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R E S E A R C H A R T I C L E

In-Vitro Anti Cataract Activity of *Adenia Cissampeloides* against Glucose -Induced Cataractogenesis

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A B S T R A C T

Cataract is defined as opacity within the clear natural crystalline lens of the eye, which gradually results in vision deterioration. The World Health Organization (WHO) estimated that in 1990, out of the 38 million blind people worldwide, cataract accounted for 41.8% - almost 16 million people. With a projected increase in the geriatric population, WHO has estimated that there will be 54 million blind people aged 60 years or older by the year 2020. Accordingly, cataract surgery will continue to weigh heavily on health care budgets in the developed nations. In the United States, cataract-related expenditure is estimated to be over $3.4 billion annually. In the developing world, the number of new cataract cases supersedes the number of new cataract cases supersedes the rate of surgical removal. In Africa alone, only about 10% of the 5,00,000 new cases of cataract blindness each year are likely to have their sight restored surgically. It is estimated that if onset of cataract could be delayed by 10 years the annual number of cataracts could be delayed by 10 years, the annual number of cataract surgeries performed would be reduced by almost a half. This calls to question the risk factors of this multifactorial disease, which have been a litany of genetic, environmental, socioeconomic, and biochemical factors working in an interlaced fashion. The purpose of this work is tp determine the catratogenesis of plant extract of Adenia Cissampeloides to treat against glucose induced catratogenesis.

Keywords: Cataract, blindness, Adenia Cissampeloides,

A R T I C L E I N F O

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C O N T E N T S

1. Introduction

- The work involved the following steps,
- Collection and authentication of the leaves of *Tamarindus indica* Linn.
- Preparation of hydro methanolic extract
- Phytochemical screening
- Induction of cataract by incubation of goat lenses with glucose (55mM) for 72hr with and without the plant extract
- Estimation of tissue protein, malondialdehyde and lipid hydroperoxides in lens homogenate
- Estimation of enzymatic antioxidants like superoxide dismutase, catalase, glutathione peroxidase, peroxidase and glutathione reductase and the nonenzymatic antioxidant, reduced glutathione in lens homogenate
- Statistical analysis

2. Materials and Methods

Plant material

The plant material consists of dried powdered leaves of *Adenia cissampeloides*. belonging to the family Passiflorine.

Plant collection and authentication

The leaves of Adenia cissampeloides were collected from Coimbatore district in Tamil Nadu, India during the month of jan 2023. The plant was identified and authenticated by Mr. G.V.S. Murthy, Joint Director, Scientist, C-I/C, Botanical survey of India, Tamil Nadu Agricultural University Campus, Coimbatore bearing the reference number BSI/SC/5/23/08-09/Tech-659.

Preparation of extraction

The fresh leaves of Adenia cissampeloides are collected, dried in shade under room temperature, powdered mechanically and sieved through No. 20 mesh sieve. The finely powdered leaves were kept in an airtight container until the time of use. The extraction was carried out by continuous hot percolation method using Soxhlet apparatus. The solvent used was a mixture of methanol: water in the ratio of 7:3. About 100 g of powder was extracted with 600 ml of solvent. The extract was concentrated to dryness under controlled temperature between 40-50 ºC.

Drugs and chemicals

Glucose and vitamin E were obtained from SD fine chemicals, Mumbai. Liquid paraffin was obtained from Fisher Chemicals Ltd., Chennai. Thiobarbituric acid, Trichloro acetic acid, Butylated hydroxyl toluene, oxidized glutathione, epinephrine and 5 5'Dithiobis-2 nitrobenzoic acid were obtained from Sisco Research Laboratories Pvt. Ltd., Mumbai. 2 – 2' dipyridyl and O-dianisidine were obtained from Himedia Laboratories Ltd., Mumbai. Goat lenses were obtained from the slaughterhouse Coimbatore. All other drugs and chemicals used in the study were obtained commercially and were of analytical grade.

In vitro experimental model of cataract

In vitro experimental model of cataract was induced in goat lenses using glucose at a concentration of 55 mM, incubated for 72 h at room temperature. At high concentrations, glucose in the lens was metabolized through sorbitol pathway and accumulation of polyols (sugar alcohols), causing overhydration and oxidative stress. This leads to cataractogenisis.

Experimental protocol

A total of 30 goat lenses were used and divided into the following 5 groups (n = 6 in each group),

Group I: Artificial aqueous humor alone (solvent control)
Group II: Glucose 55 mM alone (Negative control)
Group II: Glucose 55 mM alone (Negative control)
Group III: Plant extract (100 µg/ml) + glucose 55 mM
Group IV: Plant extract (200 µg/ml) + glucose 55 mM
Group V: Vitamin E(100µg/ml) +glucose55mM (Standard drug)

In vitro lens culture

Fresh goat eyeballs were collected from slaughterhouse, immediately after slaughter and transported to the laboratory. The lenses were removed by extracapsular extraction and incubated in artificial aqueous humor (NaCl 140 mM, KCl 5 mM, MgCl2 2 mM, NaHCO3 0.5 mM, NaoH(PO4)2 0.5 mM, CaCl2 0.4 mM and Glucose 5.5 mM) at room temperature and pH 7.8 for 72h. Penicillin 32 mg% and streptomycin 250 mg% were added to the culture media to prevent bacterial contamination (Langade, 2006).

Preparation of lens homogenate

After 72 h of incubation, homogenate of lenses (10% w/v) was prepared in Tris buffer (0.23 mM, pH 7.8) containing 0.25x10-3 M EDTA. The homogenate was centrifuged at 10,000 g for 1 h and the supernatant was used for estimation of total protein (TP), determination of the end products of lipid peroxidation namely malondialdehyde (MDA) and lipid hydroperoxides (LH), enzymatic antioxidants like superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GSSH), peroxidase (Px), and glutathione peroxidase (GPx), and the non- enzymatic antioxidant reduced glutathione (GSH).

Estimation of total protein (TP)

The amount of total protein present in the tissue homogenate was estimated by the method of Lowry et al., 1951. To 0.1 ml of tissue 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 in room temperature for 30 min for colour 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 (Lowry et al., 1951).

Estimation of malondialdehyde (MAD)

Lipid peroxidation as evidenced by the formation of thiobarbituric acid reactive substances (TBARS) and hydroperoxides (HP) were measured by the method of Nieshus and Samuelsson, 1986. About 0.1 ml of tissue homogenate (Tris HCl buffer, pH 7.4) was treated with 2 ml (1:1:1 ratio) of TBA – TCA-HCl reagent (Thiobarbituric acid 0.37%, 0.25N HCl and 15% TCA) and placed in a water bath for 15 min, cooled and centrifuged at 1000 g at room temperature for 10 min. The absorbance of the clear supernatant was measured against a reference blank at 535 nm. The values are expressed as nmoles of MDA /min/mg lens protein.

Estimation of lipid hydroperoxides (LH)

About 0.1 ml of tissue homogenate was treated with 0.9 ml of Fox reagent (188 mg butylated hydroxytoluene (BHT), 7.6 mg xylenol orange and 9.8 mg ammonium ion sulphate were added to 90 ml of methanol and 10 ml 250 mM sulphuric acid) and incubated for 30 min. The colour developed was read at 560 nm using a colorimeter. The values are expressed as nmoles/mg lens protein (Nieshus and Samuelsson, 1986).

Determination of enzymatic antioxidants

Estimation of superoxide dismutase (SOD)

SOD activity was determined by the inhibition of auto catalyzed adrenochrome formation in the presence of the homogenate at 480 nm. The reaction mixture contained 150 µl of lens homogenate, 1.8 ml of carbonate buffer (30 mM, pH 10.2), and 0.7 ml of distilled water and 400 µl of epinephrine (45 mM). Auto oxidation of epinephrine to adrenochrome was performed in a control tube without the homogenate (Kakkar et al., 1984).

Estimation of catalase (CAT)

The catalysis of H2O2 to H2O in an incubation mixture adjusted to pH 7.0 was recorded at 254 nm. The reaction mixture contained 2.6 ml of 25 mM potassium phosphate buffer pH 7.0 and 0.1ml of tissue homogenate and was incubated at 370C for 15 min and the reaction was started with the addition of 0.1ml of 10 mM H2O2. The time required for the decrease in absorbance from 0.45 to 0.4 representing the linear portion of the curve was used for the calculation of enzyme activity. Activity was expressed as µmoles/mg tissue protein (Abei, 1984).

Estimation of glutathione reductase (GSSH)

The enzyme activity was determined spectrophotometrically by the decrease in absorbance of NADPH at 340 nm. The reaction mixture contained 2.1 ml of 0.25 mM, potassium phosphate buffer pH 7.6, 0.1 ml of 0.001 M NADPH, 0.2 ml of 0.0165 M oxidized glutathione and 0.1 ml (10 mg/ml) of bovine serum albumin (BSA). The reaction was started by the addition of 0.02 ml of tissue homogenate with mixing and the decrease in the absorbance at 340 nm was measured for 3 min against a blank. Glutathione reductase activity was expressed as nmoles NADPH oxidized /min/mg lens protein at 300C (Racker, 1955).

Estimation of peroxidase (Px)

Peroxidase activity was measured spectrophotometrically by following the change in absorbance at 460 nm due to O-dianisidine oxidation in the presence of H_2O_2 and enzyme. Reaction mixture contained 0.2ml of 15 mM O-dianisidine, 0.1ml of tissue homogenate and 2.5 ml of 0.1M potassium phosphate buffer pH 5.0 and were incubated at 370C for 15 min and the reaction was started with the addition of 0.2 ml of hydrogen peroxide and the absorbance at 460 nm was followed against a blank, spectrophotometrically for about 3-5 min at 370C. Unit of enzyme activity defined as µmoles of O-dianisidine/min at 370C (Lobarzewski and Ginalska, 1995).

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Glutathione peroxidase activity was measured by the procedure of Paglia and Valentine, 1967. The reaction mixture consists of 0.2 ml of 0.4 M Tris buffer, 0.1 ml of sodium azide, 0.1 ml of hydrogen peroxide, 0.2 ml of glutathione and 0.2 ml of supernatant incubated at 370C.

for 10 min. The reaction was arrested by the addition of 10 % TCA and the absorbance was measured at 340 nm. Activity was expressed as nmoles/min/mg lens protein.

Determination of non-enzymatic antioxidant Estimation of reduced glutathione (GSH)

The method was based on the reaction of reduced glutathione with dithiobisnitrobenzoic acid (DTNB) to give a compound that absorbs at 412 nm. To the homogenate, 0.1 ml of 10% TCA was added and centrifuged. About 0.1 ml of supernatant was treated with 0.5 ml of Ellmans reagent (19.8 mg of 5, 5'-dithiobisnitro benzoic acid DTNB in 100 ml of 0.1% sodium nitrate) and 3.0 ml of phosphate buffer (0.2 M, pH 8.0) and the absorbance was read at 412nm. Activity was expressed as nmoles/min/mg lens protein (Ellman, 1959).

Statistical analysis

Statistical analysis was carried out by one-way analysis of variance (ANOVA) followed by Dunnett's test. Results are expressed as mean ± SEM of six lenses in each group. P values < 0.05 were considered significant.

The plant material consists of dried powdered leaves of *Adenia cissampoloides* belonging to the family Passifloraceae.

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The leaves of *Adenia cissampoloides* were collected from Chittoor district in A.P, India during the month of Jan 2022. The plant was identified and authenticated by Mr. G.V.S. Murthy, Joint Director, Scientist, C-I/C, Botanical survey of India, Nagarjuna University Campus, Coimbatore bearing the reference number BSI/SC/5/23/08-09/Tech-659.

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The method was based on the reaction of reduced glutathione with dithiobisnitrobenzoic acid (DTNB) to give a compound that absorbs at 412 nm. To the homogenate, 0.1 ml of 10% TCA was added and centrifuged. About 0.1 ml of supernatant was treated with 0.5 ml of Ellmans reagent (19.8 mg of 5, 5'-dithiobisnitro benzoic acid DTNB in 100 ml of 0.1% sodium nitrate) and 3.0 ml of phosphate buffer (0.2 M, pH 8.0) and the absorbance was read at 412nm. Activity was expressed as nmoles/min/mg lens protein (Ellman, 1959).

Statistical analysis

Statistical analysis was carried out by one-way analysis of variance (ANOVA) followed by Dunnett's test. Results are expressed as mean ± SEM of six lenses in each group. P values < 0.05 were considered significant.

3. Results and Discussion

Phytochemical screening: Phytochemical screening of the powdered leaves of *Adenia Cissampeloides* showed the presence of phenolics, tannins, saponins, flavonoids and alkaloids.

Table:1 Phytochemical screening

Phytochemicals	*Adenia cissampeloides*
Alkaloids	+
FlavonoidsC-glycosides	+
Saponins	+
Tanninsandphenolics	+
Terpenoids	+

Effect of the extract of *Adenia Cissampeloides* leaves on lens protein and lipid peroxidation in experimental groups: There was a significant (P<0.01) decrease in the level of total protein and an increase in the level of malondialdehyde and lipid hydroperoxides in glucose-induced cataractous lenses when compared to normal control. Incubation with the hydroethanolic leaf extract of *Adenia Cissampeloides* at doses of (100 & 200 µg/ml) and Vitamin E (100 µg/ml) simultaneously with glucose for 72 h caused a significant (P<0.01) increase in the total protein and a decrease in the level of malondialdehyde and lipid hydroperoxides (Table 2).

Table 2. Effect of *Adenia Cissampeloides* hydro methanolic leaf extract on lens protein, MDA and LH in control and experimental groups

GROUP	DOSE	PROTEIN	MDA	LH
Normalcontrol	_	103.6±8.89	0.68±0.024	4.13±0.37
Glucosecontrol	55mM	36.64±1.26[a]	1.98±0.017[a]	10.98±0.60[a]
Plantextract	100µg/ml	74.38±2.12[b]	0.84±0.014[b]	5.16±0.37[b]
Plantextract	200µg/ml	76.22±3.12[b]	0.78±0.032[b]	4.88±0.73[b]
Vitamin-E	100µg/ml	84.24±3.62[b]	0.72±0.08[b]	4.54±0.23[b]

Valuesaremean±SEM;n=6ineach

[a]P<0.01whencomparedtonormalcontrol;

[b]P<0.01 when compared to glucose control (one way ANOVA followed by Dunnett's test).
Protein = mmoles/min/mg, MDA = nmoles/min/mg protein, LH = nmoles/min/mg protein.

Table 3. Effect of *Adenia Cissampeloides* hydromethanolileaf extract on lens enzymatic and non-enzymatic antioxidants in control and experimental groups

Group	Dose	Catalase	GPx	SOD	GSSH	Peroxidase	GSH
Normalcontrol	_	1.73±0.17	2.88±0.29	4.53±0.23	1.99±0.09	2.47±0.32	2.99±0.32
Glucose control	55mM	0.49±0.04[a]	1.12±0.11[a]	1.23±0.37[a]	0.08±0.016[a]	0.86±0.19[a]	1.11±0.20[a]
Plantextract	100µg/ml	1.12±0.09[b]	2.26±0.21[b]	3.59±0.73[b]	0.94±0.26[b]	1.89±0.04[b]	2.17±0.23[b]
Plantextract	200µg/ml	1.38±0.09[b]	2.43±0.24[b]	3.84±6.37[b]	1.13±0.08[b]	1.96±0.13[b]	2.29±0.12[b]
Vitamine-E	100µg/ml	1.42±0.07[b]	2.54±0.25[b]	3.92±0.60[b]	1.24±0.08[b]	2.10±0.21[b]	2.37±0.21[b]

Values are mean ± SEM; n = 6 in each group.
aP<0.01 when compared to normal control;
bP <0.01 when compared to glucose control (One way ANOVA followed by Dunnett's test). CAT = µmoles/min/mg protein
GPx = nmoles/min/mg protein, GSH = nmoles/min/mg protein,
SOD =nmoles/min/mg protein, GSSH=nmoles/min/mg protein and Peroxidase = nmoles/min/mg Protein

Discussion

The present thesis entitled "In vitro anticataract activity of *Adenia cissampeloides*. Against glucose-induced cataractogenesis" deals with the evolution of phytochemical screening and the pharmacological action of the selected Indian medicinal plant *Adenia cissampeloides*. belonging to the family Fabaceae, is used normally by the tribes of India and African countries and is mainly used for the treatment of swelling, tumours ringworm inflammation which is useful in disease of blood, small pox, ophthalmia and other eye diseases, earache, snake-bike (Krithikar and Basu, 1981).

Blindness is majorly seen as the result for the cataract formation which is linked with the age and more over it is related to the oxidative stress as a key factor. These situations can be remedied surgically by extirpation of the cataractous lens. The limits of cataract surgery have stimulated experimental cataract research in laboratory animals and epidemiological studies to determine the incidence, prevalence and risk factors for the development of cataract so as to focus on the preventive aspects of cataract (Gupta et al., 1997a).

Cataract was induced in vitro with glucose at a concentration of 55mM in aqueous humour media and incubated for 72 h at room temperature (Langade 2006). After incubation the lens homogenate was used for the estimation of total protein (TP) content, determination of end products of lipid peroxidation namely malondialdehyde (MDA) and lipid hydroperoxides (LH), enzymatic antioxidants like superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GSSH), peroxidase (Px), glutathione peroxidase (GPx), and non-enzymatic antioxidant reduced glutathione (GSH).Lipid peroxidation is an autocatalytic process, which is a common cause of cell deth (Bandhyopadhyay et al., 1999). In order to elucidate the protective mechanism of the leaves of Tamarindus indica, glucose-induced goatlens was examined for lipidperoxide levels. Decomposition of lipid peroxides initiate the chain reactions that produce reactive carbonyl compounds. The by-products of lipid peroxidation are the toxic compounds malondialdehyde (MDA) and lipid hydroperoxides (LH) whose involvement in cataractogenesis has been suggested, mainly due to its cross linking ability. Lens MDA may be the result of lipid peroxidation of the lens cell membrane or may represent the consequence of its migration from the readily peroxidizable retina or from the central compartment. In our studies, glucose-induced goat lenses showed an increase in malondialdehyde and lipid hydro peroxide levels in lens. Incubation at different concentrations (100 & 200 µg/ml) of extract of Tamarindus indica, simultaneously with glucose (55mM) for 72h caused a significant ($P<0.01$) decrease in the lens malondialdehyde and lipid hydroperoxides and an increase in total protein level. This effect was almost similar to the vitamin E treated group.

Several varieties of toxic species of oxygen are formed in the lens milieu, including superoxide anion, hydrogen peroxide, hydroxyl radical and lipid hydroperoxides. The enzyme catalyses the reduction of oxygen (during reperfusion phase), leading to the formation of superoxide and H2O2 as well as hydroxyl radicals. It has been proposed as a central mechanism of oxidative injury in some situations (Nijveldt et al., 2001). Thus the determination of the lens in vitro antioxidant enzymes like SOD, CAT, GPx, GSSH, peroxidase (Px) and non-enzymatic antioxidant enzyme, GSH were carried out. Catalase is present in almost all the mammalian cells localized in the peroxisomes. It catalyses the decomposition of H2O2 to water and oxygen and thus proctects the cell from oxidative damage by H2O2 and hydroxyl radical. The dichromate/acetic acid reagent can be thought of as a 'stop bath for catalase activity. As soon as the enzyme hits the acetic acid, its activity isdestroyed, any H2O2 which is not split by the catalase will react with the dichromate to give a blue precipitate which is then decomposed on heating to give a green solution. Tamarindus indica extract significantly increased ($P<0.01$) the catalase level in glucose-induced cataractous lenses. The first enzyme involved in the antioxidant defence is superoxide dismutase. It is metalloprotein found in both prokaryotic and eukaryotic cells. The oxygen radicals, generated by intraction of Fe2+ and H2O2 are the species responsible for the oxidation of epinephrine at pH 10.2 and was strongly inhibited by superoxide dismutase (Misra et al., 1972).GPx has a major role in degrading the levels of H2O2 in cells. Since GPx acts on hydroperoxides of unsaturated fatty acids, the enzyme plays an important role in protecting membrane lipids, and thus the cell membranes from oxidative disintegration (Rotruk et al., 1973). Theenzymatic antioxidant levels in glucose-induced cataract groups were decreased when compared to normal control group. The leaf extract of Tamarindus indica significantly ($P<0.01$) increased the level of antioxidant enzymes. Which is almost similar to the vitamin E treated group.GSH is an intracellular reductant which plays major role in catalysis, metabolism and transport. It protects cell against free radicals, peroxides and other toxic compounds. Indeed, GSH depletion increases the sensitivity of cells to various aggressions and also has several metabolic effects (Gupta et al., 1997a). Extract of Tamarindus indica significantly ($P<0.01$) increased the level of GSH when compared to glucose-induced cataractous lenses.

4. Conclusion

To conclude, the study suggested that the leaf extract of Tamarindus indica possess anticataract and antioxidant activities, which might be helpful in preventing or slowing the progress of cataract. Further investigations on the isolation and identification of active components in the leaves may lead to chemical entities with potential for clinical use in the prevention and treatment of cataract.

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