Potential of traditional food supplement, Soya bean as a novel anti cataract agent

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Abstract
Soya bean (SB) is a source of protein and nutritional antioxidant. The present study evaluated for it’s anti cataract potential in galactose and selenite induced experimental cataractogenesis in vitro and in vivo. In vitro osmotic and oxidative stress was induced by galactose and sodium selenite respectively in the culture medium. After 24 h of incubation, lenses were subjected to the estimation of biochemical parameters and anti oxidant enzymes. In vivo 10 animals were taken in each group; galactose cataract was induced in rats by 30% galactose in the diet and in the treated groups were received SB diet along with 30% galactose up to 30 days. In vivo selenite cataract was induced in rat pups by subcutaneous injection of sodium selenite (25 μmole/kg body weight). Treatment group animals received SB extract (10, 20, 30 mg/kg body weight, ip) 4 h before the selenite challenge. The incidence of cataract was seen at 18th postnatal day. A fall in the glutathione level, rise in malondialdehyde and polyol content were observed in control as compared to normal lenses. SB significantly restored glutathione level and decreased the malondialdehyde and polyol content. A significant restoration in the activities of superoxide dismutase, catalase, glutathione-S-transferase, glutathione peroxidase was found in the SB supplemented group. A significant delay in the onset and progression of cataract was observed with 10% SB diet. At the end of 30th day none of the eyes developed mature cataract as compared to 100% in the control group. SB 20 mg/kg also reduced the incidence of selenite cataract. Only 14.3% of the eyes in the test group developed nuclear cataract as compared to 85.8% control. SB protects against experimental cataract development by virtue of its antioxidant properties.

Keywords: Glutathione, Malondialdehyde, Oxidative stress, anti oxidant.

INTRODUCTION
Cataract occur when the lens of the eye becomes clouded or opaque, resulting in poor vision or vision loss; is the leading cause of blindness and contributes to 50% of blindness worldwide (WHO, 2005). In the United States for about 60 percent of all medicare costs related to vision (Congdon et al., 2004). This is a very serious issue as cataract is easily curable through a surgery which is today available the world over. Yet cataract remains a public health problem in many developing countries. Traditionally, the cataract intervention programme is evaluated by the number of cataract operations performed per million populations per year (Vision 2020, 2006). However, the surgery has its own limitations; the inflammatory response is more pronounced post-operatively, loss of vitreous humor, posterior capsule opacification (Shamanna and Muralikrishnan, 2004) and expensive (Kyselova et al., 2004). So there is a need to look at the impact of treating cataracts and relate it not just to surgery but also to scholastic achievements and development. In recent times, more research is on herbal drugs and natural products to develop more safe, effective and economical treatment for prevention or delay the cataract. (Gupta et al., 2002, Gupta et al., 2003, Gupta et al., 2005).

Legumes have played an important role in the traditional diets of many regions throughout the world. Soya bean (Glycine max; Family: Leguminosae) is also known as soy bean, unique among the legumes because they have many...
important nutritious components, such as proteins, fats, carbohydrates and α-tocopherol, long term continuous in take of this food might effectively prevent senility (Ishii and Tanizawa, 2006). Soya bean contains concentrated source of isoflavones, well established and known (Hellendoorn, 1976; Barnes, 1998). The primary isoflavones are genistein, daidzein and glycitein (Coward et al., 1993) and they have estrogen-like structures (Xu et al., 1994; Julia, 2006). It contains other bioactive components, including saponins, protease inhibitors, phytic acid, and amino acids (Lasztity et al., 1998).

A considerable amount of research is being done and investigated that Soya beans are potential antioxidants (Tikkennen et al., 1998; Corinna et al., 2006; Kulling, 2001) and effective in the management of diabetes (Salmeron et al., 1997; Foster-Powell and Miller 1995; Wolever et al., 1992). The studies also demonstrate that Soya bean can offer protection against diseases such as cancer (Pei et al., 2003), coronary heart disease (Anthony et al., 1996) and osteoporosis (Arjmandi et al., 1998). However, information regarding the anticataract activity of Soya bean is limited.

Therefore, the present study was undertaken to evaluate the anticataract potential of Soya bean against galactose and selenite-induced cataract in vitro and in vivo experimental models. These two models were selected as it resembles some of the biochemical characteristics of senile cataract and galactose produces more number of polyols per unit as compared to other sugar-induced models. In addition, the effect of Soya bean on various biochemical parameters was also observed to elucidate the mechanisms of protection.

**MATERIALS AND METHODS**

**Chemicals**

The chemicals were obtained from the sources mentioned as follows: Galactose was purchased from SD fine-Chem Limited (Mumbai). Chemicals required for the enzyme assay were obtained from sigma Chemical Co., USA. The oxidative stress inducing agent, sodium selenite was purchased from Central Drug House (P) LTD. New Delhi.

**Animals**

Wistar rats of either sex (60-80g) were procured from the animal house, Delhi Institute of Pharmaceutical Sciences and Research, after getting the approval from Institutional Animal Ethical Committee. The animals in the current study were treated in accordance with the institutional guidelines and Association for Research in Vision and Ophthalmology statement for the use of animals in research. The mother and suckling pups were left undisturbed to acclimate for 4 days before the experiment.

**Preparation of aqueous extract of Soya bean**

The Soya bean flour was purchased from ‘Whole Foods’ (an approved center of American Soybean Association), Friends Colony, New Delhi. The accurately weighed 100 g of Soya bean flour was soaked in one liter of double distilled water for 24 h with 2 ml of chloroform to prevent bacterial contamination. Thereafter, the extract was decanted and the flour was again re-soaked. This procedure was repeated 3 times under the similar procedure. The total filtrate was collected and lyophilized. Yield was calculated. Lyophilized powder thus obtained was stored in desiccators at 4°C.

**In vitro studies with the lowest effective concentration of Soya bean**

Rats were anesthetized with ether. The lenses were carefully enucleated from eyes with a posterior approach. Each isolated lens was placed in a Falcon plastic culture plate (24-well) containing 2 ml of Dolbico’s Modified Eagle Medium (DMEM) supplemented with 20% fetal bovine serum, 100µg/ml of streptomycin, and 100 IU/ml penicillin. The lenses were incubated at 37°C under 90% moisture, 95% air, and 5% CO₂ gas atmosphere for 2 hr. The damaged lenses that developed artificial opacities were discarded and only transparent lenses were taken for the subsequent in vitro experiment.

**Galactose-induced osmotic stress in vitro**

Transparent cultured lenses were randomly divided into normal, galactose only and two treatment groups each comprising six lenses. Normal lenses were incubated in DMEM alone, whereas control group lenses were incubated in...
Soya bean DMEM supplemented with 30mM of galactose. Medium in the treated groups was additionally supplemented with different concentrations of Soya bean (100, 200 and 300 µg/ml) along with galactose. All the lenses in different groups were maintained for 24 hr at the above-mentioned experimental conditions of incubation. Post incubation, the lenses were examined for the presence of any opacity, and photo documentation was done. Thereafter, lenses were washed, weighed, and processed for the estimation of biochemical parameters. Each lens was homogenized in 1ml of 0.1M-phosphate buffer (pH 7). The homogenate was divided into two equal parts. One part was used for the estimation of glutathione (GSH) and the other for polyols.

Selenite induced oxidative stress

The same experimental procedure was followed as mentioned in galactose induced osmotic stress in vitro, instead of galactose, 100 µM of sodium selenite was supplemented in control and treated groups. The homogenate was used for the estimation of GSH and malondialdehyde (MDA).

Estimation of Glutathione (GSH)

The GSH content was estimated by the method of Moron et al (1979). The homogenate was centrifuged at 5000 rpm for 15 min at 4°C. To the supernatant, 0.5ml of 10% trichloroacetic acid was added and recentrifuged. The protein-free supernatant thus obtained was reacted with 4ml of 0.3 M of Na2HPO4 (pH 8.0) and 0.5ml of 0.04%(wt/vol) 5,5'-dithiobis-2-nitrobenzoic acid. The absorbance of the resulting yellow color was measured in spectrophotometer at 412nm. A parallel standard was also maintained.

Estimation of polyols

Polyol estimation was done by the method described by West and Rapoport (1949). The homogenate was reacted with 0.6 M perchloric acid. Precipitate was removed by centrifugation and the supernatant was neutralized with 2N NaOH. Again the precipitate was removed by centrifugation and clear supernatant was reacted with, freshly prepared 0.125 M stannous chloride and 0.2% chromotropic acid. The absorbance of purple colored complex was measured spectrophotometrically at 570 nm. Parallel standard was subjected to the above-mentioned steps for the calculation of polyol in the samples.

Estimation of malondialdehyde (MDA)

Estimation was done by the method described by Satoh with modification (1978). The homogenate was mixed with 0.15M KCl and centrifuged at 10,000 rpm for 10 minutes. 0.2 ml of the supernatant was reacted with 0.2 ml of 8.1% of SDS, 1.5ml of 20% acetic acid (pH 3.5) and 1.5 ml of TBA. All the samples were heated in a boiling water-bath for 60 minutes. After cooling, 5 ml of n-butanol:pyridine mixture was added to each sample. The solution was shaken vigorously in a vortex and centrifuged at 5,000 rpm for 10 minutes. Organic layer was separated and absorbance was observed in spectrophotometer at 515 nm. Simultaneously various amount of 1,1’3, 3’-tetra methoxy propene (TMP) was used as a standard to obtain a standard curve for the calculation of unknown MDA in the samples.

Enzyme assay

A separate set of experiments was conducted under the same experimental conditions as described above. After incubation for 24 h lenses of each group were processed for the measurement of enzyme activities such as, Superoxide dismutase (SOD), Catalase (CAT), Glutathione peroxidase (GSHPx), and Glutathione-S-transferase (GST). 10% (w/v) lens homogenate was prepared in 50mM of phosphate buffer (pH 7.0) after centrifugation at 5,000 rpm for 15 min at 4°C, and the supernatant was used for the measurement of enzyme activities.

SOD

The activity of SOD was assessed by monitoring the ability of the enzyme spectrophotometrically at 480 nm to inhibit the oxidation of epinephrine (Misra and Fridovich, 1976). One unit of SOD activity is defined as the amount of enzyme required to produce 50% inhibition of epinephrine auto-oxidation.

CAT

The enzyme activity was measured spectrophotometrically at 240 nm by following the decomposition of H2O2 (Aebi, 1974). One unit of CAT activity is defined as nmol H2O2 decomposed per min/mg protein.
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GSHPx
Activity was monitored at 340 nm. One unit of enzyme activity is defined as 1 nmol of NADPH used per minute at 37 °C (Paliga and Valentine, 1967).

GST
The conjugation of GSH with 1 chloro, 2-4 dinitro benzene (CDNB), a hydrophilic substrate, was observed spectrophotometrically at 340 nm to measure the activity of GST. One unit of GST is defined as the amount of enzyme required to conjugate 1 μmol of CDNB with GSH/min (Hebig, 1974). Protein content in each sample was estimated by the method of Lowry et al (Lowry et al., 1951).

In vivo studies

Galactose cataract
Wistar rats of either sex in the weight range of 80 to 100 g were used for induction of cataract. They were divided into test and control groups each group was comprised of 10 rats. All the groups were fed 30% galactose in diet and water ad libitum. Soya bean 5%, 10% and 20% diet was given to the test groups. The treatment was started one week before galactose challenge and was continued until end of the experiment i.e. up to 30 day. At regular intervals, cataract stages in all the groups were observed with a slit-lamp. 1% tropicamide (Tropicacyl, Sunways (L) Pvt. Ltd) eye drops was administered to dilate the pupil. Different stages of cataract were graded: stage I, faint peripheral opacity; stage II, irregular peripheral opacity and slight involvement of the lens at the center; stage III, irregular opacity involving the entire lens; and stage IV, pronounced opacity readily visible as a white spot.

Selenite cataract
Nine-day-old Wistar rat pups were divided into a control and treated groups. In each group (n=10), pups of the same litter were housed with the mother. Acute stress was produced by a subcutaneous injection of 25 μmol sodium selenite per kg body weight to all the pups in the control and the treated groups. Soya bean extract at the dose of 20 mg/kg body weight was injected intraperitoneally to the pups in the treated groups 4 h prior to the selenite challenge. Incidence of cataract was observed through a slit lamp on the 16th postnatal day when the pups first opened their eyes.

Statistical analysis
All data were expressed as mean ± SD. The groups were compared by one-way ANOVA using post-hoc Dunnett’s test and Chi Square test with a p<0.05 considered as significant.

RESULTS

Effect on lens morphology
All the lenses in DMEM alone were transparent. However, lenses after 24 h of incubation in the presence of sodium selenite developed dense cortical opacity. Incorporation of 200 μg/ml of SB in the medium of the treated group offered significant protection. Only 33% of the lenses showed cortical opacity in the treated group (p<0.01 compared with control).

![Figure 1: Effect of Soya bean on GSH levels in galactose induced osmotic stress *in vitro*

Normal: DMEM, Control: DMEM+30mM galactose, Treated: DMEM+30mM galactose + Soya bean 200 μg/ml. Values are mean±SD. *p<0.01 (control vs treated), #p<0.05 (control vs normal) as compared to control. n=6.

Effect on GSH and polyols in galactose induced osmotic stress: *In vitro*

The GSH level was observed in control group 0.16±0.06 μmol/g of lens and in normal group it was found to be 1.61±0.06 μmol/g of lens. The Soya bean at the concentration of 200 μg/ml significantly restored the GSH level (1.50±0.07μmol/g) as compared to control (Fig. 1). Increased polyol level was observed in control (1.23±0.15 μg/mg of lens). However, G. max at the concentration of 200 μg/ml was found
Table 1: Effect of Soya bean on anti-oxidant enzymes in rat lens

<table>
<thead>
<tr>
<th>Groups</th>
<th>SOD (IU/mg protein)</th>
<th>CAT (IU/mg protein)</th>
<th>GPX (IU/mg protein)</th>
<th>GST (IU/mg protein)</th>
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<tr>
<td>Normal</td>
<td>2.24±0.14</td>
<td>1.15±0.09</td>
<td>10.76±0.91</td>
<td>2.04±0.19</td>
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<td>Control</td>
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<td>1.07±0.10*</td>
<td>0.16±0.01*</td>
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<td>Treated</td>
<td>1.09±0.01*</td>
<td>0.40±0.02*</td>
<td>6.43±0.17*</td>
<td>1.06±0.13*</td>
</tr>
</tbody>
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Normal: DMEM, Control: DMEM+Sodium selenite, Treated: DMEM+Sodium selenite+Soya bean 200μg/ml. Values are mean±SD. *P<0.005 (treated vs control); #P<0.005 (control vs normal).

Effect on GSH and MDA in selenite induced oxidative stress: In vitro

GSH level in the normal group was found to be 1.19±0.24 μmol/g. There was a significant reduction in GSH level in presence of selenite stress, and the level was found to be 0.11±0.05 μmol/g lens wt in the control group. Supplementation of Soya bean at the concentration of 200μg/ml significantly restored the GSH level by 1.00±0.25 μmol/g of lens (Fig. 3). MDA level in the normal group was found to be 1.29±0.11 nmol/mg of lens wt. There was a significant rise in MDA level in presence of selenite stress, and the level was found to be 30.47±2.63 nmol/mg of lens wt. in the control group. Incorporation of 200 μg/ml of Soya bean in culture medium significantly prevented the rise in MDA level. A decreased level of 7.56±2.16 was observed with 200 μg/ml of Soya bean in test groups (Fig. 4).

Effect of Enzyme activity

The effect of 200μg/ml of Soya bean on different antioxidant enzymes (SOD, CAT, GPX and GST)
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Normal: DMEM, Control: DMEM +Sodium selenite Treated: DMEM+ Sodium selenite+ Soya bean 200μg/ml. Values are mean ± SD. *P<0.005 (treated vs control); #P<0.005 (control vs normal

is presented in Table 1. It was observed that in presence of selenite stress, antioxidant enzymes were significantly reduced as compared with the normal group. In presence of Soya bean, there was a significant positive modulation of enzyme activities.

Effect of galactose cataract: In vivo

All eyes showed cataractogenic lenticular changes in the control animals. Whereas, in the Soya bean treated groups of 5%, 10% and 20% diets, in 10% diet fed animals, 95% eyes were normal on 7th day. At the end of 30th day, 100% of the control eyes developed mature cataract, whereas none of the eyes were found mature cataract in 10% diet fed animals. The opacity index in all the groups on various interval of time (on 7th, 14th, 21st and 30th day) was calculated to study the progression of cataract development (Fig. 5). The onset of cataract in both control and treated groups was observed within 7 days of galactose feeding.

![Figure 5](image-url): Effect of Soya bean on progression of galactose cataract in rats

Effect on selenite cataract: In vivo

In control group 85.8% of the eyes was developed nuclear cataract and 14.2% eyes developed diffused nuclear opacity on the postnatal day 16. In contrast among the all treatment groups of Soya bean, in 20mg/kg dose, 57.2% of the eyes were clear, 28.5% eyes were pinpoint opacity and 14.3% of the eyes developed nuclear cataract (Fig. 6).

DISCUSSION

Cataract is a multifactorial disease associated with several risk factors and it is responsible for 50% of blindness worldwide. At present, the only remedy for cataract is surgery. However the incidence is so large that the available surgical facilities are unable to cope up with the problem because of postoperative complications such as posterior capsular opacification, endophthalmitis and uncorrected residual refractive error (Varma and Hegde, 2004). It has been estimated that a delay in cataract onset by 10 years could reduce the need for cataract surgery by as much as half (Suryanarayana et al., 2005). Any intervention that prevents or slows the progression of cataract has a significant health impact. In recent years, a great emphasis has been laid on exploring the possibility of using our natural resources to delay the onset and progression of cataract. In our earlier studies, we have reported *Ocimum sanctum* and *Camellia sinensis* to possess antioxidant properties and offer protection against cataract (Gupta et al., 2005 & 2002). In the present study, Soya bean diet delayed the progression of cataract in rats and also soya bean extract prevented the cataract development in rat pups. Although, multiple mechanisms may contribute to these effects, the antioxidant effect
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<table>
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<tr>
<th></th>
<th>Clear</th>
<th>Fine point opacity</th>
<th>Nuclear cataract</th>
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<tbody>
<tr>
<td>Control</td>
<td>85.8%</td>
<td>14.2%</td>
<td></td>
</tr>
<tr>
<td>Treated 1</td>
<td>16.8%</td>
<td>41.6%</td>
<td></td>
</tr>
<tr>
<td>Treated 2</td>
<td>28.5%</td>
<td>57.2%</td>
<td></td>
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<tr>
<td>Treated 3</td>
<td>14.4%</td>
<td>42.8%</td>
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</table>

Figure 6: Effect of Soya bean on selenite cataract in rat pups
Control: Sodium selenite, Treated 1: Sodium selenite+ Soya bean 30mg/kg, Treated 2: Sodium selenite+ Soya bean 20 mg/kg, and Treated 3: Sodium selenite+ Soya bean 10mg/kg. **P<0.005, and NS Non significant (Control vs treated) n=12.

The predominant mechanism of action of Soya bean appears to be the predominant mechanism of action.

Oxidative stress plays an important role in the pathogenesis of cataract. In previous studies, we found that lycopene protects against oxidative stress induced experimental cataract (Gupta et al., 2003). Also, bioflavonoids are the common dietary constituents and have been reported to possess potential antioxidant property (Bors et al., 1990). An anticataract effect of flavonoids such as quercetin and myricetin has been reported (Mohan et al., 1988). Soya bean is the rich source of flavonoids and is consumed as a traditional food by a large population on a daily basis. Bioflavonoids like genistein, daidzein and glycitein, have been extensively evaluated for their therapeutic potential in various pathological conditions (Kulling et al., 2001). The strong antioxidant property of soya bean extract also has been demonstrated in various in vitro assays (Corinna et al., 2006).

In the present study, to initiate cataractogenic changes, isolated rat lenses were maintained in a physiologically balanced medium supplemented with high selenium and galactose. Selenite overdose cataract is an extremely rapid and convenient in vivo model. Several biochemical processes that occur during the production of the selenite cataract include altered epithelium metabolism, calcium accumulation, calpain-induced proteolysis, insolubilization of proteins, phase transition and opacification (Shearer et al., 1997). Strong evidence that, the role of calpain in precipitation of crystallins in selenite cataract has been provided by an in vitro model of proteolytic precipitation (Shearer et al., 1995). Galactose model is reasonable to assume that the factors initiating the galactose cataract in young rats are very similar to those involved in the human galactose cataract and produces more number of polyols when compared to other sugars (Kinoshita, 1965).

The most important function of GSH is to deactivate and render excess free radicals harmless. GSH is composed of the amino acids such as cysteine, glutamic acid, and glycine. It keeps the protein (crystallin) in reduced form. The GSH is depleted due to the toxic effect of selenite and converted to glutathione disulfide (GSSG). The basic effect of selenite on cellular membranes is believed to be the peroxidation of membrane lipids through the conversion of sulfhydryl group. Most interestingly, treatment with soya bean offered substantial protection against this depletion and maintained close to the normal level. The mechanism for the effect of Soya bean extract in the lens is unknown. However, from previous work, it is hypothesized that bioflavonoids (genistein and daidzein) from Soya bean stabilize...
the antioxidant defense mechanism preventing its oxidation (Corinna et al., 2006). In the present study, we have demonstrated that the aqueous extract of soya bean showed a concentration dependent protection against osmotic and oxidative stress and the best activity was seen a low dose of 200 μg/ml concentration. With the further increase in the drug concentration, a gradual decline in the activity was observed. The anti oxidant effect of soyasaponins from Soya bean was also observed by Ishii and Tanizawa (2006).

Dietary intervention, particularly the use of traditional foods and medicines derived from natural sources, plays a vital role in the prevention of secondary complications of diabetes particularly cataract. Therefore, we have been interested in investigating Soya bean for their potential to prevent cataract in vivo. In this study, we have demonstrated the effect of Soya bean on galactose-induced cataract in rats at three different doses (5%, 10% and 20% diets). However, the onset and progression of cataract was significantly delayed by a dosage of 10% Soya bean diet compared to control. The anti cataract potential of Soya bean might be due to its anti diabetic action, which was reported by Foster and Miller (1995).

CONCLUSION

In conclusion, Soya bean showed anticataract activity against galactose and selenite cataract in experimental rats. This effect is attributed to the protection of antioxidant defense system. This preliminary study is encouraging, but further study is required to extrapolate the use of Soya bean in human beings for the prophylaxis or the treatment of human cataractogenesis.

References


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