

Research Article**Antioxidant and anti-cataract activity of ethanolic extract of *Epipremnum aureum* leaves: *In vitro* and *in vivo* study**KSSL Ramya¹, Tajuddin Shaik^{2*}, B. Ramesh³, Kakaraparthi Ravi Shankar⁴^{1,2}Assistant Professor, Malla Reddy College of Pharmacy, Hyderabad, Telangana³Student, Jawaharlal Nehru Technological University, Anantapur, Andhra Pradesh, India⁴Principal & Professor, Aditya College of Pharmacy, Andhra Pradesh, India

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Abstract

Background: *Epipremnum aureum* is an ayurvedic medicinal plant, leaves and roots of this plants are traditionally used in treat various diseases from ancient times. Here in, we have evaluated its phytochemical composition with antioxidative and anticataract potency of *Epipremnum aureum* leaves. **Objectives:** To evaluate effectiveness of *Epipremnum aureum* ethanolic leaves extract *in vitro* as an antioxidant and anticataract. To carry out the preliminary screening and free radical scavenging activity of phytochemicals of ethanolic leaves extract of *Epipremnum aureum*. To evaluate the *In vitro* and *in vivo* Anticataract Activity of ethanolic leaves extract of *Epipremnum aureum* in Glucose induced cataractogenesis using eye lens of Goat and in Galactose induced Cataractogenesis in Rats respectively. **Materials and Methods:** The antioxidant and anticataract evaluation of the ethanolic extracts of *Epipremnum aureum* leaves were carried out by using free radical scavenging activity by the Nitric oxide Scavenging Assay, Reducing power method and Phosphomolybdenum antioxidant assay. The *in vitro* anticataract activity was studied on goat eye by comparing there reducing ability of plant extract and standard drug Enalapril in the opacity of cataract lenses. The Galactose model is implemented here to study the *in vivo* anticataract. By placing the rat on water and Galactose diet for 30 days to induce the cataract and then treated with plant extract. **Results:** The result of DPPH radical scavenging activity shows a concentration dependent antioxidant activity of *Epipremnum aureum* which is comparable with a standard ascorbic acid and IC50 was found to be 87.09 µg/ml. The nitric oxide scavenging assay IC50 was found to be 24.5 µg/ml. Phosphomolybdenum anti-oxidant assay at 50µg/ml found to be 0.06±0.23. Aldose reductase (AR) activity was found to be 97.28±0.0032 in group I. Photographs of Lens in Galactose cataract *in vivo* model clearly indicates the anti-cataract activity. Level of MDA in cataract lenses (group 2) was significantly decreased compared with regular controls (Group 1). The results of *in vitro* and *in vivo* studies indicate that the selected plant extract has a positive effect on the anti-cataract potential, with the opacity of cataract lenses being reduced. **Conclusion:** Our findings from experiment provide evidence that the crude ethanolic extract of *Epipremnum aureum* is a potential source of natural antioxidants, and it justify the uses of *Epipremnum aureum* in folkloric medicines.

Keywords: *Epipremnum aureum*, phytochemical investigation, antioxidant, anticataract activity

Introduction

The primary causative factor for cataract is free radical induced by oxidative stress. Antioxidant compounds are essential in the scavenging of free radicals and the control of diseases linked to oxidative stress (Wu and Hansen, 2008). Natural antioxidants

and herbal drugs were proven to have anti-cataract properties. *Epipremnum aureum* is a medicinal plant belongs to family Araceae. It commonly called as Money plant. It is distributed throughout the tropical and sub-tropical forests worldwide, extends from Northern Australia to China, Japan, India and Indochina. In India, *Epipremnum aureum* is used from long back in various fields of medicine and to treat various diseases like jaundice, diabetes, leprosy, ulcer etc (Frankel, 1991). The plant is documented to possess beneficial effects as analgesic, Diuretic, anti-inflammatory, Anti-cancer, Anti-bacterial, anti-helminthic, Anti-fungal and

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aphrodisiac. Here in, we are studying its possibilities as an antioxidant and anticataract properties by in vitro and in vivo methods (Farjouetal. 1987).

Antioxidants: They are less active by scavenging or giving an electron to a radical, they protect the cell components and biomolecules against oxidation.

Antioxidant's mode of action are by follows four ways.

- Response to chain breakage: for example: α -tocopherol; acts to pull "ROS" radicals in Lipid Phase.
- Reduction of "ROS" levels: for example: Glutathione. Glutathione.
- Radical initiators of scavenging: for example: SOD
- Catalysts for Transition Metal Helming.

Medicinal applications of antioxidants are protection from cancer, beneficial in the immune system proper functioning, they exhibit anti-ageing, and reduction of cardiovascular diseases risks.

Cataract: In the lens of the eye, a cataract may appear as dense, white and cloudy area. A cataract begins when protein clumps within the eye shape prevent clear images from being transferred to the retina (Kinoshita, 1990). The retina works by translating sunlight into signals through the lens. Signals are transmitted to the brain's nerve optic to process it further. With the accumulation of protein clumps the signal transmission is interferes with your vision eventually.

Symptoms & causes of Cataracts: Vision of Blurry, Night vision difficulties, Look for the faded colors and enhanced light sensitivity. There are several causes underlying cataracts are an overproduction of oxidants, oxygen molecules chemically modified because of ordinary daily life, Smoking, Radiation from ultraviolet, Long-term steroid and other medicines and Some conditions, like diabetes.

Pathophysiology: The lens which causes refraction and focuses light on the retina, may be a transparent biconvex object. The human lens surrounded by a skinny capsule which is formed from fibers and is persisted each side by zonules. The lens fibres are made of the lens epithelium and move from the edge to the centre. The lens nucleus is now made of older lens fibres. Recent lens fibres are located inside the cortex's outer layers, called the lens (Guptaetal). The opacity of the lens is directly affected by oxidative stress. Age-related cataracts are divided into three types, depending on lens position: cortical, nuclear and posterior cataracts. The cells of the lens are highly metabolically active, lens cells and are oxidated, binding and insolubilized. Later, the cells migrate to the lens centre to produce lens fibres that are progressively compressed and lead to nuclear opacity

(Kinoshita, 1990). Cortical cataracts usually take the wing shape from the cortex to the centre of the lens. Plaque-like opacity rises in an axial rear cortical plate within the rear subcapsular cataract (Fatimah et al., 1998).

Materials and Methods

Collection of plant material

The leaves of *Epipremnum aureum* were collected near Peddapuram, Andhra Pradesh, India country. The plant was authenticated by Dr. T. Raghuram, Taxonomist, Maharani College, Peddapuram and voucher specimen number given is 23113.

Preparation of extract

The newly collected *Epipremnum aureum* leaves were washed by water to remove contamination and sand particles and then dried under shade for forty days. The powder was extracted for 3 days with ethanol and then for 3 hours with hot percolations. The filtering and distillation then took place on 80°C. It was then transferred into an empty Chinese dish, evaporated to obtain an ethanol extract, and stored in anhydrous desiccating calcium chloride.

Preliminary Phytochemical Screening

The preliminary phytochemical tests were carried out to check for secondary metabolites such as flavonoids, tannins, phenolic compounds, alkaloids, fixed oils, saponins and fats by ethanol extracts from *Epipremnum aureum* leaf extract.

Quantitative Phytochemical Testing

The quantitative phytochemical test like Estimation of Phenolic content were also carried out and the Phenolic content was estimated and results obtained in units of gallic acid equivalent to mg/100 mg of dried weight extract from the standard Gallic acid in methanol calibration curves. The alkaloid content of ethanol extracts in *Epipremnum aureum* was estimated by Fazel et al., method (Patil et al., 2003). The calibrate curve has been used to estimate the alkaloid content by standard atropine and to express the results as atropine mg/100mg extract equivalents.

In vitro antioxidant activity

Phosphomolybdenum antioxidant assay

Epipremnum aureum leaf extract antioxidant activity has been evaluated using the phosphomolybdenum method accordingly (Prieto et al., 1999). The test is based on extract reduction in Mo (VI)–Mo (V), with the result that a green phosphate/Mo (V) complex is formed at acidic pH. The reagent solution combined with 3 ml extract 0.3 ml (0.05, 0.1, 0.3 and 0.5 mg/ml) Extracts (0.6 M vitriol, 28 mM

sodium orthophosphate and 4 mM ammonium molybdate). The solution was incubated for 90 minutes at 95°C. The solution absorbance was determined at 695 nm.

Assay of nitric oxide scavenging/ Nitric oxide generation

In physiological solutions, sodium nitroprusside spontaneously generates gas interacting with oxygen in order to supply nitrite ions estimating the reactive Griess. Oxygen is competitive with gas scavengers, thus reducing gas production (Maccocci et al., 1994). The extracts (0.05, 0.1, 0.3 and 0.5 mg/ml) dissolved in adequate solvent system at a concentration of 25 °C for 150 min were mixed into 30 sodium nitroprusside of 5 mM with phosphate-buffered saline. The above samples have received a reaction from Griess (1 percent sulfanilamide, 2 percent H₃PO₄ and 0.1 percent naphthylethylenediamine dihydrochloride). During the diazotization of nitrite with sulphanilamide, the absorption of chromophorus and the subsequent combination with naphthylethylenediamine was measured at 546 nm and the absorption of normal ascorbic acid solutions was mentioned in the same way as the Griess reagent. The inhibition formulation was calculated by Nitric oxide Scavenged (%) = $[(A_o - A_1) / A_o] \times 100$

where A_o is control reaction absorption (with all reagents except sample extract) and A₁ is sample extract absorption. Positive controls were used for ascorbic acid.

Hydroxyl radical scavenging assay

The scaling capability of 5 sample extracts for hydroxyl radicals was determined by some modifications described by (Smirnov and Cumbes, 1989). Sample extracts of 1 mL (0.05, 0.1, 0.3, and 0.5 mg/mL) at different levels of the reagent have shortly been added Containing sodium salicylate 1 mL 1.5 mM, 0.7 mL 6 mM H₂O₂ and 0.3 mL 20 mM. The reaction mixture absorbance is at 562 nm following incubation at 37°C for 1 h. The formula for calculating the inhibition percentage was Scavenging ability on hydroxyl radicals:

$$\text{Inhibition (\%)} = [(A_o - A_1) / A_o] \times 100$$

Where A_o is control reaction absorption (with all reagents except sample extract) and A₁ is sample extract absorption. Positive controls were used for ascorbic acid.

In vitro Anticataract Activity

Collection of Goat Eye Balls

In vitro, a goat eye lens in glucose induced cataractogenesis studied the anti- Cataract potential of the plant extract. Goat balls were taken from the Peddapuram Slaughter House and transported to the laboratory immediately after slaughter.

Preparation of Lens Culture

Extra capsular lenses were extracted and incubated in artificial water humour, with a temperature of 7.8 and a temperature of 72

hours (NaCl: 140 mM, CCl: 5 mM, MgCl₂ (2 mM) NaHCO₃: mM, CaCl₂: 0.5mM, NaH(PO₄)₂: 0.5 mM, and Glucose: 5.5 mM). To stop bacterial contamination the culture media were added penicillin G 32 mg and Streptomycin 250 mg percent. For cataract induction, glucose 55 mM was used in the concentration.

Experimental Design

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

Group II: Objective + 55 mM of glucose (Negative control)

Group III: Objective + 55 mM glucose + leaf extract from *Epipremnum aureum*;

Group IV: lens + 55 mM glucose + Enalapril (10 ng/ml) Standard medication

Photographic Evaluation of Lens Opacity

The opacity of lenses was observed after incubation of 72 hours, and images of lenses were captured on the wire mesh and the posterior surface touching the net was affected, and the mesh pattern as an opacity measurement was thus observed by the lens.

Preparation of Lens Homogenate

In the Tris buffer (0.23 M pH 7.8) and 0.25 x 10⁻³ M EDTA lenses were homogenised. The homogeneous content has been modified to 10% w/v. At ten thousand minutes the homogeneous was centrifuged.

Study of anticataract potential of the plant extract

The anticataract potential of the plant extracts determined. The subsequent biochemical parameters were analyzed like.

Estimation of total protein content

4.0 ml of alkaline copper solution were added to the homogenous 0.1 ml lens which could stand up 32 to 10 minutes. The reagent was then very fast added to 0.4 ml of phenol and quickly mixed. 30 minutes for colour development incubated at temperature. Lecture was made against white prepared in the UV-visible spectrophotometer, with distilled water at 610 nm. A standard curve made from bovine albumin expressed as µg/mg lens tissue has been used to calculate the protein content.

Estimation of malondialdehyde (MDA)

In 10% (w/v) 0.1 M Tris-HCl buffer lenses were homogenised (pH 7.5). Two ml TCA-TBA-HCl reagent, 15% trichloroacetic acid (TCA) and 0.375% thiobarbituric acid (TBA) were combined into one millilitre of homogene, boiled in 0.25 N HCl and boiled for 15 min. After the centrifugation cooled the precipitate for 10 minutes, and the

absorption was measured at 535 nm against a white without homogeneous tissues. The values are given as lens protein MDA/min/mg.

Determination of aldose reductase (AR) activity

AR activity has been tested in accordance with the Rajesh described amended protocol¹². The 1-ml assay mixture consisted of 0.7 ml (0.067 M), 0.1 ml NADPH (25×10^{-5}), 0.1 ml supernatant lens, 0.1 ml D L-glyceraldehyde (substrate) (5×10^{-4} M). For corrections, other than substrate, D-L-glyceraldehyde, appropriate reference blanks were used. The enzymatic reaction was begun by adding the substrate and, therefore, at a minimum of 3 minutes, at 30 seconds, absorption was recorded in UV spectrometers of 340 nm. AR activity has been described by using the following formula:

$$\% \text{ Inhibition} = \left[1 - \frac{\Delta A \text{ Sample/min} - \Delta A \text{ Blank/min}}{\Delta A \text{ Control/min} - \Delta A \text{ Blank/min}} \right] \times 100$$

In vivo anticataract activity

Galactose cataract in vivo model (Sippel, 1966)

In vivo study with Wistar rats were split into controlled and treated groups of each sex, weighing 250g (n = 6). All ad-libitum induced cataracts have been fed with 300g/L galactose. A dose of *Epipremnum aureum* is given in distilled water (as a vehicle) 7 days prior to the start of galactose dieting of 50 mg/kg & 100mg/kg body weight dose Oral to the treated group once a day and continued until the experiment was completed. Only the water and the galactose diet are distributed in the control group. After the rat pupil was dilated with 10g/L tropicamide, eyes were examined by a slit lamp. The cataract phases were classified by Sippel's ranking. Acute toxicity studies were performed as per OECD guideline no. 423. There was no outrageous evidence of observation of any abnormalities and the maximum tolerated dose level of 1000mg/kg body weight per orally. So the pharmacological screening were carried out with two dose ranges of 50mg/kg & 100mg/kg body weight.

A normal, negative control, reference standard and 2 test groups

were used each comprising of 6 rats.

Group I: Received only saline (Normal) 5 ml/kg by p. o

Group II: Normal Saline + Galactose/kg body weight (Negative Control) orally

Group III: *Epipremnum aureum* ethanolic extract Test dose 2 + single dose of Galactose 25 μ mole/kg b. w. orally

Group IV: *Epipremnum aureum* ethanolic extract Test dose 1 + single dose of Galactose 25 μ mole/kg b.w orally.

Group V: Vitamin E 50 mg/kg body weight per oral + single dose of Galactose 25 μ mole/kg b. w. orally

Results and Discussion

Preliminary Phytochemical Screening

The results of preliminary phytochemical evaluations of *Epipremnum aureum* leaves ethanolic extract consist of secondary metabolites such as alkaloids, tannins, and triterpenoids.

In vitro antioxidant activity

It has been reported that the biological base of chronic condition is oxidative stress when free radical formation more than the body's ability to protect itself (Fatimah et al., 1998). Leaves extract *Epipremnum aureum* react by ending the chain reaction with free radicals which are the main cause for the fat self- extraction chain (Frankel, 1991). Therefore, it is apparent that the *Epipremnum aureum* extract is a free radical inhibitor or spinning agent, and a primary antioxidant that reacts with free radicals that can reduce damage in the human body due to free radicals.

The power reduction tests assess the ability of antioxidants to donate electron by a method of reducing potassium ferricyanide. The ion/ferricyanide complex is decreased by antioxidants into the iron form, the Prussian blue complex in Perl (Chou et al., 2003). The reduction of a compound's potential antioxidant activity may be a significant indicator of (Meir et al., 1995). Different mechanisms, such as chain

Table 1. In vitro antioxidant potential of ethanol extract of *Epipremnum aureum* and ascorbic acid by Phosphomolybdeum and reducing power assay

Concentrations	Nitric oxide method			Hydroxy radical scavenging method				Phosphomolybdeum Method		
	Ethanolic extract	IC50	Standard	IC50	Ethanolic extract	IC50	Standard	IC50	Ethanolic extract	Standard
50	46.71±0.482	62.845	57.85±0.028	27.531	49.31±0.045	58.182	55.14±0.36	38.414	0.0178±0.009	0.0294±0.0003
100	54.84±0.29		68.46±0.568		56.14±0.499		62.18±0.34		0.0309±0.0012	0.0481±0.005
300	66.84±0.368		83.31±0.211		64.62±0.621		72.18±0.536		0.0631±0.002	0.0893±0.0005
500	72.06±0.807		92.32±0.209		78.54±0.482		88.91±0.465		0.143±0.0017	0.168±0.0009

All the values are expressed as Mean ± SEM, n= 3; * P< 0.001 when compared with standard values

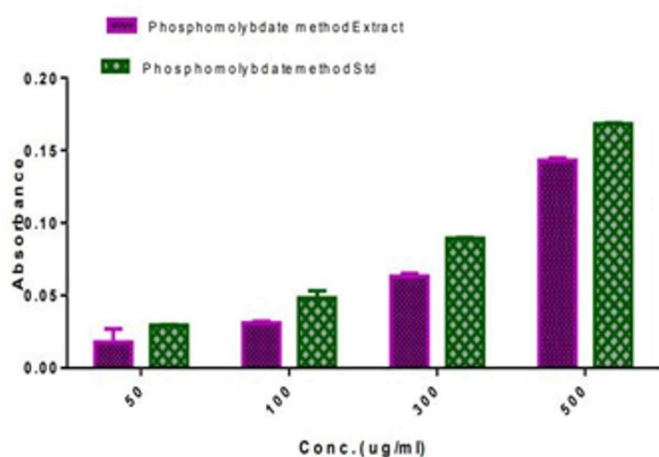


Figure 1. In vitro antioxidant potential of ethanol extract of *Epipremnum aureum* and ascorbic acid by Phosphomolybdenum and reducing power assay

starting prevention, connecting the transition metal ion catalytic systems, peroxide decay, continued hydrogen abstraction prevention, reduction of capacity and radical spraying was attributed to the antioxidant activity in extracts (Table 1 and Figure 1) and ascorbic acids (Diplock, 1997).

The most reactive free radical in biology systems is OH radical, and in the presence of metal ions such as copper and iron, the superoxide anion and hydrogen peroxide may form it. As a highly damaging species in free radical pathology, Hydroxyl radical has been involved, which can damage nearly every molecule in living cells. For example, OH-radicals, especially thiamine and guanosine, react with lipids, polypeptides, proteins, and DNA. The radical can be combined in DNA with nucleotides, cause a breakdown in the strand and lead to cancer, mutagenesis, and cytotoxicity (Madhava et al., 2008). DNA, lipids, proteins are oxidative damaged by highly reactive OH radicals (Spencer et al., 1994).

Radical nitroxides generated from the physiological pH sodium nitroprusside have been found to be inhibited by *Epipremnum aureum* and Ascorbic acid extract in the ethanol leaves. In aqueous solution, sodium nitroprusside spontaneously produces nitric oxide at physiological pH (Marcocci et al., 1994),

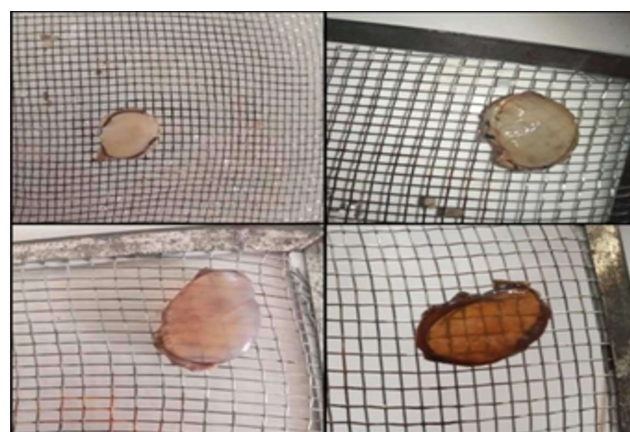


Figure 2. In vitro anticataract activity photographic evaluation of lens opacity

interacting with oxygen, producing nitrite ions estimated with the use of the Griess reagent. Nitric oxide scavengers competition with oxygen, thereby reducing nitric oxide production.

Antioxidant capacity of ethanolic leaves extract from a sample analyte and a consequent formation of green phosphate/mo(V) compounds with a maximum absorption at 695 nm was measured in a phospho-molybdenum method based on the reduction of Mo(IV) to Mo(V) (Table 1 and Figure 1).

In vitro anticataract Activity

Figure 4 shows normal lenses incubated with artificial aqueous humor and glucose (5.5 mM). The results show complete transparency. Lens are shown with normal and experimental photographs. A complete opacification was observed for the negative control in which the lens was incubated with glucose (55 mM). The opacity of lenses, similar to that of Group 4, treated by standard drugs, was significantly lower in groups 3 where the lens were incubated simultaneously with glucose (55 mM) and the ethanolic leaves of extract of *Epipremnum aureum* (500 microgm/ml). The results indicate that the selected plant extract has a positive effect on the anti-cataract potential, with the opacity

Table 2. Observation of in vitro anticataract activity photographic evaluation of lens opacity

Groups	Protein (mg/ml)	MDA (MDA/ min/ mg lensprotein)	AR-Inhibition Activity (%)
Group 1	16.6 ± 0.317	0.0006 ± 0.0023	97.28 ± 0.0032
Group 2	2.23 ± 0.162	0.0026 ± 0.0033	65.23 ± 0.0054
Group 3	11.81 ± 0.234*	0.0018 ± 0.0224*	82.44 ± 0.0062*
Group 4	12.84 ± 0.224	0.0008 ± 0.0154	90.43 ± 0.0041

All the values are expressed as Mean ± SEM, n= 3; * p< 0.001 when compared with standard values

of cataract lenses being reduced. Table 2 and figure 2 shows that the level of MDA and level of total protein the in cataract lenses (group 2) was significantly decreased compared with regular controls (Group 1). The plant extract lens (Group 3) and the standard drug enalapril lens have resulted in a significant raise in the total protein and a reduction in the MDA.

In vivo anticataract Activity

The galactose model is common in several experimental models because it results in a larger increase in the decreased form of galactitol than glucose and because galactitol does not further metabolise the decreased form of glucose as sorbitol does (Kinoshita et al., 1980). The models of galactose are reasonable to suppose that galactosis cataract factors in young rats are very similar to those in the cataract of human galactoses (Kinoshita, 1965). When rats are placed on Galactose-free diets, the lens opacities in rats, similar to those in people with Galactosemics, slowly disappear. The polyol pathways, oxidation and non-enzymatic glycation include three possible mechanisms that may be involved in the formation of cataracts through hyperglycemia or hyperglycosemia (Spector, 1995).

Alkaloids, tannins, and Triterpenoids in *Epipremnum aureum* leaves were found to be associated with the anti-cataract activity associated with extract of this plant (Farjou et al., 1987). Diabetics are associated with sugar cataract formation and galactosemia has been associated with the catalytic aldose reductase production of glucose, sorbitol, and galactitol, respectively. The accumulation of high polyol concentrations in the lens leads to excess hydration, sodium gain and potassium ion loss due to an increase in intracellular ion strength (Kinoshita, 1990). Free amino acids, glutathione, myoinositol, and other molecular small substances lose their membrane permeability and their leakage. Oxidative insults are the main cause for the development of diabetic complications, like cataract, because of hyperosmic stress (Williamson et al., 1992). Polyol levels of

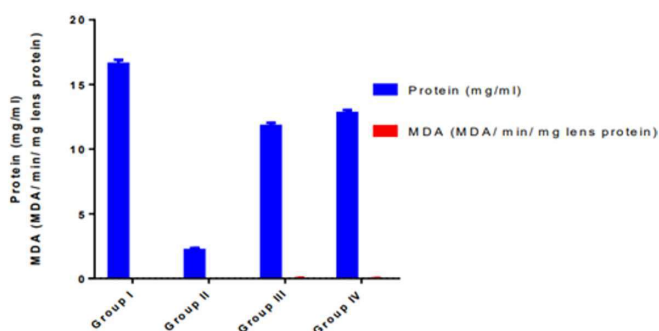


Figure 3: In vivo Anticataract Activity: Photographs of Lens in Galactose cataract in vivo model

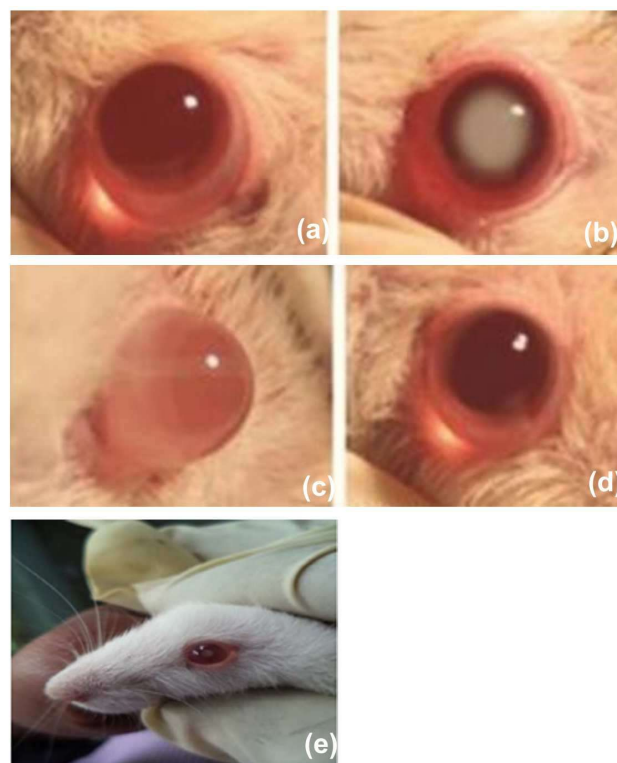


Figure 4. a) Normal control, b) negative control, c & d) Ethanolic extract 50mg/kg & 100mg/kg exhibiting stage II peripheral opacity e) Vitamin E - Showing Stage 2 faint peripheral opacity

ethanolic extracts from *Epipremnum aureum* leaf extract treated rat lenses were significantly lowered in the present investigation (Figure 3 and 4).

Epipremnum aureum leaves were confirmed from studies results to have an anti-cataractogenic effect. *Epipremnum aureum* leaves seems to have an anti- cataract potential associated with its antidiabetic effect, which is evident in the results of antioxidant activity.

Conclusion

The in vitro and in vivo anticataract activity was observed in this experimental study together with good antioxidant with the ethanolic minute extract of the *Epipremnum aureum* leaves. We got encouraging preliminary study, but a further study is needed for human prophylaxis or treatment of human cataractogenesis to extrapolate *Epipremnum aureum* leaves.

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