Indian J.Sci.Res. 7(1) : 81-84, 2016

ISSN : 0976-2876 (Print)
ISSN : 2250-0138 (Online)

EVALUATION OF ANTICATARACT POTENTIAL OF *Abutilon indicum*: AN IN *Vitro* STUDY

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ABSTRACT

Cataract, a leading cause of blindness and poor vision, is a major public health problem worldwide. Diabetes and hyperglycemia have long been recognized as risk factors for cataract. Because there is sufficient evidence that oxidative stress plays a role in the mechanisms of cataractogenesis, there is an increasing interest in developing suitable antioxidant nutrients, both of synthetic and plant origin that could be effective in delaying or preventing the formation of cataracts. Large number of medicinal plants and synthetic compounds has been reported to possess anticataract properties. The present study evaluated the in vitro anticataract potential of hydro-ethanolic leaf extract of *Abutilon indicum* against glucose-induced cataractogenesis using goat lenses. The results showed a reduction in the opacity of the lens incubated with the plant extract. The biochemical parameters were studied for the lens group treated with the plant extract (500µg/ml) which showed highest reduction in malondialdehyde level, increase in the total protein content and SOD activities and reduced the lens opacity (formation of cataract by glucose), as evidence by the photographic evaluation. Hence, the study suggested that the hydro-ethanolic leaf extract of *A. indicum* possesses the anticataract activity.

KEYWORDS : Cataract, Diabetes, Oxidative Stress, Anticataract *Abutilon indicum*

Cataract renders millions in the world blind and is notably a disease that largely afflicts the elderly. In the world, management of cataract is a significant strain on health care budgets. Delaying the onset of cataract is therefore a major health care priority across the globe. Free radical production leading to oxidative stress is an initiating factor in the development of maturity-onset cataract. Free radicals are atomic or molecular species with at least one unpaired electron in the outermost shell and any free radical involving oxygen can be referred to as reactive oxygen species (ROS). Unpaired electrons cause the free radicals to be highly reactive and likely to take part in chemical reactions. ROS molecules include superoxide anion (O^{2-}), hydroxyl radical (-OH), and hydrogen peroxide (H_2O_2). At present, the only means to treat cataract is by surgical intervention3 and it is predicted that 32 million operations will be performed annually by 2020 (Hanruo et al., 2013).

Medicinal plants are the nature's gift to human beings to make disease free healthy life. It plays a vital role to preserve the health. In India different parts of medicinal plants have been used for curing various diseases from ancient times. In this regard, one such plant is *Abutilon indicum*. *A. indicum* (Linn.) Sweet belonging to family Malvaceae commonly called as 'Country mallow' (English), 'Kanghi' (Hindi) and 'Atibala' (Sanskrit). It is a perennial shrub, softly tomentose and up to 3 m in height. The plant is found in India, Sri Lanka, tropical regions of America and Malaysia. Leaves are ovate, flowers are yellow in colour. The stems are stout, branched, 1-2 m tall, pubescent. The seeds are 3-5 mm, reniform, tubercled or minutely stellate-hairy, black or dark brown. The fruits are capsule, densely pubescent, with conspicuous and horizontally spreading beaks (Archna et al., 2013; Kumari et al., 2015).

The objective of the present study was to determine the in vitro anticataract potential of the hydro-ethanolic leaf extract of *A. indicum* against glucose-induced cataractogenesis using goat lenses.

MATERIALS AND METHODS

Collection of Plant Material

The plants chosen for the present study was *Abutilon indicum*. The selected plant was collected from various places in Coimbatore. The plant was authenticated by Dr. G. V. S. Murthy, Scientist 'F' and Head of Office, Botanical Survey of India, Tamil Nadu Agricultural University, Coimbatore.

Preparation of The Plant Extract

The leaf materials of the plant were collected and washed in tap water, shade dried and powdered. About 20 g of the air-dried powdered leaf material of *A. indicum* was extracted using Soxhlet apparatus with 200 ml volumes of ethanol, water and hydro-ethanol solvents. The solvent fractions of the extracts were evaporated by a vacuum rotary evaporator (Srinivas et al., 2015).

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Anticataract Activity of the Selected Medicinal Plants

The in *vitro* anticataract activity was carried out using the modified protocol from Shabeer et al, 2011.

1. Collection of Goat Eye Balls

The anticataract potential of the plant extracts was studied in vitro in glucose induced cataractogenesis using goat eye lens. Goat eye balls were used in the present study. They were obtained from the slaughter house at Peelamedu immediately after slaughter and transported to laboratory at 0 - 4˚C.

2. Preparation of Lens Culture

The lenses were removed by extra capsular extraction and incubated in artificial aqueous humor (NaCl: 140 mM, KCl: 5 mM, $MgCl_2$: 2 mM, $NaHCO_3$: 0.5 mM, $NaH(PO_4)_2$: 0.5 mM, $CaCl_2$: 0.4 mM, and Glucose: 5.5 mM) at room temperature and pH 7.8 for 72 hours. Penicillin G 32 mg% and Streptomycin 250 mg% were added to the culture media to prevent bacterial contamination. Glucose at the concentration of 55 mM was used to induce cataract.

3. Experimental Design

Group I: Lens + Glucose 5.5 mM (Normal control)

Group II: Lens + Glucose 55 mM (Negative control)

Group III: Lens + Glucose 55 mM + *Abutilon indicum* (500 µg/ml)

Group IV: Lens + Glucose 55 mM + Standard drug Enalapril (10 ng/ml)

4. Photographic Evaluation of Lens Opacity

After 72 hours of incubation, lenses were observed for opacity and photographs were taken by placing the lenses on the wire meshes with posterior surface touching the mesh, and the pattern of mesh was observed through the lens as a measure of lens opacity.

5. Preparation of Lens Homogenate

Lenses were homogenized in Tris buffer (0.23 M pH 7.8) and 0.25 x 10-3 M EDTA. The homogenate was adjusted to 10% w/v. The homogenate was centrifuged at 10,000 rpm at 4°C for 1 hour. The supernatant was used for studying various biochemical parameters.

6. Study of Anticataract Potential of *Abutilon indicum*

a. Estimation of Total Protein Content

The total protein content was determined using the standard protocol by Lowry, (1951).

b. Estimation of Malondialdehyde (MDA)

Lenses were homogenized in 10% (w/v) 0.1 M TrisHCl buffer (pH 7.5). 1 ml of the homogenate was combined with 2 ml of TCATBAHCl reagent, 15% trichloroacetic acid (TCA) and 0.375% thiobarbituric acid (TBA) in 0.25 N HCl and boiled for 15 min. Precipitate was removed after cooling by centrifugation at 1000 rpm for 10 min and absorbance of the sample was read at 535 nm against a blank without tissue homogenate. The values are expressed as MDA/ min/ mg lens protein (Umamaheshwari et al., 2012).

c. Assay of Superoxide dismutase (SOD)

The assay mixture contained 1.2 ml sodium pyrophosphate buffer (0.052 M, pH 8.3), 0.1 ml of 186 µM phenazonium methosulphate (PMS), 0.3 ml of 300 µM NBT, 0.2 ml of 780 µM NADH, 1.0 ml homogenate and distilled water to a final volume of 3.0 ml. Reaction was started by the addition of NADH and incubated at 30ºC for 1 min. The reaction was stopped by the addition of 1.0 ml glacial acetic acid and the mixture was stirred vigorously. Precisely 4.0 ml of n-butanol was added to the mixture and shaken well. The mixture was allowed to stand for 10 min, centrifuged, the butanol layer was taken out and the absorbance was measured at 560 nm against a butanol blank. A system devoid of enzyme served as the control (Umamaheshwari et al., 2012)

d. Determination of Aldose Reductase (AR) Activity

The assay mixture in 1 ml contained 0.7 ml phosphate buffer (0.067 M), 0.1 ml of NADPH (25×10^{-5}), 0.1 ml of lens supernatant, 0.1 ml of D L-glyceraldehydes (substrate) (5×10-4 M). Appropriate reference blanks were employed for corrections containing except the substrate, D L-glyceraldehydes. The enzymatic reaction was started by the addition of substrate and the absorbance was recorded in UV-Spectrophotometer at 340 nm for at least 3 min at 30 sec interval. AR activity was expressed as Δ OD /min/mg protein and the % inhibition activity was found (Umamaheshwari et al., 2012).

RESULTS

Anticataract Activity of *Abutilon indicum*

1. Photographic Evaluation of Lens Opacity

Figure 1 showed the normal lens incubated with the artificial aqueous humor solution and glucose (5.5 mM) that showed complete transparency. Figure 2 showed the negative control in which the lens was incubated with

glucose (55 mM) and complete opacification of lens had taken place. Figure 3 showed the lens incubated with glucose (55 mM) and the hydro-ethanolic extract of *A. indicum* which showed reduction in the lens opacity. Figure 4 consist of lens and standard drug (Enalapril) and it showed almost normal transparency when compared to cataractous lenses. The result indicates a positive effect of the selected plant extract on anticataract potential by exhibiting reduction in the opacity of cataractous lenses.

2. Study of Anticataract Potential of *Abutilon indicum*

Table 1 shows the anticataract potential of *Abutilon indicum*. Group 1 with Normal lens contained the highest amount of protein, AR inhibition % and SOD activity compared to negative control. The groups treated with the plant extract and the enalapril restored the level of protein, AR inhibition and SOD. The MDA was found to be the highest in the negative control and least in the normal control. The groups treated with the plant extract and the

Figure 1: Normal Control

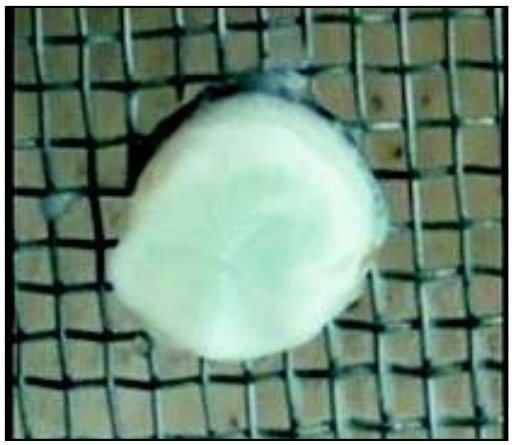

Figure 2: Negative Control

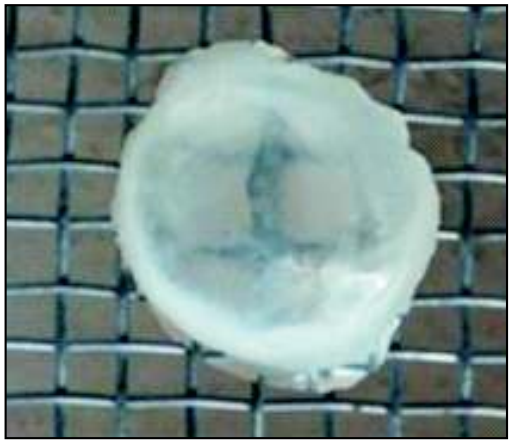

Figure 3: *A. indicum*

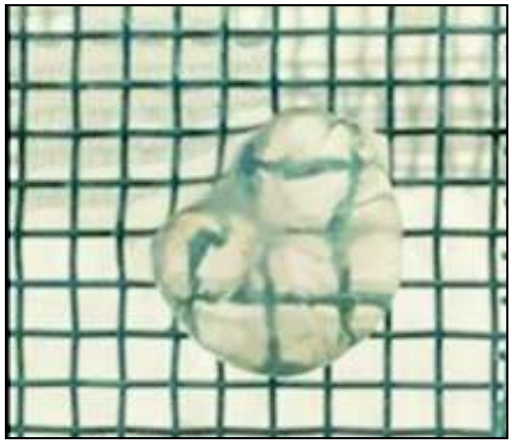

Figure 4: Standard Drug

Table 1 : Effect of the Hydro-ethanolic Leaf Extracts on Lens Protein, MDA, AR Inhibition %, SOD Activity

Groups	Protein (mg/ml)	MDA (MDA/ min/ mg lens protein)	AR – Inhibition Activity (%)	SOD (units/mg tissue)
Group 1	16.6 ± 0.316	0.0003 ± 0.00158	98.98 ± 0.0509	9.21 ± 0.01
Group 2	2.2 ± 0.158	0.0026 ± 0.000354	69.22 ± 0.0316	3.11 ± 0.0223
Group 3	8.8 ± 0.412	0.0021 ± 0.0001	91.40 ± 0.0509	7.81 ± 0.10024
Group 6	12.8 ± 0.224	0.0005 ± 0.000316	91.40 ± 0.0509	8.50 ± 0.10024

The values are given as mean ± standard deviation

enalapril reduced the level of MDA in the lens (Ahmed et al., 2011.)

DISCUSSION

As the role of oxidative stress in cataract development had been established, and thus the importance of antioxidants in prevention of cataract has, also been accepted in human ophthalmology. Three molecular mechanisms may be involved in the development of diabetic cataract: nonenzymatic glycation of eye lens proteins, oxidative stress, and activated polyol pathway in glucose disposition. All of these changes accelerate generation of reactive oxygen species (ROS) and increases in oxidative chemical modification of proteins in the lens of diabetic patients (Pritom et al., 2015). In the present study, opacity in the lens (cataract) occurred due to the incubation of lens in the media containing high concentration of glucose (55mM) which was because of the formation of free radicals like superoxide, H_2O_2, MDA inside the cataractous lens (that led to the increase in the oxidative stress). These free radicals are inhibited by enzymatic antioxidants such as SOD and CAT (Pritom et al., 2015).

The presence of phytochemicals such as phenolic groups and flavonoids in *A. indicum* has been found in the study conducted by Ramasubramaniaraja, (2011). Flavonoids contain high antioxidant activity thereby playing a major role in cataract prevention (Stefek, 2011). The amount of protein, SOD and AR inhibition were found to be increased in the lens incubated with the plant extract and enalapril and were found to suppress the formation of cataract which is evident from the above results. These results were in agreement with other similar studies carried out by Umamaheshwari et al. (2012) and Pritom et al. (2015) Naik et al., 2015.

ACKNOWLEDGEMENT

I would like to acknowledge my sincere thanks to the Head, Department of Biotechnology and my guide, Dr G R Damodaran College of Science, Coimbatore for their complete guidance, help and constant support during the time of my research.

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