Prevention of Selenite-Induced Cataractogenesis in Wistar Albino Rats by Aqueous Extract of Garlic

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Abstract

Purpose: The main aim of this study was to evaluate the inhibitory impacts of the aqueous extract of garlic (Ga) on the formation of cataract induced by sodium selenite (Se).

Methods: Thirty-two Wistar albino rat pups (4 equal groups: G1, G2, G3, and G4) were treated as follows: G1, subcutaneous (s.c.) and intraperitoneal (i.p.) injection of normal saline (0.3 mL) on postpartum (day 10); G2, i.p. injection of aqueous extract of garlic (1 mL/kg body weight) and s.c. injection of normal saline (0.3 mL) on postpartum (day 10); G3, s.c. injection of sodium selenite (20 nmol/g body weight) and i.p. injection of normal saline (0.3 mL) on postpartum (day 10); and G4, s.c. injection of sodium selenite (20 nmol/g body weight) and i.p. injection of aqueous extract of garlic (1 mL/kg body weight) on postpartum (day 10). Daily i.p. injections of aqueous extract of garlic (in G2 and G4) and normal saline (in G1 and G3) were continued for 14 days. The development of cataract was assessed over a period of 2 weeks after injection of sodium selenite. For further examination, the rats’ lenses were removed and analyzed for glutathione (GSH), malondialdehyde (MDA) levels, and superoxide dismutase (SOD), glutathione peroxidase (GPX) activities.

Results: In G3 (Se-treated group), all rats developed grade 3 cataract in both eyes. However, in G1 (untreated control group), G2 (Ga-treated group), and G4 (Ga–Se-treated group), the lenses in both eyes of all rats remained clear (P < 0.0001). This clinical finding was associated with higher GSH level and GPX, SOD activities and lower level of MDA in the Se- and Ga-treated group (G4) compared with SS-treated group (G3) rat lenses (P < 0.003).

Conclusions: Intraperitoneal injection of the Ga in rat model appeared to effectively prevent Se-induced cataract, thus such herbal remedy may be considered for treatment of cataract.

Introduction

SeLENITE-INDUCED CATARACT is a cataract model mainly dependent on oxidative stress, in which oxidation of the critical sulfhydryl groups is essential for the initiation of cataractogenesis.1 This is a rapid and convenient model of nuclear cataract produced in young rats by an overdose of the essential trace mineral selenium. It has been used in many in vivo rodent models since 1978.2 Selenite induces bilateral nuclear cataract within 4 to 6 days when administrated to suckling rat pups before completion of the critical maturational period of the lens. Cataractogenesis is a multifactorial pathological process in which many risk factors and different causes were involved.3 Nevertheless, it has been shown that free radicals and, in consequence, oxidation damage are increasingly responsible for the opacification of the lens.5,6 Free radical-induced oxidative stress has been found to be one of the major inducing factors for senile cataract formation.5,7 This hypothesis has been supported by the protective effect of various nutritional as well as pathological antioxidants such as pyruvate, iodide and nutritional antioxidants such as ocimum sanctum, ascorbate, vitamin E, and carotenoids in delaying the development of experimental cataract have been reported by some investigators.8–12 The aqueous extract of garlic is deemed to confer potent antioxidant activities.13,14 The intrinsic antioxidant activity of garlic,15 garlic extracts,16,17 and some garlic constituents19 has been widely documented in vivo and in vitro.13,18 It has been shown that the garlic extracts can increase antioxidant enzymes such
was performed by slit lamp biomicroscopy on a scale of 4 to 0 as follows: grade 4, mature dense opacity involving the entire lenses; grade 3, strong nuclear cataract with perinuclear area opacity; grade 2, if nuclear cataract was observed; grade 1, if subcapsular opacity was observed; and grade 0 was normal clear lens. All rats were killed 14 days after the first injection, and the lenses were removed intracapsularly via an incision 2 mm posterior to the limbus under surgical microscope magnification. Removed rat lenses were weighted carefully and after adding 0.2 mL saline normal to each pair, they homogenized with glass–glass homogenization, then centrifuged at low Rmn (3,000/g) for few seconds, and 0.1 mL of the supernatant was analyzed for GSH, MDA level and SOD, GPX activities. All these assays were done in standard condition. SOD and GPX activities were spectrophotometrically measured with RANSCEL and RANSOD kits, respectively (Randox Laboratories Ltd., Crumlin, UK). The GSH level was measured with Cayman Chemical’s glutathione assay kit. In order to assay MDA, thiobarbituric acid-based method was used. All statistical analyses were carried out using SPSS statistical software (SPSS version 12, Chicago, IL). Results were expressed as mean ± SD. All data were analyzed statistically using the Mann–Whitney or the Wilcoxon test. 

P value <0.05 was regarded as significant.

Results

All rats in groups 1, 2, and 4 had clear lenses (Fig. 1A, B, and D). In group 3, grade 3 cataract developed in all rats (Fig. 1C). The difference in exhibited cataract in all rats of group 3 compared with groups of 1, 2, and 4 was significant (P < 0.0001). The mean GSH level in group 3 (6.44 ± 2.04 μmol/g) was significantly lower than that in group 4 (10.06 ± 1.43 μmol/g; P = 0.003) and in controls (11.18 ± 1.02 μmol/g; P = 0.001; Fig. 2A). The mean MDA level in group 3 (7.29 ± 0.80 nmol/g) was significantly higher than that in group 4 (3.91 ± 0.69 nmol/g; P = 0.0001) and in controls (4.04 ± 0.91 nmol/g; P = 0.0001; Fig. 2B). The mean SOD activity in group 3 (144.30 ± 20.36 IU/g) was significantly lower

Methods

Thirty-two Wistar albino rat pups were divided into 4 equal groups (G1, G2, G3, and G4) and each group housed with 1 weight mother rat. Experiments were conducted in accordance with the ARVO Statement for Use of Animals in Ophthalmic and Vision Research and Guiding Principles in the Care and Use Animals. In G1, normal saline (0.3 mL) was injected (s.c. and i.p.) on postpartum (day 10). For treating G2, 30 g peeled garlic was crushed with distilled water; the crushed material was carefully decanted by pressing; and 60 mL of aqueous extract was extracted. The previous studies have shown 1 mL of this extract contained material from 500 mg of garlic. And accordingly in G2, the Ga (1 mL/kg body weight, via i.p.) and normal saline (0.3 mL, s.c.) were injected on postpartum (day 10). In G3, sodium selenite (Sigma Chem. Co., St. Louis) 20 nmol/g body weight (via s.c.) along with normal saline (0.3 mL, via i.p.) was injected on postpartum (day 10). In G4, the Se (20 nmol/g body weight, via s.c.) and the Ga (1 mL/kg body weight, via i.p.) were injected on postpartum (day 10). Daily i.p. injections of the Ga in groups 2 and 4, and normal saline in groups 1 and 3 were continued for 14 days (till postpartum day 24). The development of cataract was assessed weekly for 2 weeks and its density was graded and photographed with a photo slit lamp (Topcon Digital camera Unit DC-I). One person masked to treatment group performed all examinations. At the final examination, the pupils were dilated with 0.5% tropicamide and staging of cataract

FIG. 1. Topcon photo slit lamp images of rats’ eyes 14 days after injection. Clear lenses in control (A), garlic (B), and selenite (Se) + garlic (D) groups. Grade 3 cataract in Se group (C). The photographs were taken by digital photo slit lamp (Topcon, DC-I), and pupils of rats, eyes dilated prior to each photographs with 1% tropicamide drop.
Senile cataract is a multifactorial disease associated with several risk factors. Biochemical evidence suggests that oxidation of the lens proteins is involved in the genesis of human senile cataract as well as experimental selenite-induced cataract in rats. Selenite-induced oxidative stress (20–30 nmol/kg body weight of selenite) causes nuclear opacity through the calpain proteolysis of lens proteins. It is a strong sulfhydryl oxidant and is considered as a model for those cataracts caused by oxidative stress. Previous studies have shown that antioxidant agents can protect the rat lenses from experimental selenite-induced cataract. 

In fact, in past 2 decades, most studies on garlic have been primarily in the fields of cardiovascular and cancer research. Of these findings, diminishing effects of garlic bioactive constituents on cholesterol and triglycerides levels in patients have been evidenced. Further, significant antithrombotic actions both in vitro and in vivo systems have been reported upon use of the garlic extracts and/or several garlic constituents. For example, the allicin and adenosine are the most potent antiplatelet constituents of garlic. Recent identification of potent enzyme-inhibiting activities of adenosine deaminase and cyclic AMP phosphodiesterase in garlic extracts may elucidate its various clinical impacts such as antithrombotic, vasodilatory, and anticancer actions. Arhan et al. investigated the oxidant/antioxidant status in hepatic tissues from cholesterol-fed rabbits and the establishment of possible protective effects of aqueous garlic extract on cholesterol-induced hepatic steatosis. They observed significant increase in antioxidant potential and accordingly profound protection against peroxidation damage in the hepatic tissue upon treatment with garlic extract. This, together with significant reductions in the cholesterol levels of blood and hepatic tissues, may explain its effectiveness as an antioxidant. Besides, many studies have been carried out to investigate the effect of garlic active principle “diallyl disulfide” on cancer, DNA damage, intestinal damage in rats injected with endotoxin and acetaminophen-induced cataract. Of these studies, intriguingly, Zhao and Shichi reported prevention of acetaminophen-induced cataract in C57BL/6 mice. They showed that the major organosulfides in garlic oil “diallyl disulfide (DADS)” (200 mg/kg body weight) can prevent cataract development and prolonged survival time. Such treatment...
once combined with N-acetyl l-cysteine, a prodrug that stimulates GSH synthesis, completely prevented cataractogenesis. Upon assaying the plasma glutamate–pyruvate transaminase activity as an indicator of liver necrosis, these researchers claimed that such combined modality can effectively protect the liver. Despite this interesting investigation, to our best knowledge, no substantial works have been conducted to examine the clinical effects of garlic extract in ocular disease. Block et al. showed that aged garlic extract contains unique organosulfur compounds, which provide its characteristic flavor and odor and most of its potent biological activity. The major of these unique organosulfur compounds are water-soluble S-allylcysteine and S-allylmercaptocteyline, which have potent antioxidant activity. We investigated the protective effect of aqueous garlic extract against experimental selenium-induced cataract in rat lenses that is not studied so far.

We have previously reported the effects of onion juice on sodium selenium-induced cataract formation. In the present study, we found that the i.p. injection of the aqueous extract of garlic could protect the model animals against Se-induced cataract (up to 100%). This effect was associated with higher GSH level and GPX, SOD activities as well as lower level of MDA in Se- and Ga-treated group (G4) compared to the Se-treated groups. It could be postulated that aqueous garlic extract against as a potent source of antioxidant can provide an additional support to the elevation of GSH level and GPX, SOD activities and decrease of MDA level. Previous studies showed that nutritional antioxidant or ingestion of IH636 grape seed proanthocyanidin extract can prevent selenium-induced cataract formation by 41% and 50% at the 15th day of the experiment Wistar rats, while we did not observe cataract at the same day of the experiment within the group treated with Se and Ga.

In conclusion, upon our present biochemical and morphological results, it seems that the aqueous extract of garlic can inhibit the formation of the Se-induced cataract, perhaps through its potent free radical scavenging and antioxidant properties. Thus, the aqueous extraction of garlic is suggested to be used as an anti-cataract agent, although such therapeutic modality requires further clinical trials prior to its application.

References


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