a.s.) University of Medical Sciences, Tehran, IR IRAN

3- Endocrinology & Metabolism Research Center, Tehran University of Medical Sciences Tehran, IR IRAN

4- Applied Neuroscience Research Center, Baqiyatallah University of Medical Sciences, Tehran, IR IRAN

\*Corresponding author: Department of Biochemistry, Faculty of Medicine, Baqiyatallah (a.s.) University of Medical Sciences, Tehran, IR IRAN

Tel & Fax: +98-21-22219981-3

E-mail: alizare80@yahoo.com

Receive: 18 Jan. 2009

Acceptance: 21 Feb. 2009

### 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



## 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



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  $\pm$  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  $\pm$  SD) days of cataract progression in control and silymarin treated groups.**

|                          | Groups | Grade of cataract progression |             |             |             |
|--------------------------|--------|-------------------------------|-------------|-------------|-------------|
|                          |        | 1                             | 2           | 3           | 4           |
| Control                  | 10     | 8.0 ± 0.8                     | 11.0 ± 0.8  | 19.0 ± 1.4  | 31.0 ± 1.8  |
| Silymarin<br>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  $\pm$  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 $\pm$ 12.3            | 30.4 $\pm$ 3.2                    |
| Control               | 60.4 $\pm$ 7.3              | 60.8 $\pm$ 5.3                    |
| Silymarin 200 mg/kg/d | 75.3 $\pm$ 11.2*            | 45.6 $\pm$ 4.2*                   |

(The results of control and silymarin treated groups were analyzed statistically)

\*p<0.05

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.

## Acknowledgements

We thank to Gillben J Frank Eye Research Institute, Oakland University, Rochester, Michigan and Ohta Y et al. Department of Chemistry and Ophthalmology, School of Medicine, Fujita Health University, Aichi, Japan for sending us a copy of their article on antioxidant and cataract. We also thank the administration of the Institute of Medicinal Plants for their support in providing the necessary facilities for conducting this study.

## References

1. Abraham AG, Condon NG, West Gower E. The new epidemiology of cataract. *Ophthalmol Clin. North Am.* 2006; 19 (4): 415 - 25.



2. Bao YZ, Cao XG, Li XX, Chen J, Hu JX, Zhu T. Prevalence of age-related cataract among adults aged 50 and above in four rural areas in western China. *Zhonghua Yi Xue Za Zhi* 2008; 88 (24): 1697 - 702.
3. Dal Pizzol MM, Esteves JF, Scococo CA, Roggia MF, da Rosa CM, Lambert JH, Canani LH. Cataract and type 1 diabetes mellitus. *Arq Bras Ophthalmol.* 2008; 71 (4): 564 - 7.
4. Meyer LM, Dong X, Wegener A, Söderberg P. Dose dependent cataractogenesis and Maximum Tolerable Dose (MTD (2.3:16)) for UVR 300 nm-induced cataract in C57BL/6J mice. *Exp. Eye. Res.* 2008; 86 (2): 282 - 9.
5. Tan JS, Wang JJ, Younan C, Cumming RG, Rochtchina E, Mitchell P. Smoking and the long-term incidence of cataract: the Blue Mountains Eye Study. *Ophthalmic Epidemiol.* 2008; 15 (3): 155 - 61.
6. Reichle ML. Complications of intravitreal steroid injections. *Optometry* 2005; 76 (8): 450 - 60.
7. Spector A. Review: Oxidative stress and disease. *J. Ocul. Pharmacol. Ther.* 2000; 16 (2): 193 - 201.
8. AuTan AG, Mitchell P, Flood VM, Burlutsky G, Rochtchina E, Cumming RG, Wang JJ. Antioxidant nutrient intake and the long-term incidence of age-related cataract: the Blue Mountains Eye Study. *Am. J. Clin. Nutr.* 2008; 87 (6): 1899 - 905.
9. Maurya OP, Mohanty L, Bhaduri G, Chandra A. Role of anti-oxidant enzymes superoxide dismutase and catalase in the development of cataract: study of serum levels in patients with senile and diabetic cataracts. *J. Indian Med. Assoc.* 2006; 104 (7): 396 - 7.
10. Richer S. Antioxidants and the eye. *Int Ophthalmol Clin* 2000; 40 (4): 1 - 16.
11. Wu SY and Leske MC. Antioxidants and cataract formation: a summary review. *Int. Ophthalmol Clin.* 2000; 40 (4): 71 - 81.
12. Ohta Y, Torii H, Yamasaki T, Niwa T, Majima Y and Ishiguro I. Preventive action of vitamin E-containing liposomes on cataractogenesis in young adult rats fed a 25% galactose diet. *J. Ocul. Pharmacol. Ther.* 1997; 13 (6): 537 - 50.
13. Song Z, Deaciuc I, Song M, Lee DY, Liu Y, Ji X, McClain C. Silymarin protects against acute ethanol-induced hepatotoxicity in mice. *Alcohol. Clin. Exp. Res.* 2006; 30 (3): 407 - 13.
14. Kiruthiga PV, Shafreen RB, Pandian SK, Devi KP. Silymarin protection against major reactive oxygen species released by environmental toxins: exogenous H<sub>2</sub>O<sub>2</sub> exposure in erythrocytes. *Basic Clin. Pharmacol. Toxicol.* 2007; 100 (6): 414 - 9.
15. Muzes G. and Deak M. Effect of the bioflavonoids silybum marianum on the in vitro activity and expression of superoxide dismutase (SOD) enzyme. *Acta Physiol. Hungarica.* 1991; 78: 3 - 9.
16. Gerster, H. Antioxidant vitamins in cataract prevention. *Z. Ernährungswiss* 1989; 28: 56 - 75.
17. Gupta SK, Joshi S, Tandon R, Mathur P. Topical aspirin provides protection against galactosemic cataract. *Indian. J. Ophthalmol.* 1997; 45 (4): 221 - 5.
18. Griffith O. W. Determination of glutathione and glutathione disulfide using glutathione reductase and 2-vinylpyridine. *Anal. Biochem.* 1980; 106: 207 - 12.
19. Ohkawa H, Ohishi N, Yagi K. Assay for

lipid peroxides in animal tissues by thiobarburic acid reaction. *Anal. Biochem.* 1979; 95: 351 - 8.

**20.** Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* 1976; 72: 248 - 54.

**21.** Ohta Y, Yamasaki T, Niwa T, Goto H, Majima Y, Ishiguro I. Cataract development in 12-month-old rats fed a 25% galactose diet and its relation to osmotic stress and oxidative damage. *Ophthalmic Res.* 1999; 31 (5): 321 - 31.

**22.** Ozmen D, Mutaf I, Ozmen B, Menten J, Bayindir O. Lens lipid peroxides and glutathione concentrations in diabetic cataract. *Ann Clin. Biochem.* 1997; 34 (Pt 2): 190 - 2.

**23.** Karslioglu I, Ertekin MV, Kocer I, Taysi S, Sezen O, Gepdiremen A, Balci E. Protective role of intramuscularly administered vitamin E on the levels of lipid peroxidation and the activities of antioxidant enzymes in the lens of rats made cataractous with gamma-irradiation. *Eur. J. Ophthalmol.* 2004; 14 (6): 478 - 85.

**24.** Palla S, Kamala K, Bhanuprakash GR. Effect of curcumin on galactose-induced

cataractogenesis in rats. *Molecular Vision* 2003; 9: 223 - 30.

**25.** Raju TN, Kumar CS, Kanth VR, Ramana BV, Reddy PU, Suryanarayana P, Reddy GB. Cumulative antioxidant defense against oxidative challenge in galactose-induced cataractogenesis in wistar rats. *Indian J. Exp. Biol.* 2006; 44 (9): 733 - 9.

**26.** Choudhary S, Xiao T, Vergara LA, Srivastava S, Nees D, Piatigorsky J, Ansari NH. Role of aldehyde dehydrogenase isozymes in the defense of rat lens and human lens epithelial cells against oxidative stress. *Invest Ophthalmol. Vis. Sci.* 2005; 46 (1): 259 - 67.

**27.** Reddy VN, Giblin FJ. Metabolism and function of glutathione in the lens. *Ciba. Found. Symp.* 1984; 106: 65 - 87.

**28.** Taylor A, Davies KJ. Protein oxidation and loss of protease activity may lead to cataract formation in the aged lens. *Free Radic. Biol. Med.* 1987; 3 (6): 371 - 7.

**29.** Zhang JQ, Mao XM, Zhou YP. Effects of silybin on red blood cell sorbitol and nerve conduction velocity in diabetic patients. *Zhongguo. Zhong. Xi Yi Jie He Za Zhi* 1993; 13 (12): 725 - 6.