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Original Research

Effect of *Aloe vera* gel on antioxidant enzymes in streptozotocin-induced cataractogenesis in male and female Wistar rats

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Abstract

Background: There is increasing evidence that complications related to diabetes are associated with increased oxidative stress. *Aloe vera* (AV) gel has several biological properties, including antioxidant activity.
Purpose: This study was undertaken to evaluate the effects of AV gel extract on oxidative stress in streptozotocin (STZ)-induced diabetic rats.
Methods: Wistar albino male and female rats were divided into the following four groups: normal control rats (N), AV-treated normal (N + NT), diabetic (DU), and AV-treated diabetic (DU + DT). The AV-treated normal (N + NT) and AV-treated diabetic (DU + DT) groups received oral administration of AV gel extract (300 mg/kg body weight) for 60 days. Diabetes was induced experimentally by an intraperitoneal injection of STZ at a dose of 55 mg/kg body weight.
Results: By the end of the experimental period, levels of various biochemical parameters such as superoxide dismutase, catalase, lipid peroxidation (LPO), protein oxidation (POD), glutathione peroxidase (GPx), glutathione reductase, Aldose reductase (AR), were increased, whereas the level of reduced glutathione (GSH) and Sorbitol dehydrogenase (SD), soluble protein, and insoluble protein were decreased under diabetic conditions. Oral administration of AV gel extract at a dose of 300 mg/kg body weight for 60 days resulted in the prevention of the aforementioned abnormalities.
Conclusion: In conclusion, our data demonstrate the protective role of AV leaf extract in inhibiting STZ-induced diabetic oxidative stress, and therefore, this plant could be used as an adjuvant agent for the prevention and/or management of diabetes and aggravated antioxidant status.

Keywords: *Aloe vera*; antioxidants; glutathione; oxidative stress; streptozotocin

1. Introduction

Diabetes is currently growing at a rapid rate throughout the world. In addition, diabetes is the 16th leading cause of global mortality,[1] and diabetic cataract is reported to be an underlying cause of blindness in nearly half of the world's blind population.[2] Cataract is characterized by cloudiness or opacification of the crystalline eye lens. Traditionally, the cataract intervention program is evaluated by the number of cataract operations performed per million population per year (Vision 2020). However, the surgery has its own limitations, including more pronounced postoperative inflammatory response, loss of vitreous humor, posterior capsule, opacification,[3] and in addition it is expensive.[4] Thus, there is a need not only to look at the impact of treating cataracts and relate it just to surgery but also to look at scholastic achievements and development in the management of cataract due to diabetes. Sex differences have also been reported in the severity of experimentally induced diabetic cataract. However, there are some studies in which this case has not been observed, perhaps due to differences in the site of streptozotocin (STZ) administration or

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dosage. In recent years, the role of alternative therapeutic approaches has become very popular[5] and because various plant extracts may have many pharmacological activities (e.g., antidiabetic, antioxidant, and antistress), they can be effectively used to delay or counter diabetic complications such as cataract.

For this study, we selected *Aloe vera* (AV) as this was already mentioned in ayurveda for the treatment of diabetes mellitus and has been reviewed elsewhere.[6] In brief, AV (kalabanda in Telugu and coastal aloe in English) belongs to the Liliaceae family, of which there are approximately 360 species.[7] Over the years, the plant has been known by a number of names such as "the wand of heaven", "heaven's blessing", and the "silent healer". They are succulent perennials, which secrete a watery juice from the tubular cells that run lengthwise throughout the stout and fleshy leaves. The mucilaginous tissue in the center of the AV leaf is called gel.

The objective of this investigation was to evaluate the effects of AV leaf extract in delaying or preventing diabetic cataract. Therefore, this study was designed to investigate the protective effect of AV leaf gel extract on the levels of lens lipid peroxide (LPO) and enzymatic antioxidants in male and female rats with STZ-induced diabetes.

2. Materials and methods

2.1. Chemicals

STZ, DL-glyceraldehyde, and 2-thiobarbituric acid were obtained from Sigma-Aldrich (St. Louis, MO, USA). All other chemicals and solvents were of analytical grade and were obtained from Sisco Research Laboratories (P) Ltd. (Mumbai, India).

2.2. Preparation of AV gel extract

AV leaves were collected from the Department of Botany, Sri Krishnadevaraya University (Anantapur, Andhra Pradesh). Mature, healthy fresh leaves were washed with water and cut transversely into pieces. The thick epidermis was selectively removed. The solid gel was then homogenized and the resulting mucilaginous, thick, straw-colored homogenate was stored in dry sterilized small containers at 4°C until further use. The gel was freshly prepared every time and administered orally. The dosing schedule was once per day.

2.3. Animals

Male and female Albino Wistar rats (130–160 g) used in the present study were procured from Sri Venkateshwara Enterprises (Bangalore, India). The animals were acclimatized for 7 days in our animal house (Registered number: 470/01/a/CPCSEA) before dietary manipulation. All the animals had free access to water. Food intake (daily) and body weight (weekly) were monitored regularly. Animal care and study protocols were in accordance with and approved by the Institutional Animal Ethics Committee.

2.4. Experimental design

At the time of dietary manipulation, all the animals were 6 weeks of age, weighing approximately 200 g. The rats were divided into four groups, with 10 animals in each group as follows: Group N, normal rats (N); Group NT, normal rats treated with AV leaf gel (300 mg/kg/day; NT), Group DU, rats with STZ-induced diabetes that received a single intraperitoneal injection of STZ (55 mg/kg) in 0.1 mL of 0.05M citrate buffer at pH 4.5 (DU), and Group DT, diabetic rats treated with AV leaf gel (300 mg/kg/day; DT).

2.5. Lens collection and processing

After the experimental period, the animals were fasted overnight and euthanized by cervical decapitation. The lenses were dissected by the posterior approach and stored at −70°C until further analysis. A 10% homogenate mixture was prepared from three to four pooled lenses in 50mM potassium phosphate buffer at pH 6.2. All the biochemical parameters were analyzed in the soluble fraction of the lens homogenate (centrifuged at 16,000*g* at 4°C) except for lens malondialdehyde (MDA) and reduced glutathione (GSH), which were determined in the total homogenate.

2.6. Biochemical estimations

The extent of lipid peroxidation was determined by assaying MDA formation according to the method suggested by Utley et al.[8]

The protein carbonyl content was measured by forming hydrazone derivatives using 2,4-dinitrophenylhydrazine, which were quantified spectrophotometrically at 370 nm according to the method suggested by Levine et al.[9]

The total GSH content was measured by following the method suggested by Ellman.[10] This method is based on the development of a yellow color, which is due to the reaction of 5,5′-dithio-2-nitrobenzoic acid with the compounds containing sulfhydryl groups. The maximum absorbance was observed at 412 nm.

Tyrosine and tryptophan present in the proteins react with Folin–Ciocalteu reagent in the presence of alkaline copper to give a colored complex with maximum absorbance at 750 nm.[11]

The lysate was prepared from the lens of rats from different experimental groups. The enzyme preparation was allowed to react with H_2O_2 in the presence of GSH for a specified period according to the method recommended by Rotruck et al,[12] and the remaining GSH was measured by following the method suggested by Ellman.[10]

Glutathione reductase (GR) catalyzes the reduction of oxidized glutathione by nicotinamide adenine dinucleotide phosphate-reduced (NADPH) to GSH. The activity of the enzyme was measured by following the oxidation of NADPH spectrophotometrically at 340 nm according to the method suggested by Pinto and Bartley.[13]