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Original Article

Cataractostatic Activity of *Drynaria quercifolia* Tuber Extract

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ABSTRACT: The cataractostatic activity of methanolic and ethylacetate extracts of *Drynaria quercifolia* tuber were evaluated here as a prospective treatment for lens cataracts. Glucose-induced cataract formed in isolated goat lenses *in vitro* was used as a model in this investigation. When tested with extracts, it was observed that the ethylacetate extracts of the tuber was able to inhibit cataractogenesis when the lenses were treated at a final concentration of 20 µg/ml. However, methanolic extract of the tuber showed a moderate anticataract activity at 80 µg/ml concentration. When justified quantitatively, the cataract formation was inhibited 51.61±9.68% by methanolic extract at 80 µg/ml. On the contrary, the ethylacetate extract could inhibit cataract formation by 62.37±15.24% at 20 µg/ml and 100.00±3.23% at 80 µg/ml. These data indicated that, the ethylacetate extract of *Drynaria quercifolia* tuber is a potent cataractostatic agent and may be able to prevent diabetic cataract.

KEYWORDS: Cataractostatic activity, *Drynaria quercifolia,* goat lens, *in vitro*.

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INTRODUCTION

Cataracts are opaque formations in the lens that limit the amount of light entering the eye, thereby leading to diminished vision[1]. It may develop in one or both eyes which may lead to complete blindness[2]. The sorbitol-aldose reductase (polyol) pathway has been indicated to be the primary cause of cataract formation[1]. In this pathway glucose is converted to sorbitol[3]. Excess sorbitol induces osmotic and oxidative stress in lens leading to degradation and denaturation of lens proteins, which in fact occludes the lens and forms cataract[4]. According to the CDC, cataracts are the leading cause of blindness in all countries of the world[5]. Cataracts account for severe loss of vision in about 17 million people worldwide[6,7]. The present WHO standard strategy for the treatment of cataracts is simple ocular surgery, but the surgery has some degree of complications[8]. Therefore, scientists are looking for possible prophylactic measures. Medicinal plants have been mentioned in longstanding folklores to indicate their usage in treatment of several diseases. Plants are known to produce a number of important biological and medicinal compounds. Many of these compounds could have anticataract activity and could potentially be used to treat cataracts[9,10]. *Drynaria quercifolia,* commonly known as oak-leaf fern or pankhiraj, is an epiphytic fern with dimorphic digitate fronds[11]. The plant is native to Southeast Asia and usually found in Bangladesh. Several important compounds such as epifriedelinol and friedelin have also been isolated from this plant[12]. Also, *D. quercifolia* has been used as an ethnic medicine to treat cataracts in Bangladesh[13]. With these ideas in mind, the investigation was carried out to evaluate the efficacy of *D. quercifolia* in treating cataracts in goat lenses. The investigation

was further validated by measuring the intensity of refracted light through the lens.

MATERIALS AND METHODS

Plant material

The tubers of *D. quercifolia* were collected from the University of Dhaka in the month of May, 2016 and were identified by Bangladesh National Herbarium (accession number 37592). Tuber samples were separated from the plant and were dried under shadow for 10 days. Then the samples were crushed into powder using mechanical grinder. 700 g of dried tuber powder was soaked in 2,800 ml of ethylacetate (ACS grade, BDH, USA), or 3000 ml methanol (ACS grade, BDH, USA) for 5 days with gentle shaking. The extracts were collected as filtrate through Whatmann filter paper (Grade 4) and evaporated through rotary evaporator under reduced pressure. Later, the extracts were dissolved in dimethyl sulfoxide (DMSO) (ACS grade, BDH, USA).

Collection of eyeballs

Fresh eyeballs of young and healthy goats were collected from the slaughter house, Ananda Bazar, Dhaka immediately after the slaughter. The eyeballs were then transported in a cold chain at 0 ºC to the laboratory. The corneas were sliced and the lens was the carefully extracted and placed in glass vials containing artificial aqueous humor. The lenses were then rinsed several times with artificial aqueous humor.

Lens culture

The lenses were incubated in 5 ml of artificial aqueous humor or basal lens culture media (140mM NaCl, 5mM KCl, 2mM $MgCl_2$, 0.5mM $NaHCO_3$, 0.5mM Na_2HPO_4, 0.4mM $CaCl_2$, 5.5mM Glucose, pH 7.8) in glass vials for 72 hours at 37 ^{0}C in a standard incubator[14,15]. To prevent the growth of microorganism, penicillin G, streptomycin and amphotericin B were added to the culture media to final concentrations of 100 U/ml, 100 µg/ml and 0.5 µg/ml respectively. 55 mM glucose was added to the culture media to induce cataract[15]. The glass vials were left a little open to allow air to enter. The lenses were then treated with different concentrations of *D. quercifolia* extracts (at final concentrations of 20, 40, and 80 µg/ml). At least 5 lenses were tested for each of the negative control, positive control, and individual treatment.

Photographic Evaluation

After proper incubation, the lenses were placed on top of wire mesh. Photographs were then taken to observe the visibility of the wire mesh patterns to determine the opacity of the lenses.

Quantitative cataractostatic activity analysis

The lenses were placed on the top of a coherent light bed on a modified black film with a pinhole in the middle. The lenses were then enclosed in a circular dark cylinder and a standard lux meter (LX1010B, Digital Lux Meter, China) was used to measure the light intensity passing through the lenses.

RESULTS

Qualitative anticataract activity of the tuber extracts

It was observed that the basal lens culture media alone or with 100 µl DMSO did not induce cataracts (negative control). When the lenses were treated with glucose (55mM), cataract was evident

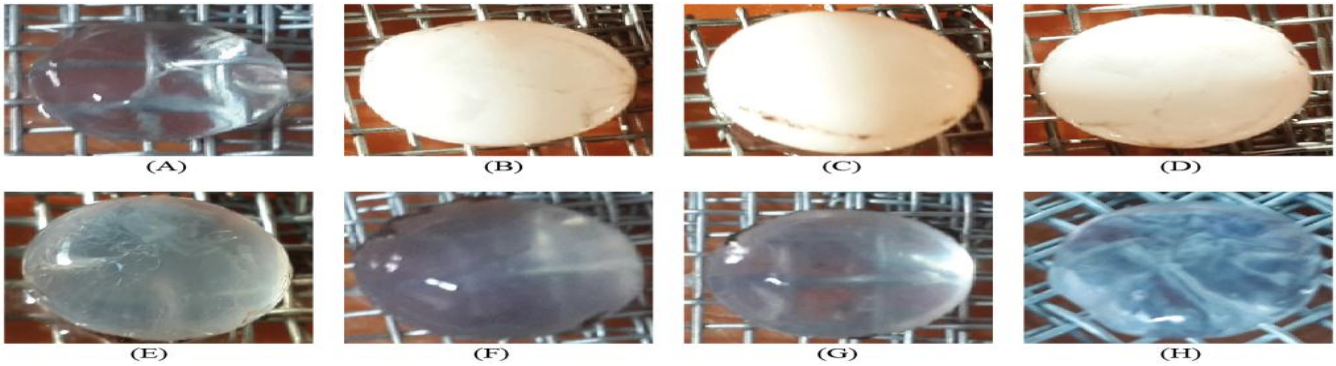

Figure 1. Visual observation of cataract formation in goat lenses. (A) Negative control: Lens without 55mM glucose treatment, (B) Positive control: Lens with 55mM glucose treatment, (C)-(E): treated with methanolic extract of D. quercifolia tuber, (F)-(H): 80 µg/ml.

(positive control). The methnol extract of *D. quercifolia* tuber was capable to reduce cataractogenesis at the concentration of 80 µg/ml (Figure 1). Lower concentrations of the methanol extract failed to show any anticataract activity. The ethylacetate extract of *D. quercifolia* tuber was the most potent anticataract agent. At 20 µg/ml, it could prevent the formation of cataract upon glucose treatment, but the best activity was

obtained with increased concentration. At the concentration of 80 µg/ml, the ethylacetate extract of *D. quercifolia* tuber completely inhibited the formation of cataract **(Figure 1).**

Quantitative anticataract activity of the tuber extracts

As a quantitative approach, we determined the intensity of the refracted light passed through the individual lenses to numerically express the formation of cataract. Here, we have hypothesized that the intensity of the refracted light through an isolated lens is inversely proportional to cataractogenesis. It was observed that the intensity of refracted light through normally cultured lens was 35.33±0.58 lux. When cataract was formed, the intensity was lowered to 4.33±0.58 lux. Thereby, we used this system to determine the intensity of refracted light through the treated lenses (Table 1). It was observed that the intensity of refracted light through lenses treated with lower concentrations of methanolic extract was comparable with positive control (5-6 lux), but the intensity was increased at higher concentration (20.33±2.52 lux). For ethylacetate extract treated lenses, the intensity was 23.67±4.93 to 35.33±0.58 lux. Thereby, it can be confirmed that the ethylacetate extract treated lenses were not cloudy enough to be considered as cataract.

Table 1. Intensity of refracted light through the lenses as determined using simple light meter.

Group Name	Light intensity (lux)
Negative control (without 55mM glucose)	35.33±0.58
Positive control ((with 55mM glucose))	4.33±0.58
20 µg/ml methanolic extract	5.33±0.58
40 µg/ml methanolic extract	5.67±0.58
80 µg/ml methanolic extract	20.33±2.52
20 µg/ml ethylacetate extract	23.67±4.93
40 µg/ml ethylacetate extract	30.67±0.58
80 µg/ml ethylacetate extract	35.33±0.58

Relative cataractostatic activity of the tuber extracts

Considering 100% inhibition of cataract in negative control, the potency of the extracts as anticataract agent was determined (Figure 2). It was found that the ethylacetate extracts of *D. quercifolia* tuber can inhibit the formation of cataract completely ($p<0.05$). The inhibition of cataract formation was 62.37±15.24%, 84.95±1.86% and 100.00±3.23% for 20 µg/ml, 40 µg/ml, and 80 µg/ml concentrations, respectively, of the ethylacetate extracts of *D. quercifolia* tuber. But the methanol extract could inhibit cataract formation up to 51.61±9.68% at 80 µg/ml. These data were consistent with the visual inspection. Based on these observations, it can be concluded that the ethylacetate extracts of *D. quercifolia* tuber is a potent anticataract agent.

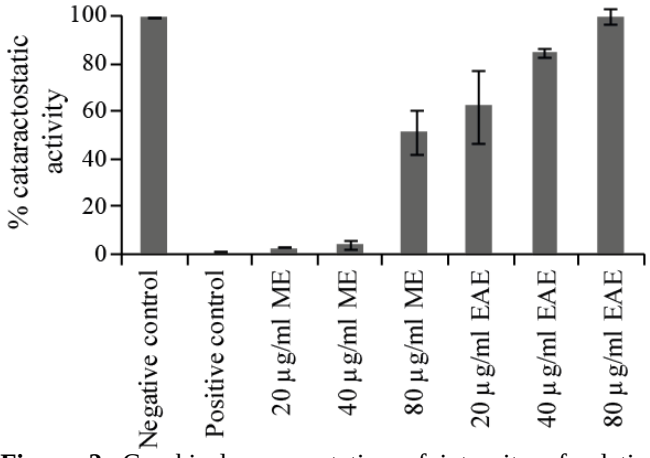

Figure 2. Graphical representation of intensity of relative cataractostatic activity of *D. quercifolia* extracts. Negative control: Lens without 55mM glucose treatment, Positive control: Lens with 55mM glucose treatment, ME: methanolic extract of *D. quercifolia* tuber, EAE: ethylacetate extract of *D. quercifolia* tuber. Anticataract activity of ethylacetate extract of *D. quercifolia* tuber was comparable to negative control ($p<0.05$).

DISCUSSION

Cataract is one of the most common visual impairment leading to decrease in vision and blindness[1,6]. Current practice to treat such condition is the surgical removal of cataract[8]. However, the prospect of pharmacological intervention to inhibit or to delay the onset of cataract is still at the experimental stage and only N-acetylcarnosine has some promise[16]. To find an alternative cure, we have studied the anticataract activities of *D. quercifolia* extracts in this research. And, it was observed that the ethylacetate extract of the tuber of this plant has potent anticataract activity. This effect was manifested as amelioration of glucose-induced lens opacity. Also, we have proposed a method to quantitate cataract in isolated lens. When lenses are cultured at higher concentration of glucose (55mM), the excess glucose is converted to sorbitol and cataract is formed. We have observed that the tuber extract could inhibit cataract successfully. When the concentration of the ethylacetate extract was increased from 20 µg/ml to 80 µg/ml, the anticataract activity was increased from 62.37±15.24% to 100.00±3.23%. Thus, the *in vitro* cataractostatic activity of the tuber extracts on isolated goat lenses incubated in a high glucose medium was dose-dependent. Hence, the tuber

extract might posses some compounds with anticataract activity. Since the ethylacetate extract of the tuber was more active compared to the methanol extract, the active compound in the ethylacetate extract could be polar or semi-polar in nature. Also, the ethylacetate extracts possess excellent antioxidant activities; such activity might have advocated the anticataract activity as well[11]. In future the anticataract activity will be evaluated on different biochemical activities such as the Na^+ K^+ ATPase activity, total protein content, malonaldehyde content, and glutathione GSH content[10]. There is a potential that the results may correlate *in vivo* as well. Therefore, animal models will be used up to further validate the anticataract activity of *Drynaria quercifolia*. In conclusion, we can say that the ethylacetate extract of the *D. quercifolia* is a potent anticataract agent.

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