Research Article

Phytochemical Screening and *in-vitro* Anti-cataract Activity on the Leaves of *Ipomoea batatas* (L) LAM Ethanolic Extract

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Received: 22-10-2022; **Revised:** 23-12-2022; **Accepted:** 30-12-2022; **Published on:** 15-01-2023.

ABSTRACT

The clouding of the eye's lens, called as a cataract, which leads to impairs vision. Cataract often develops slowly and can affect one or both eyes. When proteins in the eye aggregate to create clumps that stop the lens from focusing, a cataract develops. Older persons frequently develop cataracts. Natural products are more useful for body because they promote the repair mechanism in nature way. The anti-cataract activity may be attributed to the presence of different phytoconstituents present in the ethanolic extract of leaves on *Ipomoea batatas* (L.) *lam,* especially Vitamin A, ascorbic acid, β - carotene, carotenoids, and flavonoids. This phytoconstituents possess the antioxidant property to reduce the development of cataract. The present investigation was aimed to evaluate the efficacy of ethanolic extracts of *Ipomoea batatas (L) lam* leaves against glucose induced cataract in goat lens. In this *in-vitro* study goat lenses were subjected to photographic evaluation and subjected to biochemical parameters such as total proteins, water soluble proteins. Photographic examination of the eyes showed that treatment with ethanolic extracts of *Ipomoea batatas* (L) lam leaves retarded the progression of lens opacification. The total proteins and water-soluble proteins activity is increased in the extract treated lenses when compared with the standard drug ascorbic acid. From this study we conclude that ethanolic extract of leaves on *Ipomoea batatas* (L.) *lam* was significant reduction of cataract at the dose of 80µg/ml in goat lens.

Keywords: Anti-cataract, *Ipomoea batatas*, Goat lens, Vitamin A, Total proteins, Water soluble proteins.

QUICK RESPONSE CODE →

DOI: 10.47583/ijpsrr.2023.v78i01.012

DOI link: http://dx.doi.org/10.47583/ijpsrr.2023.v78i01.012

INTRODUCTION

The Cataracts were undoubtedly very common in antiquity. The word cataract which means both an opacity of the lens and a torrent of water, comes from the Greek word υπόχυσις (kataráktēs) meaning the fall of water. The Latins called it suffusio, an extravasation and coagulation of humors behind the iris[1]. Opacity of the lens is a direct outcome of oxidative stress. Based on location of opacification within the lens, age-related cataracts are classified into three types: cortical, nuclear, and posterior subcapsular cataracts [2]. Cataract is associated with old age and cataract is a major complication of diabetes Mellitus. It is a multifactorial disease occurs mainly due to the formation of large protein aggregates in the lens. The lens Na - K^+- ATPase activity plays an important role in maintaining lens transparency, and its impairment causes accumulation of Na+ and loss of K+ with hydration and swelling of the lens fibers leading to cataractogenesis. Normal lens contains containing glutathione and ascorbic acid as antioxidants. The old age people have less effective in anti-oxidative mechanism and there is the increase in inactive insoluble proteins and semi-permeability of the lens capsule which may lead to cataract formation[3].

A wide range of drugs like aldose reductase inhibitors, non-steroidal anti-inflammatory drugs (NSAIDs) are being tried for their anticataract activity [4]. There has been a growing interest in the various activities of indigenous plants. Many indigenous plants have been explored as potential promising sources of antioxidants. *Ipomoea batatas* (L.) *lam* is a dicotyledonous plant that belongs to the family, Convolvulaceae[5]. The plant locally known as "Sarkaraivallikizangu or sweet potato"[6]. The nutritionally important compounds like phenolic, flavonoids, alkaloid, sterols, terpenoids, glycosides and many other metabolites have been isolated from different parts of *Ipomoea batatas* (L.) *lam* and possess various pharmacological activities [7-10].

Sweet potato is an extremely versatile and delicious vegetable that possess high nutritional value. Sweet potato has been grown in tropical and subtropical regions. Due to its versatility, sweet potatoes now are recommended over other vegetables. The medicinal properties of sweet potato include antioxidant, antimicrobial agent, anti-inflammatory, anti-arthritic, hypolipidemic, antidiabetic activity, hematinic, anti-proliferative, cytotoxic, diuretic, wound healing, hepatoprotective, anti-mutagenic and anti-carcinogenic, immunomodulatory activities. Magnesium, an essential mineral contained in sweet potatoes that assists in

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relaxing, provides approximately. In Ayurvedic, Sweet potato leaves applied to boils and acne, boiled roots used for diarrhoea, hot water infusion of the whole plant used in the management of diabetes mellitus [9, 11-26]. Hence, this study has been taken with an aim to evaluate anti-cataract activity on the leaves of *Ipomoea batatas* (L.) *lam* ethanolic extract on glucose induced cataract in goat lens and ascorbic acid served as standard. Glucose induced cataract in goat lens model was practiced to assess the inhibition cataract formation.

MATERIALS AND METHODS

Plant collection and identification

The Leaves on *Ipomoea batatas* (L.) *lam* was collected from their natural habitats in Thasanayakkanpalayam, Anthiyur, Erode District, Tamil Nadu. In the month of January 2020. It was authenticated by Professor. P.Jayaraman, Ph.D. Director, Institute of Herbal Botany Plant Anatomy Research Centre, West Tambaram, Chennai- 45.

Preparation of plant extracts

The Leaves on *Ipomoea batatas* (L.) *lam* was cleaned and chopped into small pieces. It is dried under shade and pulverized. Extraction is carried out by using soxhlet apparatus with ethanol as solvent. The extract was concentrated on a water bath and residue was dried in a desiccators.

Phytochemical Analysis [27-29]

Qualitative analysis

1. Test for alkaloids

A small portion of the extract was stirred with few drops of dilute hydrochloric acid and filtered. The filtrate was tested carefully with various alkaloidal reagents such as **Mayer's reagent** (cream precipitate), **Dragendroff's reagent** (orange brown precipitate), **Hager's reagent (**yellow precipitate), and **Wagner's reagent** (reddish brown precipitate).

2. Test for flavonoids

NaOH test: A small amount of extract was treated with aqueous NaOH and HCl, observed for the formation of yellow orange colour indicated presence of flavonoids.

H_2SO_4 test: A fraction of extract was treated with concentrated H_2SO_4 and observed for the formation of orange colour indicated presence of flavonoids.

Lead acetate test: A small amount of extract was treated with lead acetate and observed for the formation of white precipitate indicated presence of flavonoids

3. Test for tannins

Few ml of extract was treated with 10% alcoholic ferric chloride solution and observed for formation of blue or greenish colour solution indicated presence of tannins.

4. Test for phenols

Ferric chloride test: The fraction of extract was treated with 5 % ferric chloride and observed for formation of deep blue or black colour indicated presence of phenols.

5. Test for terpenoids

Liebermann – Burchard test: Extract (1ml) was treated with chloroform, acetic anhydride and drops of H_2SO_4 was added and observed for the formation of dark green colour indicated presence of terpenoids.

6. Test for anthraquinones

Borntrager's test: About 50 mg of powdered extract was heated with 10% ferric chloride solution and 1ml concentrated HCl. The extract was filtered, cooled, and mixed with diethyl ether subsequently. Strong ammonia is also used to further extract the ether extract, producing deep pink or deep red colourations of aqueous layer indicate the presence of anthraquinone.

7. Test for anthocyanin

NaOH test: A small amount of extract was treated with 2M NaOH and observed for the formation of blue green colour indicated presence of anthocyanin.

8. Test for proteins

Biuret test: The extract is treated with an equal volume of 1% strong sodium hydroxide followed by a few drops of copper (II) sulphate, formation of purple colour indicated the presence of protein.

Million's test: To the extract million's reagent is added, a white precipitate is produced, while heating it turns brick red colour indicated the presence of protein.

9. Test for sterols Liebermann-Burchard test:

Extract (1ml) was treated with chloroform, acetic anhydride and drops of H_2SO_4 was added and observed for the formation of dark pink or red colour indicated presence of sterols.

10. Test for saponins

Foam test: The extract was diluted with 5ml distilled water. The suspension was shaken in graduated cylinder for 15 min. A 2cm layer foam indicated the presence of saponins

11. Test for mucilage

Aqueous potassium hydroxide can be used to treat the extract. Mucilage has been observed, as indicated by swelling.

12. Test for carbohydrates

Molish's test: To the extract few drops of α-naphthol solution in alcohol, con.$H_2SO_4$is added at the side of test tube, formation of violet ring at the junction of two liquids indicated the presence of carbohydrates.

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In-vitro Anti-cataract Activity by Glucose Induced Cataract in Incubated Goat Lenses Model [30-35]

Materials requirements

- Goat lenses
- Sodium chloride
- Potassium chloride
- Magnesium chloride
- Sodium bicarbonate
- Sodium phosphate
- Calcium chloride
- Glucose
- Penicillin G
- Streptomycin
- Ascorbic Acid
- Ethanolic extract of *Ipomoea batatas* (L.) *lam* leaves.

Procedure

A. Collection of eye balls:

Goat eyeballs were used in the present study. They were obtained from the slaughterhouse. Immediately after slaughter and transported to laboratory at 0 - 4 degree Celsius.

B. Preparation of lens culture:

The slaughterhouse brought fresh goat eyeballs, that were then transported to the laboratory at 0-4°C. Extracapsular extraction was used to remove the lens, which was then cultured in artificial aqueous humor (NaCl 140 mM, $MgCl_2$ 2 mM, $NaHCO_3$ 0.5 mM, $NaHPO_4$ 0.5 mM, $CaCl_2$ 0.4 mM, KCl 5 mM, and glucose 5.5 mM) 5 mM) at ambient temperature and add $NaHCO_3$ to maintain pH 7.8. Penicillin G 32% and streptomycin 250 mg% added to the culture media to prevent bacterial contamination. At high concentration, glucose in the lens was metabolized through sorbitol pathway and accumulation of polyol causing over hydration and oxidative stress. As a result, cataractogenesis begins.

C. Induction of cataract on goat lenses

To induce cataracts, glucose at a concentration of 55 mM was used. The sorbitol pathway can be used by the lens's high concentrations of glucose to breakdown it. Accumulation of polyol (sugar alcohols) causes over hydration and oxidative stress. This leads in cataractogenesis. These lenses were incubated in artificial aqueous humor with different concentration of glucose (5.5 mM) served as normal control and 55 mM served as toxic control) for 72 hours.

D. Experimental design

Goat lenses were divided into six groups containing one lens in each and incubated as following **Table 1.**

Table 1: Experimental design of *in-vitro* anti-cataract activity

Group	Treatment
I	Goat lens + Artificial Aq. Humor (Glucose 5.5 mM)
II	Goat lens + Artificial Aq. Humor (Glucose 55 mM)
III	Goat lens + Artificial Aq. Humor (Glucose 55 mM) + 40 µg/ml of Ascorbic Acid
IV	Goat lens + Artificial Aq. Humor (Glucose 55 Mm)+ 20 µg/ml of Ethanolic extract of leaves on *Ipomoea batatas* (L.) *lam*
V	Goat lens + Artificial Aq. Humor (Glucose 55 mM)+ 40 µg/ml of Ethanolic extract of leaves on *Ipomoea batatas* (L.) *lam*
VI	Goat lens + Artificial Aq. Humor (Glucose 55 mM) + 80 µg/ml of Ethanolic extract of leaves on *Ipomoea batatas* (L.) *lam*

E. Assessment of anti-cataract activity

I. Photographic evaluation

To test lens opacity, lenses were put on a wire mesh with their posterior surfaces touching the mesh. The number of mesh squares that were clearly visible through the lens was observed.

The opacity was graded using the following system.

0 - means there is no opacity.

1 - A very slight amount of opacity.

2 - Diffuse opacity is present.

3. The presence of thick, widespread opacity

II. Analysis of biochemical parameter in homogenate lens

a) Preparation of lens homogenate

After 72 hours of incubation, homogenate of lens was prepared in Tris buffer (0.23 M, pH-7.8) containing 0.25×10^{-3} M EDTA and homogenate was adjusted to 10% w/v which was centrifuged at 10,000 G at 4ºC for 1 hour and the supernatant was used for the estimation of biochemical parameters.

1. Estimation of total protein content

4 ml of alkaline copper solution was added to 0. 1 ml of lens homogenate and allowed to stand for 10min. Then, 0.4 ml of phenol reagent was added very rapidly and mixed quickly and incubated in room temperature for 30 mins for colour development. Readings were obtained at 610 nm in the UV spectrum against a distilled water-prepared blank. The protein content was calculated from standard curve prepared with bovine serum albumin and expressed as µg/mg lens tissue.

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Statistical analysis

Results were expressed as Mean ± Standard Error of Mean (SEM). P<0.05 was considered statistically significant. Data obtained was analysed by one-way ANOVA followed by Dunnett's multiple comparisons test using Graph pad prism version 7.

RESULTS AND DISCUSSION

1. Phytochemical analysis

Qualitative analysis

Preliminary phytochemical analysis of Ethanolic extract of Leaves on *Ipomoea batatas* (L.) *lam* revealed the presence of various components like carbohydrate, glycoside, flavonoid, alkaloid, tannin and steroids (**Table 2**). Sweet potato leaves is also an important source of vitamin A, thiamine, riboflavin, niacin, ascorbic acid, β -carotene and many other functional compounds[36,37]. The leaves of the sweet potato plant were powerful antioxidants. The antioxidant activity of Ethanolic extract of Leaves on *Ipomoea batatas* (L.) *lam* has been shown to offer protection against the cataract[38].

Table 2: Phytochemical analysis of Ethanolic extracts of Leaves on *Ipomoea batatas* (L.) *Lam*

S.no	Phytochemical Test	Ethanolic extract
1.	Alkaloids	+
2.	Flavonoids	+
3.	Tannins	+
4.	Phenols	+
5.	Terpenoids	+
6.	Anthraquinones	+
7.	Anthocyanin	+
8.	Proteins	+
9.	Sterols	+
10	Saponins	-
11.	Mucilage	-
12.	Carbohydrates	+

NOTE: (+) Present (-) Absent

In-vitro anti-cataract activity by glucose induced cataract in incubated goat lenses model

A wide range of plants and plant derived products are used in folk medicine for the treatment of cataract as a prophylactic agent or as curative agent. *In-vitro* anti-cataract activity of ethanolic extract of leaves on *ipomoea batatas* (L.) *lam* was evaluated by glucose induced cataract in incubated goat lenses model. To access the inhibition of cataract by using Photographic evaluation to find out the degree of opacity on goat lenses. The results obtained illustrated **Table 3 and Figure 1**.

After 8 hours of incubation with glucose 55 mM, lenses started to be become transparent. This progressively increased towards the center, with complete opacification at the end of 72 hrs. In photographic evaluation (Fig.1), After 72 hours of incubation transparency was maintained in the Group I (normal control group) [Fig.A] but there was the complete loss of transparency in the Group II (negative control group) [Fig.B] indicating complete cataractogenesis. Group III (positive control group) [Fig.C] containing lens treated with standard ascorbic acid were squares of the graph paper were visible through the lenses. Group IV, V, and VI containing lens treated with ethanolic extract of leaves on *Ipomoea batatas* (L.) *lam* were respectively 20 µg/ml, 40 µg/ml, 80 µg/ml and squares of the graph paper were visible through the lenses indicating suppression of cataract formation [Fig.D, E, and F]. Group VI was more effective in suppressing cataract formation than Group IV and Group V (**Table 3**).

The lens showed the absence of opacity because the ethanolic extract of leaves on *ipomoea batatas* (L.) *lam* inhibits cataractogenesis and oxidative stress. Incubation of goat lenses in the media containing high glucose (55 mM) concentration has induce cataract and has shown to cause the considerable drop in Na^+/ K^+- ATPase activity, with the progression of opacity. The impairment of Na^+/ K^+-ATPase causes accumulation of Na^+ and loss of K^+ with hydration and swelling of the lens fibers leading to cataractogenesis. This alteration in the Na^+, K^+ ratio changes the protein content of the lens, leading to a decrease in total proteins causing lens opacification. The imbalance of Na^+ and K^+ was prevented due to an action of ethanolic extracts of leaves on *ipomoea batatas* (L.) *lam* which corrects imbalances in the polyol pathway by decreasing aldose reductase activity, sorbitol concentration, and intracellular glucose.

Photographic evaluation

Table 3: Effect of ethanolic extract of leaves on *Ipomoea batatas* (L.) *lam* in degree of opacity on goat lens by glucose-induced cataract

S.NO	Group	Degree of opacity
1	I (Normal control)	0
2	II (Negative Control)	3
3	III (positive control) (Standard drug Ascorbic Acid - 40 µg/ml)	1
4	IV (Test 1 -20 µg/ml of Ethanolic extract of leaves on *Ipomoea batatas* (L.) *lam*	2
5	V (Test 2 - 40 µg/ml of Ethanolic extract of leaves on *Ipomoea batatas* (L.) *lam*	1
6	VI (Test 3 - 80 µg/ml of Ethanolic extract of leaves on *Ipomoea batatas* (L.) *lam*	0

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- ✓ Normal control: Zero degree opacity occurred, clear lens was obtained.
- ✓ Negative control: The presence of extensive thick opacity, because of high concentration of glucose induced cataractogenesis.
- ✓ Positive control (Ascorbic acid 40µg/ml): The lens shows the slight degree of opacity, slightly clear lens was obtained.
- ✓ Test 1 (20 µg/ml of Ethanolic extract of leaves on *Ipomoea batatas* (L.) *lam*). The lens shows the diffuse opacity, clear lens was not found.
- ✓ Test 2 (40 µg/ml of Ethanolic extract of leaves on *Ipomoea batatas* (L.) *lam*). The lens shows the slight degree of opacity, clear lens was not found.
- ✓ Test 3 (80 µg/ml of Ethanolic extract of leaves on *Ipomoea batatas* (L.) *lam*). Zero-degree opacity is occurred, the clear lens was obtained

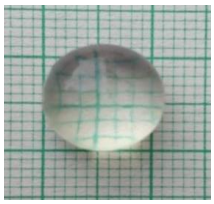

Fig (A): Normal control (group I)

Fig (B): Negative control (Group II)

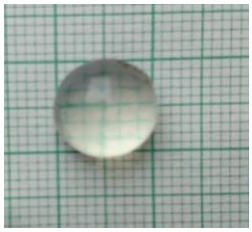

Fig (C): Positive control (Group III)

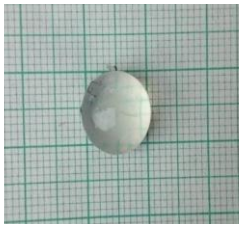

Fig (D): Test 1 -20 µg/ml (Group IV)

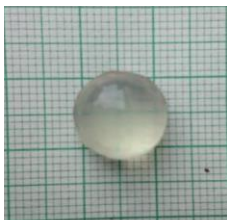

Fig (E): Test 2 - 40 µg/ml (Group V)

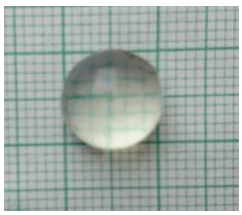

Fig (F): Test 3 - 80 µg/ml (Group VI)

Figure 1: Photographic evaluation of lens opacity

II. Analysis of biochemical parameter in homogenate lens

1. Estimation of total soluble protein content in homogenate lens

From the **Table 4 and Figure 2**, Group II was showed significantly lower concentrations of total soluble protein in the lens homogenate compared with Group I. Group III, Group V, Group VI were showed significantly higher concentrations of total soluble protein in the lens homogenate compared with Group II. Group IV was showed significantly near the concentrations of total soluble protein in the lens homogenate compared with Group II. The Ethanolic extracts of leaves on *Ipomoea batatas (L.)* lam significant increase the concentration of total protein content in homogenate lens when compared to the negative control group and ascorbic acid standard drug. The standard drug showed maximum increase the concentration of total protein content in homogenate lens of 215.37± 2.6 mg/ml at 40 µg. Though the Ethanolic extracts of leaves on *Ipomoea batatas (L.)* lam showed significant increase the concentration of total protein content in homogenate lens of 218.76± 1.6 mg/ml at 80 µg. It was found to be higher than that of standard.

Table 4: Estimation of total protein content in homogenate lens

S.No	Group	Total protein content (mg/ml)
1	I (Normal control)	222.51 ± 2.3
2	II (Negative Control)	173.40±2.0$^{\#\#}$
3	III (positive control) (Standard drug Ascorbic Acid - 40 µg/ml)	215.37± 2.6**
4	IV (Test 1 -20 µg/ml of Ethanolic extract of leaves on *Ipomoea batatas* (L.) *lam*	186.24± 2.1
5	V (Test 2 - 40 µg/ml of Ethanolic extract of leaves on *Ipomoea batatas* (L.) *lam*	209.31± 1.6**
6	VI (Test 3 - 80 µg/ml of Ethanolic extract of leaves on *Ipomoea batatas* (L.) *lam*	218.76± 1.6**

Values are expressed as Mean ± SEM. n=6,

Comparisons were made as between:

1. Group I Vs Group II, Group III, Group IV, Group V and Group VI. P values: $^{\#\#\#\#}P \le 0.0001$,$^{\#\#\#}P \le 0.001$, $^{\#\#}P \le 0.01$, $^{\#}P \le 0.05$ and ns- P >0.05

2. Group II Vs Group I, Group III, Group IV, Group V and Group VI. P values: ****P ≤ 0.0001, ***P ≤ 0.001, **P ≤ 0.01, *P ≤ 0.05 and ns - P >0.05

All the data's were statistically analyzed by one way ANOVA followed by Dennett's multiple comparison test.

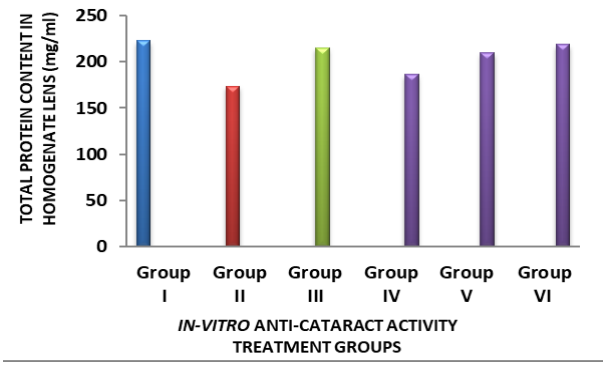

Figure 2: Estimation of Total Protein Content in Homogenate Lens

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2. Estimation of water-soluble protein content in homogenate lens

From the **Table 5 and Figure 3**, Group II was showed significantly lower concentrations of water soluble protein in the lens homogenate compared with Group I. Group III, Group V, Group VI were showed significantly high the concentrations of water soluble protein in the lens homogenate compared with Group II. Group IV was showed significantly near the concentrations of water soluble protein in the lens homogenate compared with Group II. The Ethanolic extracts of leaves on *Ipomoea batatas (L.)* lam significant increase the concentration of water soluble protein content in homogenate lens when compared to the negative control group and ascorbic acid standard drug. The standard drug showed maximum increase the concentration of water soluble protein content in homogenate lens of 79.52± 2.1mg/ml at 40 µg. Though the ethanolic extracts of leaves on *Ipomoea batatas (L.)* lam showed significant increase the concentration of water soluble protein content in homogenate lens of at 80.65±1.1 mg/ml at 80 µg. It was found to be higher than that of standard.

Table 5: Estimation of Water-Soluble Protein Content in Homogenate Lens

S.NO	Group	Water soluble protein content (mg/ml)
1	I (Normal control)	83.65± 3.0
2	II (Negative Control)	64.25± 3.1$^{\#\#}$
3	III (positive control) (Standard drug Ascorbic Acid - 40 µg/ml)	79.52± 2.1**
4	IV (Test 1 -20 µg/ml of Ethanolic extract of leaves on *Ipomoea batatas* (L.) *lam*	73.83± 1.2*
5	V (Test 2 - 40 µg/ml of Ethanolic extract of leaves on *Ipomoea batatas* (L.) *lam*	77.82± 1.6**
6	VI (Test 3 - 80 µg/ml of Ethanolic extract of leaves on *Ipomoea batatas* (L.) *lam*	80.65± 1.1**

Values are expressed as Mean ± SEM. n=6

Comparisons were made as between:

1. Group I Vs Group II, Group III, Group IV, Group V and Group VI.

P values: $^{\#\#\#\#}P \leq 0.0001$, $^{\#\#\#}P \leq 0.001$, $^{\#\#}P \leq 0.01$, $^{\#}P \leq 0.05$ and ns- P >0.05

2. Group II Vs Group I, Group III, Group IV, Group V and Group VI

P values: ****P ≤ 0.0001, ***P ≤ 0.001, **P ≤ 0.01, *P ≤ 0.05 and ns - P >0.05

All the data's were statistically analyzed by one way ANOVA followed by Dennett's multiple comparison test.

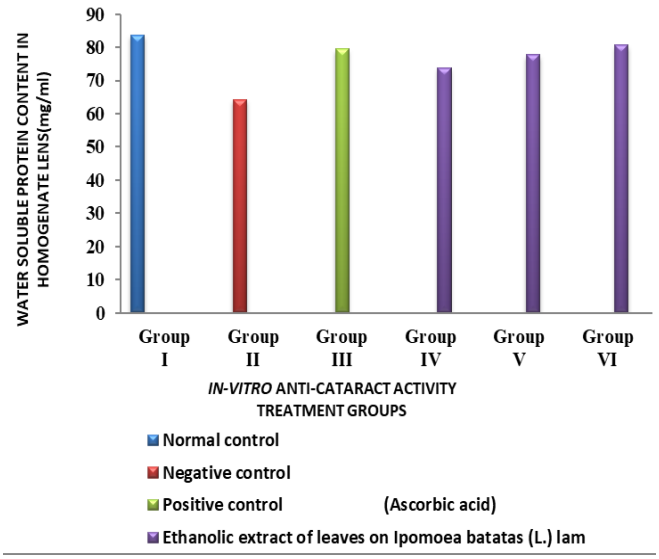

Figure 3: Estimation of Water-Soluble Protein Content in Homogenate Lens

The total protein and water-soluble protein are found in eye lenses. These proteins provide the high refractive index in eye lenses focuses light on to the retina and regulate the metabolic pathway in eye lenses. So, Ethanolic extracts of leaves on *Ipomoea batatas (L.)* lam showed significant increase the concentration of total protein and water-soluble protein content in goat lens. From all the above observation it can be concluded that ethanolic extract of leaves on *Ipomoea batatas* (L.) *lam* possess anti cataract activity to greater extent.

CONCLUSION

Ethanolic extract of leaves on *ipomoea batatas* (L.) *lam* possess anti cataract activity when compared with standard drug ascorbic acid. Thus, may be beneficial in the treatment of cataract. Further studies can be carried out in the future to *in-vivo* anti-cataract activity and elucidate the mechanism of action of ethanolic extract of leaves on *Ipomoea batatas* (L.) *lam.* This may be followed and clinical studies to establish its efficacy in humans.

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Source of Support: The author(s) received no financial support for the research, authorship, and/or publication of this article.

Conflict of Interest: The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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