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RESEARCH ARTICLE

Anti-cataract Potential of *Piper Betle* Leaf Ethanol Extract on Dexamethasone-Induced Cataract

Vijaya Ambica Durga B [1*], Priyanka Metla[2], Sneha Moka[2],Varaprasad Midde[2]

[1] *Asst. Professor, Sri Vasavi Institute of Pharmaceutical Sciences, Tadepalligudem, Andhra Pradesh, India*
[2] *Student, B. Pharmacy, Sri Vasavi Institute of Pharmaceutical Sciences, Tadepalligudem, Andhra Pradesh, India*

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Abstract:
The objective of this research was to assess the effectiveness of various ethanolic extracts derived from *Piper betle* leaves in combatting cataracts, particularly in glucose-induced goat eye lenses used as in-vitro models. Cataracts, a prominent cause of vision impairment globally, manifest as the clouding of the eye lens. Dexamethasone, a frequently employed steroid, is recognized for its propensity to induce cataract formation. *Piper betle*, a traditional medicinal plant known for its anti-inflammatory properties, was investigated in this study. The research involved inducing cataracts in goat lenses using glucose. It was established that the phytoconstituents present in piper betle possessed notable antioxidant properties, contributing to their efficacy against cataracts. Examination of photographic evidence revealed that the application of various *Piper betle* leaf extracts delayed the onset of lens opacity. Additionally, the induced cataract lenses exhibited lower catalase levels compared to the normal control group, resulting in diminished opacity. *Piper betle*, also referred to as betel vine or Nagvalli, is widely cultivated in Asia and utilized for various medicinal purposes due to its astringent taste and affordability. The promising outcomes of this study underscore the potential of *Piper betle* as a therapeutic agent against dexamethasone-induced cataracts. The protective effects observed in the ethanolic extract of *Piper betle* leaves against dexamethasone-induced cataracts in isolated goat lenses suggest its anti-cataractogenic properties. These effects are likely mediated by the rich phytochemical composition of *Piper betle*, including polyphenols and flavonoids, which act as antioxidants, mitigating oxidative stress and preserving lens clarity. While this study provides valuable insights into the potential therapeutic benefits of *Piper betle* against cataracts, further research involving in vivo experiments and clinical trials is necessary to confirm these findings and assess the applicability of *Piper betle* as a cataract-preventive agent in humans.

Keywords: *Piper betle*; Anticataract activity; Dexamethasone-induced cataract; Phytochemical composition; Ocular disorders

1. Introduction

Betle leaf, alternatively known as betel vine or Nagvalli, holds significant medicinal value across Asia, with widespread cultivation in countries like Bangladesh, India, Sri Lanka, Malaysia, Thailand, Taiwan, and other Southeast Asian nations. Its diverse applications in traditional medicine, chewing practices, vaginal douching, mouthwash preparations, and remedies for skin ailments and coughs stem from its characteristic astringent taste. The leaf's affordability and abundance underscore its potential for further exploration in both the food and pharmaceutical industries. Moreover, betel leaf has long been a staple in traditional Indian customs, particularly in aiding digestion post-meals, with numerous varieties available worldwide, particularly concentrated in India, where it boasts 45 distinct types. Rich in tannins, sugar, diastases, and essential oils endowed with antiseptic properties, betel leaf presents a versatile botanical resource. [1-3]

This extends to evaluating the *ex vivo* anti-cataract potential of piper betel leaf ethanolic extracts, particularly in combating dexamethasone-induced cataracts using isolated goat lenses. Notably, treatment with the ethanolic extract of Piper betle leaves demonstrates a significant inhibitory effect on the progression of dexamethasone-induced cataracts, showcasing a dose-dependent reduction in lens opacity and protein aggregation. [4] Furthermore, the extract augments the activities of crucial antioxidant enzymes such as superoxide dismutase and catalase, thereby mitigating oxidative stress-induced damage to lens proteins. Beyond its pharmacological applications, the botanical taxonomy and traditional uses of betel leaf, spanning millennia and diverse cultures, underscore its rich heritage and ongoing relevance in contemporary medicinal practices.[5,6]

Betel leaf exhibits a spectrum of pharmacological activities, including anti-bacterial, anti-microbial, analgesic, anti-inflammatory, anti-oxidant, anti-proliferative, hepatoprotective, wound healing, anti-cancer, and anti-diabetic effects. In studies measuring antibacterial activity, betel leaf extracts inhibited the growth of various bacteria, including *S. aureus*, *S. epidermidis*, *E. coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, and *Proteus vulgaris*, suggesting broad-spectrum antimicrobial potential. The presence of compounds

* Corresponding author: Vijaya Ambica Durga B

such as alkaloids, phenols, flavonoids, tannins, saponins, glycosides, terpenoids, steroids, and essential oils, particularly eugenol, 5-(2-propenyl)-1,3-benzodioxole, and other fatty acids, contribute to these antimicrobial effects. [7]

Moreover, betel leaf extracts have shown significant analgesic and anti-inflammatory properties in animal models, attributed to phytochemical compounds like flavonoids, tannins, phenols, and glycosides. Additionally, betel leaf extracts exhibit anti-oxidant activity, scavenging free radicals and mitigating oxidative stress, possibly through compounds such as ascorbic acid, with potential implications in disease prevention and management. Furthermore, betel leaf extracts demonstrate anti-proliferative effects on cancer cells, including breast cancer, along with hepatoprotective properties against drug-induced liver toxicity. [8] These diverse pharmacological activities underscore the therapeutic potential of betel leaf in various health conditions, from wound healing to diabetes management, suggesting its significance in traditional and modern medicine

2. Methodology

2.1. Procurement and authentication

Betle leaves were obtained from the medicinal garden of the Vasavi Institute of Pharmaceutical Sciences located in Andhra Pradesh, India. The authenticity of the leaves was confirmed by the Department of Botany at AU, and voucher specimens were generated for future reference. [9]

2.2. Preparation and preservation

Upon collection, the leaves were subjected to a drying process under shade conditions lasting for a duration of 2 weeks. Subsequently, they were finely ground into a coarse powder and stored in a tightly sealed container to maintain their integrity until further analysis. [9]

2.3. Extraction procedure

The powdered betle leaves underwent extraction procedures, initially with petroleum ether over a period of 7 days, followed by Soxhlet extraction utilizing 75% ethanol. These processes resulted in the production of a brownish-yellow extract. [10]

2.4. Phytochemical screening

2.4.1. Alkaloid Test

Mix 1ml of the extract with concentrated hydrochloric acid, then add Dragondroff's reagent. Presence of alkaloids is indicated by the appearance of a reddish-brown color. [11]

2.4.2. Flavonoid Test

Add concentrated sulphuric acid to 1 ml of the extract. The presence of flavonoids is confirmed by the stable yellowish-orange color that forms. [11]

2.4.3. Glycoside Test

Dissolve a small amount of alcoholic extract in water, then add aqueous NaOH solution. A yellow color signifies the presence of glycosides. [11]

2.4.4. Steroid Test

Mix 2 ml of acetic anhydride with 0.5gm of ethanolic extract and 2 ml of H_2SO_4. A change in color from violet to blue or green indicates the presence of steroids. [11]

2.4.5. Salkowski's Test

Combine 1 ml of the extract with chloroform and concentrated sulphuric acid. The formation of a red precipitate suggests the presence of steroids. [11]

2.4.6. Libermann Test

Mix 1 ml of the extract with chloroform, concentrated sulphuric acid, and acetic anhydride. A green precipitate formation indicates the presence of steroids. [11]

2.4.7. Tannin Test

Add 5 ml of the extract to 1% lead acetate solution. The presence of tannins is confirmed by the formation of a yellow precipitate. Alternatively, mixing with freshly prepared 10% ferric chloride results in a greenish-black coloration for tannins. [11]

2.4.8. Lead Acetate Test

Mix 1 ml of the extract with 10% Lead Acetate. Presence of tannins is indicated by the formation of a white precipitate. [11]

2.4.9. Terpenoid Test

Mix 5 ml of each extract with chloroform and concentrated H_2SO_4 to form layers. A reddish-brown coloration at the interface confirms the presence of terpenoids. [11]

2.4.10. Saponin Test

Agitate the extract with distilled water in a graduated cylinder for 15 minutes. The formation of a 1cm layer of foam indicates the presence of saponins. [11]

2.5. Assessment of anti-cataract activity

The research employed readily accessible goat lenses obtained from a slaughterhouse in Pedatadepalli.

2.5.1. Lens Cultivation

Fresh goat eyeballs were procured and placed in artificial aqueous humor solution at room temperature with a pH of 7.8. Additionally, Cefixime 500 mg was included to prevent bacterial contamination. Subsequently, ex vivo cataract induction was initiated. [12, 13]

2.5.2. Ex Vivo Cataract Induction

Posterior subcapsular cataract was induced in four lenses using dexamethasone 10 mg, while artificial aqueous humor served as the toxic control for a duration of 5 days. [14-16]

2.5.3. Study Groups

The four lenses were divided into distinct groups:

- Group I: Artificial aqueous humor (normal control)
- Group II: Artificial aqueous humor + dexamethasone 10 mg (toxic/model control)
- Group III: Artificial aqueous humor + dexamethasone 10 mg + EEPB 50 µg/ml
- Group IV: Artificial aqueous humor + dexamethasone 10 mg + EEPB 100 µg/ml.

2.5.4. Photographic Assessment

Following 5 days of incubation, the lenses were positioned on a wired mesh, and their opacity was evaluated using a scale ranging from 0 (no opacity) to 3 (presence of extensive thick opacity). [16, 17]

2.5.5. Preparation of Lens Homogenate

After the 5-day incubation period, the lenses were homogenized in Tris buffer supplemented with EDTA. The homogenate was then adjusted to 10% concentration and centrifuged for 1 hour to facilitate the estimation of biochemical parameters. [14, 15]

3. Results and discussion

3.1. Phytochemical screening

Positive results were obtained for alkaloids, flavonoids, glycosides, steroids, Salkowski's test, tannins, lead acetate test, terpenoids, and saponins, indicating the presence of these compounds within the sample. These findings align with previous studies suggesting the presence of bioactive constituents in similar samples. Notably, the absence of a reaction in the Libermann test and the ferric chloride test may indicate the absence of specific compounds typically detected by these assays. [12]

3.2. Anti-cataract activity

The study findings demonstrated that experimentally induced cataract lenses exhibited reduced catalase levels compared to the normal control group, leading to diminished opacity levels. Notably, the group treated with 100mg of dexamethasone in combination with EEPB showcased enhanced anti-cataract activity when compared to the 50mg dose. Photographic evaluation revealed distinct patterns among the study groups: lenses treated with dexamethasone 10 mg displayed gradual opacification over a 5-day period, progressing from moderate opacity at 2 days to complete opacity by the fifth day. Conversely, lenses treated with EEPB exhibited a retardation in opacity progression, indicating a potential protective effect against cataract formation. [14] The results are shown in Figure 1.

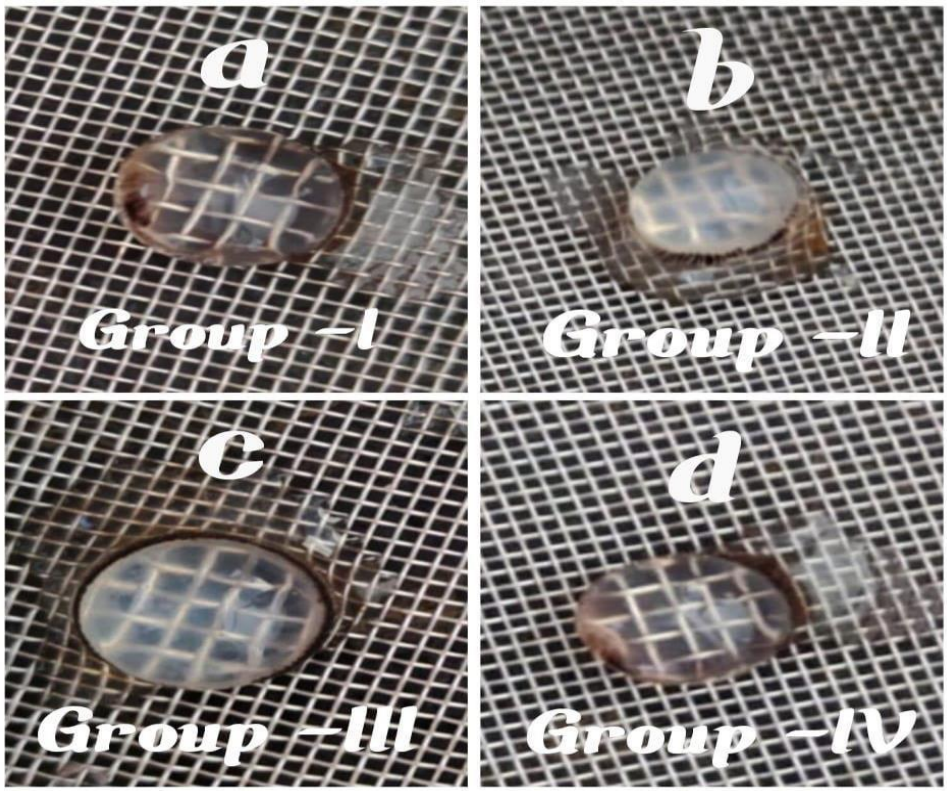

Figure 1. Evaluation of anti-cataract activity (a)Aqueous Humor (normal) (b)Aqueous Humor + Dexamethasone10mg (c)Aqueous Humor + Dexamethasone + EEPB 50(ug/ml) (d)Aqueous Humor + Dexamethasone + EEPB 100 (ug/ml)

4. Conclusion

In summary, the assessment of *Piper betle* leaf ethanolic extract's ex vivo anticataract potential against dexamethasone-induced cataracts in isolated goat lenses provides valuable insights into its therapeutic effects. The study aimed to investigate the protective properties of *Piper betle* leaves against corticosteroid-induced cataract formation. Our findings demonstrate significant protective effects against dexamethasone-induced cataracts, suggesting potential anti-cataractogenic properties attributed to the extract's rich phytochemical composition. These compounds, including polyphenols and flavonoids, likely act as antioxidants, scavenging free radicals and preventing oxidative stress-induced lens protein damage. Moreover, the extract may modulate inflammation and apoptotic pathways, preserving lens transparency. While this study offers foundational insights into *Piper betle's* potential for cataract prevention, further research, including in vivo studies and clinical trials, is essential to validate these findings and assess its translational relevance. To conclude, *Piper betle* leaf ethanolic extract shows promising ex vivo anticataract activity, warranting further investigation as a potential natural remedy for cataract prevention and contributing to the ongoing exploration of plant extracts' medicinal properties for ocular disorders.

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