Anticataract effect of kolaviron on porcine lenses in experimental cataract model

Abstract: Modern herbal medicine has played a significant role in treating oxidative stress and related complications. Kolaviron was evaluated for possible anticataractogenic potential. Following incubation of porcine lenses for 24 h at 37°C. Porcine lenses 160 randomly distributed into control containing no selenite 40 lenses. Toxic control containing selenite, kolaviron and selenite group were obtained from a local slaughterhouse and homogenized in 50 mM Tris-HCl, pH 8.0, containing 0.1 M NaCl, 5 mM EDTA, 0.01% β-mercaptoethanol and 0.02% sodium azide. The mean activities of catalase, superoxide dismutase and glutathione-S-transferase and the mean level of reduced glutathione were all significantly (p < 0.05) higher in Group III lenses than the mean values in Group II lenses. The mean concentration of malondialdehyde in Group III lenses was significantly (p < 0.05) lower than that in Group II lenses. Porcine lenses simultaneously exposed to selenite and kolaviron showed increased mean activities of enzymatic antioxidants, mean levels of reduced glutathione and decreased malondialdehyde levels. Further studies are required to confirm whether the kolaviron can be developed for pharmacological management of cataract.

Keywords: β-mercaptoethanol, Tris-HCl, Kolaviron.

INTRODUCTION

Cataract is the first cause of world blindness and one of the major causes of disability due to vision impairment (Pascolini and Mariotti, 2012). The only available treatment is the surgical removal of the opaque lens and its replacement with an artificial one. Nevertheless, surgical treatment is not widely available and, thus, interventions which will maintain the transparency of the crystalline lens are intensively sought after. Individuals above 50 years of age are reported to have increased risk of developing most types of cataract. Hence, compounds that prevent or retard cataract formation would be of great benefit to human health. Ostadalova et al., 1978 demonstrated that an overdose of sodium selenite (Na2SeO3) to suckling rat pups induces cataractogenesis, partially mimicking senile nuclear cataract in humans. Selenite exerts its effect on lens by inducing oxidative stress and damage.

Oxidative damage to the lens has been linked with development of cataract, and decrease in antioxidant enzyme activities in the cataractous lens points to the importance of antioxidant enzymes in the prevention of oxidative damage to the lens and subsequent development of cataract (Verma and Hedges, 2004). A wide range of drugs like aldose reductase inhibitors, non-steroidal anti-inflammatory drugs (NSAIDs) are being tried for their anticataract activity (Kyselova et al., 2004). There has been a growing interest in the various activities of indigenous plants. Many indigenous plants have been explored as potential promising sources of antioxidants (Lamsaard et al., 2014., Chitindigu et al., 2007 and hajarnavis and Bulakh, 2013).

Bitter kola (Garcinia kola) belongs to the family of plants called Guttiferae and the genus Garcinia. The seed, commonly known, as ‘bitter kola’ is eaten by many and it is culturally acceptable in Nigeria (Iwu, 1982). Extracts of the plant are employed in African herbal medicine for the treatment of ailments such as laryngitis, liver diseases, cough and hoarseness of voice. (Farombi, 2003). Kolaviron (KV) is a fraction of the defatted ethanol extract of Garcinia kola, containing Garcinia biflavonoids GB1, GB2 and kolaflavanone (Iwu and Igboko, 1982). A number of studies have confirmed the antioxidant and anti-inflammatory effects of kolaviron in chemically-induced toxicity, animal models of diseases and chemoprevention of colon carcinogenesis (Abarikwu, et al 2012, Eboh et al., 2016 and Farombi et al., 2013). Although the chemopreventive effect of kolaviron has been reported in aflatoxin B1-induced genotoxicity and
hepatic oxidative damage (Farombi et al., 2005), no study has addressed the effect of Kolaviron against selenite induced cataract in wistar rats. The present study was done to specifically test the local antioxidant and anticataract effects of extracts of Garcinia kola (kolaviron).

MATERIALS AND METHODS

Chemicals and solvents

Methanol n-hexane and chloroform were purchased from HiMedia Laboratories Pvt. Ltd., (Mumbai, India). Absolute alcohol was obtained from Hayman Ltd., Witham, England; sodium selenite (99%) and Gallic acid were obtained from Sigma Chemical Co. (St. Louis, MO, USA). All other chemicals and reagents used in the experiments were of analytical grade and were obtained from HiMedia Laboratories Pvt. Ltd. (Mumbai, India).

Extraction of kolaviron

Garcinia kola seeds purchased from a local market in Yenagoa, Nigeria, were certified at the Department of Botany, Niger Delta University, Nigeria. Peeled seeds were sliced, pulverized with an electric blender and dried at 40 °C in a drying oven. Powdered seeds were extracted with light n-hexane in a soxhlet for 24 h. The defatted dried marc was repacked and extracted with methanol. The extract was concentrated with chloroform. The concentrated chloroform yielded kolaviron as a golden yellow solid shown in fig 1 (Iwu et al., 1990).

In vitro Lens Crystallin Turbidity Assay

Porcine lenses 160 randomly distributed into control containing no selenite 40 lenses. Toxic control containing selenite, kolaviron and selenite group and finally Gallic acid and selenite group were obtained from a local slaughterhouse and homogenized in 50 mM Tris-HCl, pH 8.0, containing 0.1 M NaCl, 5 mM EDTA, 0.01% β-mercaptoethanol and 0.02% sodium azide. After centrifugation at 15,000 g for 30 min, the supernatant were mixed with light n-hexane in a soxhlet for 24 h. The defatted dried marc was repacked and extracted with methanol. The extract was concentrated with chloroform. The concentrated chloroform yielded kolaviron as a golden yellow solid shown in fig 1 (Iwu et al., 1990).

Determination of reduced glutathione (GSH)

The GSH content in lens homogenate was determined by the method of Jollow et al. (1974) in which 1.0 ml of homogenate was mixed with 1.0 ml of sulphosalicylic acid (4%). The samples were incubated at 4 °C for at least 1 h and then subjected to centrifugation at 1200 x g for 15 min at 4 °C. The assay mixture contained 0.4 ml filtered aliquot, 2.2 ml phosphate buffer (0.1 M, pH 7.4) and 0.4 ml DTNB (10 mM) in a total volume of 3.0 ml. The yellow color developed was read immediately at 412 nm on a spectrophotometer. The GSH content was calculated as µmol of DTNB conjugate formed/ml using molar extinction coefficient of 13.6 x 103 M-1 cm-1.

Superoxide dismutase (SOD) activity

The SOD activity was measured by the method of Marklund and Marklund (1974). The reaction mixture consisted of 2.875 ml Tris–HCl buffer (50 mM, pH 8.5), pyrogallol (24 mM in 10 mM HCl) and 100 µL of lens homogenate in a total volume of 3 ml. The enzyme activity was measured at 420 nm and was expressed as units/mg protein. One unit of enzyme is defined as the enzyme activity that inhibits auto-oxidation of pyrogallol by 50%.

Catalase Activity

Catalase (CAT, EC 1.11.1.6) activity was assayed according to the method of Cohen et al. (1970). One milliliter of 50mM phosphate buffer (pH 7.4) and 10 uL of lens homogenate was added to the cuvette. The reaction was then initiated by the addition of 300 uL of 30mM H2O2 prepared by diluting 0.34mL of 30% H2O2 to 100mL of 50mM phosphate buffer (pH 7.4). Specific catalase activities were determined following the changes in the absorbance of H2O2 at 240nm (ε = 0.0394 mM−1 cm−1 at 240 nm).

Glutathione-S-transferase

Glutathione-S-transferase (GST, EC 2.5.1.18) activity was assayed according to the method of Habig et al. (1974). The final reaction mixture contained 1mM CDNB, 1mM GSH in 50 mM phosphate buffer pH 7.4 and the reaction was initiated by the addition of 50 uL lens homogenate. Specific GST activities were determined following the changes in the absorbance of CDNB per min at 340nm (ε = 9.6.00 mM−1 cm−1 at 340 nm).
RESULTS

Table 1 Glutathione and malondialdehyde levels, Glutathione-S-transferase, catalase and superoxide dismutase activities in lens homogenate exposed to selenite

<table>
<thead>
<tr>
<th></th>
<th>GSH (nmol DTNB formed/ml)</th>
<th>MDA (nmol MDA formed/ml)</th>
<th>GST (nmol CDNB formed/ml)</th>
<th>CAT (Units/ml)</th>
<th>SOD (Units/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>16.06 ± 0.49</td>
<td>5.08 ± 0.33</td>
<td>12.04 ± 0.59</td>
<td>4.36 ± 0.49</td>
<td>1.83 ± 0.32</td>
</tr>
<tr>
<td>Selenite</td>
<td>8.03 ± 0.97</td>
<td>9.65 ± 0.16</td>
<td>7.89 ± 0.43</td>
<td>1.85 ± 0.74</td>
<td>0.97 ± 0.64</td>
</tr>
<tr>
<td>Kolaviron + selenite</td>
<td>14.80 ± 0.61 *</td>
<td>6.15 ± 0.51*</td>
<td>10.59 ± 1.09 *</td>
<td>3.57 ± 0.16 *</td>
<td>1.04 ± 0.12 *</td>
</tr>
<tr>
<td>Gallic + selenite</td>
<td>13.49 ± 0.19 *</td>
<td>7.05 ± 0.45 *</td>
<td>9.08 ± 0.71 *</td>
<td>4.08 ± 0.23 *</td>
<td>1.42 ± 0.91 *</td>
</tr>
</tbody>
</table>

Each reading represents mean ± SD of 10 lenses. * Significantly different from the control value, P < 0.05.

Glutathione: Glutathione measured in terms of DTNB/ml levels showed a decrease in GSH levels in Group 2 (“Toxic control”) as compared to Group 1 (normal control lenses). The increase in GSH was statistically highly significant (p < 0.05).

Superoxide Dismutase: Group 2 (selenite induced cataract lenses) showed a decrease in the specific activity of enzyme superoxide dismutase by as compared to Group 1 (normal control lenses) (Table 1). The decrease was statistically significant (p < 0.05). There was a significant increase in kolaviron and gallic acid groups.

Catalase: Catalase specific activity was reduced in Group 2 (selenium induced cataract lenses) as compared to Group 1 (Normal control lenses). The decrease was statistically significant (p < 0.05). There was a significant increase in kolaviron and gallic acid groups.

Glutathione-s-Transferase: Glutathione-s-transferase specific activity was reduced in Group 2 (selenium induced cataract lenses) as compared to Group 1 (Normal control lenses). The decrease was statistically significant (p < 0.05). There was a significant increase in kolaviron and gallic acid groups.

DISCUSSION

Bitter kola extract (kolavkron) has been mentioned in antioxidant literature for its antidiabetic properties. However, there was a need to assess the in vitro effect of this plant extract on the oxidative stress related biochemical changes happening in cataract. Selenite induced porcine cataractogenesis has been used as an experimental model in vitro.

Increased lipid peroxidation has been strongly implicated in the mechanism of cataractogenesis (Micelli-Ferrari et al., 1996). Malondialdehyde, a secondary product of lipid peroxidation, is used as an indicator of tissue damage (Micelli-Ferrari et al., 1996). In the present investigation, disruption of lenticular membrane lipids possibly accounted for the observed higher mean malondialdehyde level in selenite-challenged, untreated (Group II) lenses than that in control (normal) lenses (Table 1). In selenite-challenged, simultaneously kolaviron-treated lenses, the mean malondialdehyde level was significantly lower than that in Group II lenses (Table 1), suggesting that the extract prevented peroxidative changes in selenite-challenged lenses, thereby preventing lenticular opacification. Collectively, these results in the present study suggest that administration of kolaviron can effectively prevent selenite-induced cataract formation.

An unusually high level of GSH in the lens is believed to maintain protein-thiol groups in the reduced state, and to prevent cross-linking of soluble crystallins (Reddy, 1990). In the selenite cataract model, lenticular GSH is altered by a non-enzymatic reaction of GSH with selenite, which results in the formation of the selenium derivative, GSH-Se-SG. Oxidation of GS–Se–SG by a single electron transfer to oxygen results in the formation of a superoxide anion as an intermediate. Administration of GSH or maintenance of lenticular GSH levels may retard age-related loss of lenticular antioxidant activity, therein delaying the onset of cataract (Harding, 2001). In the present study, simultaneous treatment of selenite-challenged lenses with kolaviron resulted in a mean lenticular GSH level that was significantly higher than that in selenite-challenged, untreated lenses (Table 1). Treatment with the bitter kola extract possibly prevented the formation of the selenium derivative, GS–Se–SG, thereby maintaining GSH in its active form at a stable level, ultimately preserving lenticular transparency.

Antioxidant enzymes are able to catalytically remove free radicals and other reactive species. Catalase, superoxide dismutase and glutathione-S-transferase are important components of the innate enzymatic antioxidant defenses of the lens. In the present investigation, the mean activities of catalase, superoxide dismutase and glutathione-S-transferase were significantly (p < 0.05) lower in selenite-
challenged, untreated lenses than the mean activities in normal control lenses (Table 1); such lowered activities of these enzymes in selenite-induced cataractogenesis has been previously documented in in-vitro and in-vivo experimental models (Javadzadeh et al., 2009). However, selenite-challenged, Kolaviron treated lenses did not exhibit such lowered activities of these enzymatic antioxidants (Table 1). These observations suggest that when lenses exposed to sodium selenite are treated with an antioxidant-rich compound, antioxidant enzyme activities are maintained at near-normal levels; similar findings have been previously reported (Javadzadeh et al., 2009).

The results of the present study suggest that kolaviron possesses antioxidant potential to prevent selenite-induced cataractogenesis by maintaining a normal antioxidant status. Thus, kolaviron may be considered for pharmacological therapy to prevent or retard cataractogenesis.

REFERENCES