International Journal of Pharmaceutical Research and Applications
Volume 6, Issue 6 Nov-Dec 2021, pp: 1168-1173 www.ijprajournal.com ISSN: 2249-7781

# To Evaluate the Anticataract Potential of Piper Betel Leaf Extract (Ethanolic) Using Invitro Model of Goat's Lens

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**Submitted: 15-12-2021** **Accepted: 31-12-2021**

**ABSTRACT**

The cataract is defined as loss of transparency because of opacification of lens. The human lens is crystalline, transparent, and biconvex and work similar way to the lens of camera. It has unique property to produce clear passage for ray of light. The cataract causes inflammation of cell of lens so produce opacity. This directly decrease transparency and cloudiness has direct impact and we get blur image of two substance .The oxidative stress is main mechanism involved in development and progression of cataract. Surgery through phacoemulsification and intraocular lens implantation as main available treatment, but it has its own drawback like postsurgical inflammation and other injuries to delicate Eg . Causes permanent blindness. The piper betel leaves too have antioxidant property. It has presence of flavonoids and has free radicals scavenging activity. This property is used to reduce cataract .The present study evaluate that the anticataract potential on goat lens. The ethanolic extract of piper betel leaf is used against glucose induced cataract by preparing lens culture. The photographic evaluation of goat lenses is provided as evidence of anticataract potential of piper betel leaf. Hence, study proposed and proves that piper betel leaf has anticataract activity.

**Keywords**: - Antioxidant, Piper betel leaf, Ethanolic extract, Lens opacity, Cataract, Oxidative stress.

## INTRODUCTION: -

When the lenses of eye become opaque it is called as cataract. When the ray of light enters the eye it focuses on cornea so then lens and it is focus on retina and we get the clear image. But in cataract due to cloudiness of two lenses this light ray does not focus on the retina and we get blur vision.

Normally lens contain of natural crystalline material that are combined in specific quantities with the proteins and water to form transparent structure to allow the passage of light rays. Due to oxidative stress the fiber of the eye lens arrange due to metabolic changes their water content and ATP content changes and lens become cloudy instead of clear.

Based on the location of opacification of lens cataract is classified into:

1) Nuclear cataract
2) Cortical cataract
3) Sub capsular cataract

PATHOPHISIOLOGY:-

Lens opacity is occurs due to changes, mutation in the lenses which leads to physical and biochemical changes in the lens structure.

1) Oxidative stress: -

Oxidative stress is the main involved in the formation of cataract. When the level of ROS increases this denatures humor, proteins, lipids, leading to mutation and

apoptosis .The metabolic activities mostly take place in lens epithelium. This oxidative stress can cause lens damage .The SOP is the main enzyme which prevent damage cause due to oxidative stress . Studies suggest that this SOP cataract level is decrease in the aqueous humor of the eye.

2) Glucose: -

Polyol pathway is associated with diabetic patient. Enzymes implicated in the polyol pathway, sorbitol dehydrogenase and AR are responsible for the conversion of glucose to fructose. Sorbitol, an intermediate compound was found to produce cell lesion by modification cell permeability. Accumulation of sorbitol lead to osmotic stress, coagulation, liquefaction of lens fibers, resulting in loss of transparency. Inhibition of AR and sorbitol dehydrogenase can reduce cataract.

Piper betel leaf:-

Piper betel leaf is commonly called as betel leaf and is climbing shrub found in the tropical region of Asia, Australasia and the pacific and grown

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mostly in pacific, and grow mostly in Bangladesh and India, China, Bhutan, Malaysia.
Traditionally this leaves are used to treat various diseases like halitosis, boils and abscesses, conductivities, constipation, swelling of gums, cuts and injuries . The

phenolic compounds present in extract contribute to the antioxidant activity of piper betel leaf .This antioxidant property is useful to treat cataract .

**EXPERIMENTAL METHODOLOGY:-**

Selection of herb :-
The plant selected for present study is piper betel .It is a green leafy vine growing as a ground cover or small climber. Its scientific classification is as follows
Synonyms: Chavica Beta, Artanthe Hixagona Kingdom: Plantae
Order: Piperales Family: Piperaceae Genus: Piper Species: Piper Betel
Division: Magnoliphyta
Collection and extraction of leaves:-
The plant leaves was freshly collected from the nursery in the parbhani village. The leaves were 1st sterilized by using ethanol and then it is kept for air dry for 10 days. After 10 days the leaves are powdered and 30 gm of powder is macerated in 90 ml of (ethanol 1:3 proportion) for 24 hrs with occasional shaking. After 24 hrs the solvent fraction was then evaporated by a vacuum rotary evaporator.
Collection of goat eye balls:-
The anticataract activity of piper betel leaf was experimented on goat eyeballs invitro. The eyeballs are collected from slaughter house in parbhani and immediately stored at 40 c and transported to laboratory .
Preparation of lens culture
The lenses were separated from extra capsular portion and it was incubated in artificially prepared lens culture. The aqueous humor made of NaCl : 140 mM , KCl :5 mM , MgCl2
:2 mM , NaHCO3 : 0.5 mM , NaH(PO4)3 , CaCl2 :0.4mM , and glucose :5.5mM . the culture was placed in incubator for 72 Hrs And pH 7.8 . Penicillin G 32 mg% and Stryptomycin 250 mg% were added to the lens culture media to prevent bacterial contamination. Glucose at concentration of 55 Mm was used to induced cataract.
Phytochemical investigation of piper betel leaf :
Test for Alkaloids:
To the extract added 1% HCl and 6 drops of Mayer's reagent and Dragendroff's reagent. An organic precipitate indicated the presence of alkaloids in the sample 24.
Test for Flavonoids:
5 ml of dilute ammonia solution were added to a portion of aqueous filtrate of each plant extract followed by addition of conc. H2SO4. A yellow coloration is observed which confirms the presence of flavonoids and it disappears on standing 25.
Test of glycosides:
Dissolve small amount of an alcoholic extract of the fresh or dried material in one ml of water. Add a few drops of aqueous NaOH solution. Yellow color indicates the presence of glycoside 25.
Test for Steroids:
2 ml of acetic anhydride was added to 0.5gm of ethanolic extract of each sample with 2 ml of H2SO4. The color change from violet to blue or green indicated the presence of steroids 26.
Test for Tannins:
5 ml of extract was added to few drops of 1% lead acetate. A yellow precipitate indicated the presence of tannins 26.
Test for Terpenoids:
5 ml of each extract was added to 2 ml of chloroform and 3 ml of conc. H2SO4 to form a monolayer of reddish brown coloration of the interface was showed to form positive result for the terpenoids 25.
Test for Saponins:
The extract with 20 ml of distilled water was agitated in a graduated cylinder for 15 minutes. The formation of 1cm layer of foam indicated the presence of saponins 24.

**OBSERVATION ON PHYTOCHEMICAL GROUP TESTS**

| Secondary Metabolite | Name of the test | Observation | Result |
|---|---|---|---|
| Alkaloids | Mayer's test | Creamy white precipitate | ++ |
| | Hager's test | Yellow crystalline precipitate | ++ |
| | Wagner's test | Brown precipitate | ++ |
| Glycosides | General test | Yellow color | ++ |
| Cardiac | Legal's test | Pink to red color | ++ |

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| | | | |
|---|---|---|---|
| glycosides | Baljet's test | Yellow orange color | ++ |
| Anthraquinone glycoside | For O-glycoside | Rose pink in the aqueous layer | ++ |
| | For C-glycoside | Rose red coloration in aqueous layer | ++ |
| | For aglycones | Bright pink coloration | ++ |
| Terpenoids | Salkowsky test | Red color | ++ |
| Flavonoids | General test | Rose pink in the aqueous layer | ++ |
| | Specific test | Orange to red color | ++ |
| Tannins | FeCl3 test | Brownish green color | ++ |
| Saponins | Frothing test | Change observed | ++ |

NB; ++ indicates presence of secondary metabolites and –Indicates absence of secondary metabolite EXPRIMENTAL DESIGN:-

The in vitro anticataract activity of piper betel leaf was carried out in laboratory according to the modified protocol from Shabeer et al, 2011.
FORMULA 1 (F1): Lens + Glucose 5.5 mM ( normal control)
FORMULA 2 (F2): Lens + Glucose 55 mM (negative control )
FORMULA 3 (F3): Lens + Glucose 5.5 mM + extract eye drop
FORMULA 4 (F4): Lens + Glucose 5.5 mM + ethanolic leaf extract (500µg / ml) FORMULA 5 (F5): Lens + Glucose 5.5 mM + ascorbic acid (20µg ) FORMULA 6 (F6) : Lens + Glucose 55 mM + natural fresh leaf extract ( 2 drops) FORMULA 7 (F7) : Lens + Glucose 55 mM + standard drug cipnol ( 2 drops) FORMULA 8 (F8) : Lens + Glucose 55 mM + standard drug catarest ( 2 drops) FORMULA 9 (F9) : Lens + Glucose 55 mM + standard drug nepalact ( 2 drops)

**Contents in the F3 EYE DROP:-**

| SR NO | CATEGORY | QUANTITY |
|---|---|---|
| 1 | Ethanolic leaf extract | 500µg / ml |
| 2 | Preservative (cetrimide) | 0.5 % |
| 3 | Distilled water | q.s. |

**Main role of glucose in the induction of cataract:**

The high level of glucose i.e. 55mM of glucose induces cataract. Excessive glucose level induces the glycation of proteins , generating superoxide radicals and AGEs in the lenses which causes toxicity in the lens and proguce opacity in the lens. Hence 55mM glucose is used for inducing cataract artificially and 5.5 mM glucose is a normal level of glucose in the aqueous humor.

**PHOTOGRAPHIC EVALUATION:-**

The lenses were placed in separate 9 Petri dishes with artificially prepared aqueous humor. The drug is added as in the experimental design and all the Petri dishes nwere placed in the incubator for 72 hrs . After 72 hrs the lenses are removed from incubator and placed on wire meshes with posterior surface touching the mesh , the lenses were observed for opacity and compared with each other and photographs were taken.

**Post observation result of each formula was described as follows :**

**Formula (F1)**

These lenses were incubated in artificial aqueous humor with 5.5 mM glucose for 72 hrs in the incubator. Glucose in this quantity does not produce any stress on the lens and lens is clear. This is positive control on the lens as there is no

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toxicity lens is clear.

Figure 1

**Formula (F2)**

After adding glucose of conc 55mM and incubating the lenses the cytotoxic reaction takes place and the lenses become highly opaque.


Figure 2

**Formula (F3)**

The eye drop contains citrimide preservative for preventing the microbial growth in the ophthalmic solution. The given quantity of the ethanolic solution of piper betel is added and 2 to 3 drops of this eye drop is added on the lens. And after incubation the lens is more clear. showing anticataract activity of piper betel leaf.


Figure 3

**Formula (4)**

After adding ethanolic leaf extract the lens is highly clear. The antioxidant property of piper betel leaf is used to relive cataract , hence post incubation the lens is clear and no any opacity is seen.


Figure 4

**Formula (5)**

The activity if piper betel is compared with 20µg of ascorbic acid. Ascorbic acid in this quantity shows quite opacity and lens is not fully cleared.


Figure 5

**Formula (6)**

The fresh leaf extract also reduces the opacity of the lens. The ethanolic extract shows more clearance than the natural leaf extract. Ethanolic leaf extract is more capable of reducing the opacity of the lens.


Figure 6

**Formula (7)**

Cipnol is the eye drop used for antibacterial activity. The lens were clear aside and inner part of the lens is as opaque as the negative control eye lens.

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Figure 7

**Formula (8)**
Catarest eye drop is used for post operational cataract reduction. The lens opacity is reduced and lens has minimum opacity but ethanolic leaf extract has highest clarity than this standard drug.

Figure 8

**Formula (9)**
epalact eye drop is used for anti- inflammatory infection in the eye lens. The lens is clear and have some opacity.

Figure 9

## CONCLUSION:

Oxidative damage is an important effect of ionic radiation in biological membranes .it is a chain reaction of free radicals generated from radiolytic decomposition of water can attack fatty acids chain of membrans .

Presense of polyphenol compounds like catechol, alcyl ayrcatenol, charbetol in betel leaf extract inhibit to radiation include lipid peroxidation process effectively. This could be attributed to its ability to scavenge free radicals involved in initial and proparation step. The heating action was attribute to free radicals scavenging activity of plant extract.

The common treatment for cataract include cataract surgery i.e.

Phacoemulsification . This method have its own post surgical complication and high cost. Piper betel leaf exhibit very high antioxidant property hence use against cataract. Ethanol extract of piper betel leaves showed the potential to reduce cataract, at least during in vitro conditions. Cataract more efficiently than standard marketed synthetic drug.

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