Research Article

Anticataract Activity of *Abutilon hirtum* on Glucose Induced Cataract in Goat Eye Lens

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ABSTRACT

Cataract is the clouding of lens which is major secondary complication of diabetes mellitus. There are lot of medicinal plants getting its resurgence in treatment of cataract. The present study was carried out to determine the *in vitro* anticataract potential of *Abutilon hirtum* using glucose induced cataractous goat eye lens. The study included preliminary phytochemical screening, antioxidant activity and anticataract activity of the hydro ethanolic leaf extract of *Abutilon hirtum*. The phytochemical screening revealed the presence of tannins, flavonoids, saponins, alkaloids and phenols. The plant extract taken at a concentration of 100 µg/ml showed considerable antioxidant activity (total phenol content, DPPH radical scavenging activity, SOD, catalase assay and total reducing power). An *in vitro* study was conducted which reported that the lens group treated with the plant extract (500µg/ml) showed reduction in the opacity compared to the lens in the negative control. The study of the anticataract potential of *A. hirtum* showed an increase in total protein content, aldose reductase inhibition activity and SOD activity a decrease in the level of malondialdehyde compared to the negative control. The present study revealed the anticataract potential of hydro ethanolic leaf extract of *A. hirtum.*

Keywords: Cataract, diabetes mellitus, *Abutilon hirtum*, phytochemicals, antioxidant, anticataract.

INTRODUCTION

Medicinal plants contain some organic compounds which produce definite physiological action on the human body and these bioactive substances include tannins, alkaloids, carbohydrates, terpenoids, steroids and flavonoids. Medicinal plants are of great importance to the health of individuals and communities.[1] Medicinal plants continue to be a major source of drugs and natural products on the basis of their therapeutics in virtually all cultures. The plants possess potent bioactive compounds capable of preventing and treating most oxidative related diseases.[2] Cataract is the opacity of the lens that produces painless gradual loss of vision. Cataract formation is mainly an age-related phenomenon, although socioeconomic and lifestyle factors such as nutritional deficiency, sunlight, smoking, environmental factors, lack of consumption of antioxidants may also influence its occurrence.[3]

Abutilon hirtum (Family Malvaceae) commonly known as Indian mallow is perennial herb or shrub, pubescent leaves 4-14 cm across, ovate to orbicular, irregular and minute to coarsely serrate or crenate. Usually cordate at base, obtuse to acute or shortly acuminate at apex pubscent on both sides. Many branched erect, stout and aromatic herb about 1.0-1.5 m tall. Abutilon hirtum is found throughout tropical and subtropical regions of India. This is small herb found throughout India also in melghat forest.[4]

MATERIALS AND METHODS

Plant material

The plant chosen for the present study was *Abutilon hirtum* to evaluate the anticataract activity in glucose induced cataractogenic goat eye lens. The selected plant was collected from Kothampadi (Salem district, Tamil Nadu). 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 (BSI/SRC/5/23/2015/Tech./2061).

Preparation of extract

The leaf materials of the selected plant was collected and washed in tap water, shade dried and powdered. About 20 g of the air-dried powdered leaf material of *A. hirtum* and was extracted using soxhlet apparatus with 200 ml of hydro-ethanolic solvent (50 %). The solvent fractions of the extract was evaporated by a vacuum rotary evaporator.[5]

Antioxidant Activity of *A. hirtum*

Determination of Total Phenolic Content (Folin and Ciocalteu's, 1927)

The content of total phenols in leaves of *A. hirtum* plant extracts was determined spectrophotometrically using Folin–Ciocalteu reagent by using the modified protocol. Calibration curve was prepared by mixing ethanolic solution of gallic acid with 5 ml Folin-Ciocalteu reagent (diluted tenfold) and sodium carbonate solution in distilled water (4 ml, 0.7 M). The absorption was measured at 765 nm using a UV- spectrophotometer. Precisely 1 ml of plant extracts (10 g/l= 10 mg/ml) was mixed instead of 1 ml gallic acid with the same reagents as described above in three different test tubes and after 1 hour the absorption was measured to determine the total phenolic contents. The absorbance was measured against a reagent blank, which was composed of the same reagents except test extract. Total content of

phenolic in the plant extracts were expressed as gallic acid equivalents (mg of GAE/g sample).[6]

Catalase activity (CAT)

CAT activity was determined by modified protocol of Sumaira.[7] The reaction solution of CAT activities contained 2.5 ml of 50 mM phosphate buffer (pH 5.0), 0.4 ml of 5.9 mM H_2O_2 and 0.1 ml enzyme extract. Changes in absorbance of the reaction solution at 240 nm were determined after one minute. One unit of CAT activity was defined as an absorbance change of 0.01 as units/mg/min.

Superoxide dismutase activity (SOD)

The reaction mixture, which contained 50 µl of sample, 50 µl of NADH (936 µM) in phosphate buffer (100 mM, pH 7.4), 50 µl of NBT (300 µM) in phosphate buffer and 50 µl of PMS in phosphate buffer was incubated at room temperature for 5 min. The absorbance was read at 560 nm. The reaction mixture, without the extract was used as control. One unit of SOD activity is the amount of enzyme required to inhibit the reduction of NBT by 50% under the specified conditions.[8]

DPPH radical scavenging activity

The DPPH radical scavenging activity was done by using the modified protocol by Guno and Prashant. It is one of the most extensively used antioxidant assay for plant samples. This assay is based on the measurement of the scavenging ability of antioxidant test substances towards the stable radical. The radical scavenging activities of the plant extracts against DPPH radical (Sigma Aldrich) were determined by UV spectrophotometer at 517 nm. About 1 ml of various concentrations of the extracts was added to 3 ml of methanol followed by 0.5 ml of 1 mM methanolic solution of DPPH. After incubation period at room temperature, the absorbance was read against a blank (A blank solution was prepared containing the same amount of methanol and DPPH except the test compound). Ascorbic acid (Vitamins C) was used as the antioxidant standard. The radical scavenging activity (inhibition of DPPH free radical in percent) was calculated using the following formula

$$Scavenging\ Activity\ (\%) = \frac{A517nm\ (Control) - A517nm\ (Sample)}{A517nm\ (Control)} \times 100$$

where,

A_{517nm} (Control) is the Absorbance of the control at 517nm;

A_{517nm} (Sample) is the Absorbance of the plant samples at 517nm.

Total reducing power assay

The total reducing power was determined using the protocol by Sana and Chandrashekar.[9] About 100 µl of the extracts of varied concentrations were mixed with phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and 1% potassium ferricyanide (2.5 ml). The mixture was incubated at 50°C for 20 min. About 2.5 ml of 10% trichloroacetic acid (TCA) was added to the mixture and centrifuged at 3000 rpm for 10 min. The supernatant (2.5 ml) was mixed with distilled water (2.5 ml) and freshly prepared $FeCl_3$ solution (0.5 ml, 0.1 %). The absorbance was measured at 700 nm. Reducing power was expressed as ascorbic acid equivalent (AAE) in milligram per gram of extract and % reducing power was calculated using the formula:

$$Reducing\ Power\ (\%) = \frac{A700nm\ (Control) - A700nm(Sample)}{A700nm\ (Control)} \times 100$$

where,

A_{700nm} (Control) is the Absorbance of the control at 700nm;

A_{700nm} (Sample) is the Absorbance of the plant samples at 700nm.

Anticataract Activity of *A. hirtum*

The *in vitro* anticataract activity was carried out using the modified protocol of Shabeer.[10]

Collection of Goat Eye Balls

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

Preparation of Lens Culture

The lenses was 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.

Experimental Design

Group I: Normal lens + glucose 5.5 mM (Normal control)

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

Group III: Lens + Glucose 55 mM + *A. hirtum* (500 µg/ml)

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

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.

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.

Study of Anticataract Potential of the Plant Extract

The anticataract potential of the plant extracts was determined. The following biochemical parameters were analyzed using the modified protocol of Umamaheswari.[11]

Estimation of total protein content

To 0.1 ml of lens homogenate, 4.0 ml of alkaline copper solution was added and allowed to stand for 10 min. Then, 0.4 ml of phenol reagent was added very rapidly and mixed quickly and incubated at room temperature for 30 mins for color development. Reading was taken against blank prepared with distilled water at 610 nm in UV-visible spectrophotometer. The protein content was calculated from standard curve prepared with bovine serum albumin and expressed as µg/mg lens tissue.

Estimation of malondialdehyde (MDA)

Lenses were homogenized in10% (w/v) 0.1 M Tris–HCl buffer (pH 7.5). One milliliter of the homogenate was combined with 2 ml of TCA–TBA–HCl 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.

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 30°C 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.

Determination of aldose reductase (AR) activity

AR activity was assayed according to the modified protocol described by Rajesh.[12] 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 using the following formula:

$$AR\ Inhibition\ Activity\ (\%) = \frac{A340nm\ (Control) - A340nm\ (Sample)}{A340nm\ (Control)} \times 100$$

where,

A_{340nm} (Control) is the Absorbance of the control at 340nm;

A_{340nm} (Sample) is the Absorbance of the plant sample at 340nm.

RESULTS AND DISCUSSION

Antioxidant Activity

The antioxidant activity was studied by determining the total phenol content, DPPH radical scavenging activity, total reducing power, catalase assay and superoxide dismutase assay.

Table 1: Antioxidant activity of hydro-ethanolic extract of *A. hirtum*

Total Phenol content (mg GAE/g)	DPPH radical scavenging activity (100 µg/ml)	Total reducing power % (TRP)	Catalase assay (units/mg/min) (CAT)	Superoxide dismutase assay (units/mg dry weight) (SOD)
0.004	31.94, IC_{50}-290µg/ml	67.15	51.3	33.33

Table 2: Effect of the hydro-ethanolic leaf extract on lens protein, MDA, AR-Inhibition and 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	11.8 ± (0.158)	0.0018 ± (0.000158)	83.29 ± (0.0223)	8.28 ± (0.00316)
Group 4	12.8 ± (0.224)	0.0005 ± (0.000316)	91.40 ± (0.0509)	8.50 ± (0.10024)

The values are given as mean ± standard deviation

Anticataract Activity of Hydro-Ethanolic Plant Extract

Photographic Evaluation of Lens Opacity

Photographs of the lenses in normal and experimental groups are shown in Plate 1 which revealed that normal lens incubated with the artificial aqueous humor solution and glucose (5.5 mM) showed complete transparency. The negative control in which the lens was incubated with glucose (55 mM) a complete opacification of lens was noticed. Groups 3 in which lens were incubated simultaneously with glucose (55 mM) and the hydro-ethanolic extract of *A. hirtum* (500 µg/ml) showed a considerable reduction in the opacity of the lens similar to that of Group 4 treated with standard drug. The result indicates a positive effect of the selected plant extract on anticataract potential by exhibiting reduction in the opacity of cataractous lenses.

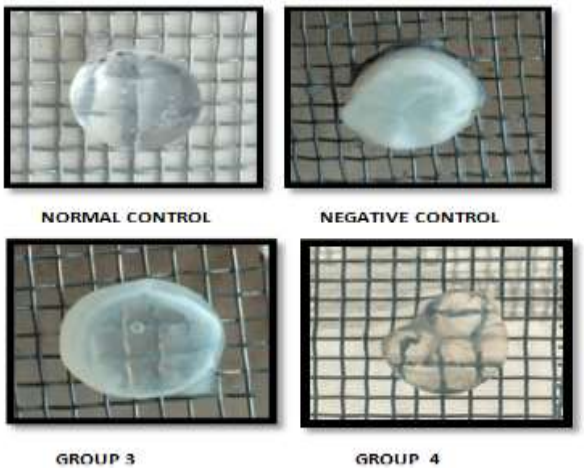

Plate 1: Photographic Evaluation of the Lens Opacity

Effect of the Hydro-ethanolic Leaf Extract on Lens Protein, MDA, AR-Inhibition and SOD activity

It is evident from table 2 that there was a significant decrease in the level of total protein and an increase in the level of MDA in cataractous lens (Group 2) when compared to normal control (Group 1). The lens with the plant extract (Group 3) and the lens treated with standard drug enalapril caused a significant increase in the total protein and a decrease in the level of MDA. Similar results were achieved in a study carried out by Umamaheshwari.

CONCLUSION

The study suggested that the hydro ethanolic extract of *A hirtum* possessed antioxidant and anticataract activity which might be due to the presence of phytochemicals such as tannins, phenols, flavonoids, alkaloids.

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