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Evaluation of *in vitro* Anti Cataract activity of Sinapic Acid

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Abstract

In homeopathic remedies, sinapic acid (SA), a powerful nutraceutical, is used to cure cataracts and other eye-related conditions. One of the main underlying causes of cataracts is diabetes. This study used fresh goat lenses and a variety of in vitro techniques to assess the anti-cataract activity of sinapic acid (SA).

Keywords: Cataract, Sinapic Acid, Naringin, Hyperglycaemia, Diabetic retinopathy, Oxidative stress.

1. Introduction

Due to the increased incidence and progression of cataract in patients with diabetes mellitus, cataract is thought to be a primary cause of visual impairment in diabetic patients [1, 2]. Clinical epidemiological and fundamental scientific investigations have demonstrated the correlation between diabetes and the development of cataracts. Worldwide, the prevalence of type 1 and type 2 diabetes is gradually rising, which contributes to an increase in diabetic cataracts. Although the most common surgical ocular procedure performed globally, cataract surgery, is a beneficial treatment, it is still difficult to understand the pathomechanisms that prevent or delay the development of cataract in diabetic patients. Moreover, complications after cataract surgery are more common in people with diabetes mellitus [3]. Diabetes and cataracts are major health and financial burdens, especially in underdeveloped nations where access to cataract surgery is generally limited and diabetes treatment often inadequate [4].

The polyol pathway, which is connected to the formation of diabetic cataract, is catalyzed by the enzyme aldose reductase (AR) and involves the conversion of glucose

to sorbitol. The central significance of the AR pathway as the beginning factor in the formation of diabetic cataracts has been the subject of extensive research.

Research has demonstrated that the build-up of sorbitol inside cells causes osmotic shifts that cause hydropic lens fibers to deteriorate and develop sugar cataracts [5, 6]. Sorbitol dehydrogenase in the lens produces sorbitol more quickly than it can be converted to fructose. Furthermore, sorbitol's polar nature inhibits diffusion-based intracellular elimination. An infusion of fluid countervails the osmotic gradient as a result of the increased sorbitol accumulation, which produces a hyperosmotic effect. According to research on animals, intracellular polyol buildup causes lens fibers to collapse and liquefy, which in turn causes lens opacities to occur [5, 7]. These results have given rise to the "Osmotic Hypothesis" of sugar cataract formation, which emphasizes that intracellular fluid increase in response to AR-mediated polyol accumulation causes lens swelling, which in turn causes intricate biochemical alterations that ultimately result in the formation of cataracts [5, 6, 8].

In recent years, there has been a lot of interest in the possibility of using our natural resources to prevent the onset and advancement of cataract. It is claimed that many medicinal plants and their preparations offer cataract prevention and antioxidant properties. For this worldwide problem, the production of nutraceutical doses from goods with remarkable anti-traumatization action may be advantageous.

Phenolic acids, which are found in food items and medicinal plants, are one type of antioxidant [9, 10]. Sinapic acid is a derivative of hydroxycinnamic acid and an example of phenolic acid. Fruits (such as strawberries or citruses) and vegetables (particularly those in the Brassicaceae family, such as broccoli or tronchuda cabbage) contain sapapic acid [11].

Sinapic acid has been used in the homeopathic system to treat cataracts since ancient times. The serum of ovariectomized rats in the early stages of estrogen shortage showed favorable effects of sinapic acid on indices associated with glucose and lipid metabolism, as well as on certain parameters of oxidative stress [12,13]. There have also been recommendations on the potential use of phenolic acids, such as sinapic acid, to prevent the development of cataracts, based on numerous experimental in vitro and in vivo animal studies [14,15,16,17].

This study aimed to assess the anti-cataract effects of sinapic acid in isolated lenses from goats and chicks.

2. Materials & Methods

Material Sinapic acid purchased from Sigma Aldrich, USA. All the other chemicals used were of analytical grade.

2.1 Lens culture preparation

We followed the procedure proposed method by Nithya S (2012), for evaluating anti-cataract activity. Goat eyeballs were obtained freshly from the nearby slaughterhouse and then immediately refrigerated at temperature of 0-5° C (210). The extracapsular extraction procedure was carried out by removing eyeball lenses and then incubated in prepared artificial aqueous humor solution (5.5 mM glucose, 0.5 mM $CaCl_2$, 0.5 mM NaH_2PO_5, 0.5 mM $NaHCO_3$, 2 mM $MgCl_2$, 5mM KCl and 150 mM NaCl) at ambient temperature and pH maintained at 7.8 by adding $NaHCO_3$ for 72 hours. The microbial contamination to isolated goat lenses was prevented by the addition of Ceftriaxone at 5mg/mL dose [18,19].

2.2 Induction of cataract by *in vitro* method

A total number of 55 goat lenses were utilized in the experimental study. These isolated lenses were then incubated in an aqueous humor solution adding different concentrations of glucose substrate (5.5 mM represented as normal control and 55 mM represented as cataract control) for a period of 72 hours. Glucose substrate at 55 mM concentration was utilized for inducing cataracts in goat-isolated lenses. At this concentration, glucose substrate was metabolized by sorbitol enzymatic pathway which results in the generation of polyols (sugar alcohols). This accumulated sugar alcohol induces over hydration followed by oxidative stress, which then generates cataractogenesis [20].

2.3 Effect of SA on isolated goat lenses

Prepared goat lenses were separated into 4 groups and every group containing 6 lenses. These lenses were incubated as mentioned below:

Experimental design

Group I : Normal control (Glucose 5.5 mM)

Group II : Cataract control (Glucose 55 mM)

Group III : Glucose 55 mM + SA (50 µg/mL)

Group IV : Glucose 55 mM + SA (100 µg/mL)

After incubating for a period of 72 hours, isolated lenses were fixed on wired mesh by placing the posterior surface touching towards the mesh (the pattern of mesh number and squares should be visible clearly through the fixed lenses) was examined and the lens opacity was estimated.

The opacity intensity was graded as mentioned below:

0 → Lack of opacity (All mesh square boxes are visible)

++++ → Dense opacity (No boxes are visible)

+++ → Moderate opacity (3/5 boxes are visible)

++ → Inter mediate diffused opacity (1/2 boxes are visible)

+ → Mild opacity (1/5 boxes are visible)

After incubating for a period of 72 hours, lenses homogenate was mixed in 0.23 M Tris buffer solution at pH 7.8, which contains $0.25X10^{-3}$M EDTA and the homogenate concentration was adjusted to 10 % w/v. Then the homogenate was subjected to centrifugation process for a period of 1 hour, speed maintained at 10,000 rpm and temperature at 5°C. The obtained supernatant liquid was utilized for estimating various biochemical parameters like ions (K^+ and Na^+), catalase and proteins.

3. Results and Discussion

3.1 Effect of SA on isolated goat lenses

Table 1- The opacity grades after administering SA

Treatment	Grade
Control	0
Cataract Control	3
SA (50 µg/ml)	2
SA (100 µg/ml)	1

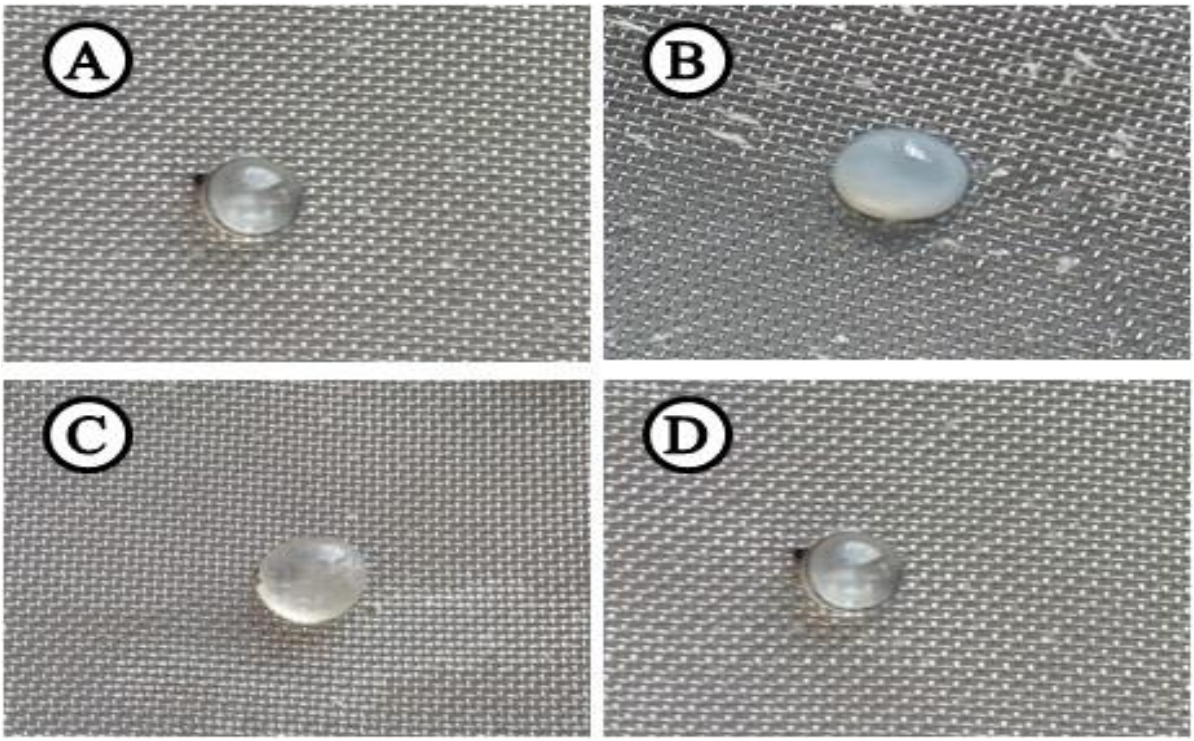

Figure 1. Photographical representation and evaluation of opacity in isolated goat lenses- a) Control b) Cataract Control c) SA (50 µg/mL) d) SA (100 µg/mL).

Table 2. Effect of SA on Sodium, Potassium, Total protein and Catalase levels in Cataract induced by glucose.

Groups	**Sodium (µg/mL)**	**Potassium (µg/mL)**	**TPC (gm/dL)**	**Catalase (µm of H_2O_2/min)**
Control	105.5±+2.10	10.8±0.44	3.25±0.01	228.3±0.85
Cataract Control	227.3±3.30	6.17±0.11	1.87±0.03	143±0.91
SA (50 µg/mL)	165.8±1.93	8.95±0.12	2.52±0.04	217.5±1.04
SA (100 µg/mL)	116±1.29	10.18±0.15	2.86±0.04	204±1.86

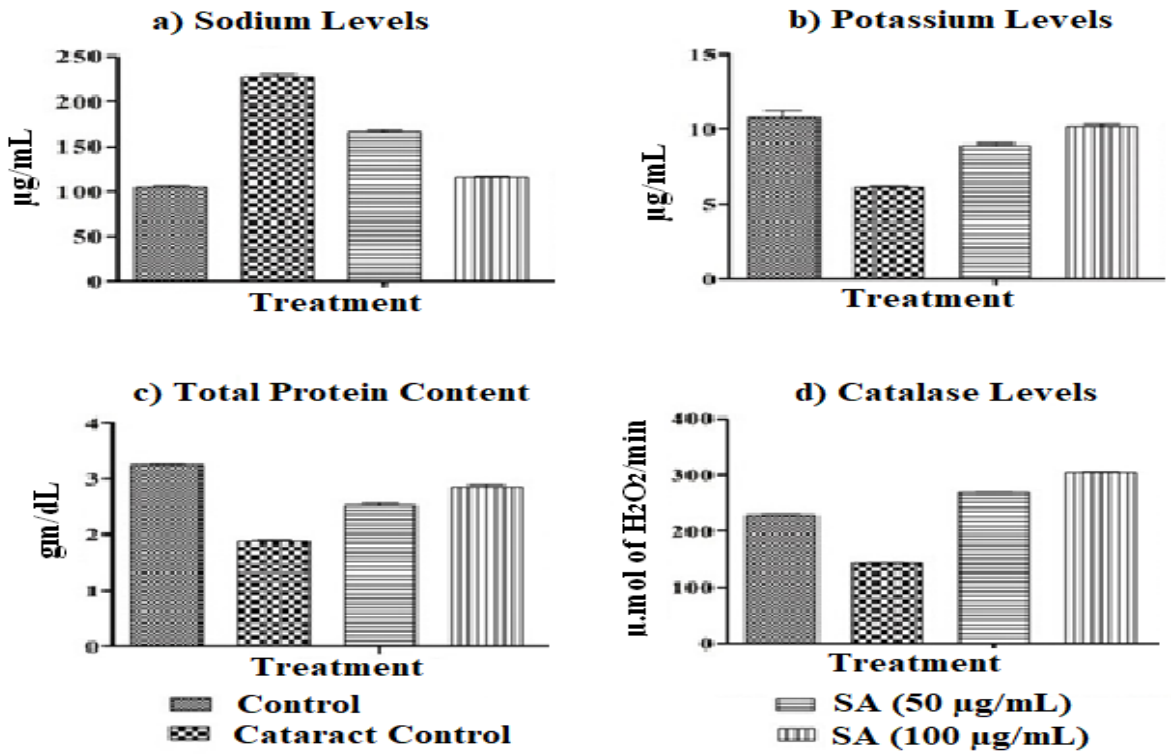

Figure 2. a) Indicates sodium levels after administering with 2 different doses of SA to cataract induced by glucose (55Mm) in isolated goat lenses. While, Potassium, Total protein content and Catalase levels were presented in **b**, **c** and **d** correspondingly. Treatment with SA at doses 50 and 100 µg/mL was done. The obtained values were demonstrated as Mean ± SEM; By using Dunnett's test, one-way ANOVA significance was calculated. The obtained results were compared with the values of cataract control.

3.2 Discussion

Isolated goat lenses in normal control group were remined transparent. Dense opacities had been noticed in goat lenses which were subjected to incubation for about 72 hours using 55.5 mM of glucose substrate solution only, was presented as Cataract Control. Lenses opacity was increased consistently towards the centre part with almost complete opacification. In the current research study, it was demonstrated that opacification was decreased after administering SA, at high concentration (100 µg/mL). Similar type of observations was demonstrated in the research study performed on rat lenses. In this rat lenses were loaded with glucose substrate, demonstrated haziness and increased opacification when correlated with control group lenses suggesting development of cataract. In the current research study, lenses opacification decreases gradually after treating with SA as presented in Tables 1 - 2 and depicted in Figures 1 - 2. SA incubated lenses homogenate Na^+ ion levels were decreased significantly from 227.3±3.30to 120±0.9 and K^+ ion levels were increased from 6.17 ±0.11 to 9.8±0.1 in cataract lenses homogenate. The catalase and total protein levels were decreased significantly from 228±0.85 to 143±0.91, 3.25±0.01 to 1.87±0.03, correspondingly in cataract lenses homogenate. Increased K^+ ion levels, along with decreased total protein, Na^+ ion levels catalase restoration of lenses by SA strongly suggested its anti-cataract activity. This clearly indicated that SA prevents the alteration of K^+ ion and Na^+ ion balance, which directly effects on Na^+K^+ATPase present in lenses membrane and indirectly through their radical scavenging capability. Numerous research studies were reported that flavonoids exhibited greater preventive capability on development of cataract.

Our present research study, demonstrated that flavonoid portion in SA, may be responsible for anti-cataract activity in cataract lenses. Additionally, reduction in lenses opacification, increased catalase, total protein and K^+ ion levels along with decreased Na^+ ion levels, after SA treatment showed that the results obtained were précised.

4. Conclusion

The obtained results of the present study show that the nutraceutical dosages support its well-prescribed and extensive use in the treatment of cataract. During the study, it observed that opacity was reduced after addition of Sinapic acid (SA) at higher concentration i.e.100 µg/mL. The findings support a protective role of Sinapic acid (SA) in pathologies involving Oxidative stress, namely cataract. However, further studies to identify and isolate the main constituents responsible for its anti-cataract activity and proper dosage form for maximum benefits of this SA to be carried out in future.

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