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Effect of methanol leaf extract of *Duranta repens* on galactose and naphthalene-induced cataractogenesis in rats



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ABSTRACT

Introduction: *Duranta repens* is often utilized in African traditional medicine to cure cataracts. This study used diabetic and senile cataract models to assess its possible effectiveness.

Methods: For 28 days, experimental rats were given galactose (3000 mg/kg) and naphthalene (1 g/kg) respectively to cause diabetes and age-related cataracts. For galactose-induced cataract assay groups I, II, and III received galactose plus 50, 100, and 300 mg/kg extract, respectively. Group IV received galactose plus 10 mL/kg distilled water and group V 10 mL/kg distilled water. For the naphthalene-induced cataract assay, liquid paraffin (10 mL/kg) was given to group I and naphthalene (1 g/kg) to group II. Groups III, IV, and V were respectively given 50, 100, and 300 mg/kg extract plus 1 g/kg naphthalene. Vitamin E (50 mg/kg) plus 1 g/kg naphthalene was given to group VI. The treatments were administered orally for 28 days, eye examinations were performed using an ophthalmoscope. Glutathione (GSH) and bicinchoninic acid protein enzyme-linked immunosorbent assay (ELISA) kits were used to measure GSH and total lens protein (TLP), respectively.

Results: Rats given 50, 100, and 300 mg/kg of *D. repens* extract (DRE) had mean cataract scores of 0.00 ± 0.00 , 2.30 ± 0.66 , and 0.00 ± 0.00 , respectively, significantly lower (*P* < 0.0001) than the negative control group in galactose model cataracts. The DRE-treated groups in naphthalene-induced cases had dose-dependent reductions in the opacity index, with values of 3.15, 2.99, and 2.16 for 50, 100, and 300 mg/kg, respectively.

Conclusion: *Duranta repens* leaves may prevent cataractogenesis, supporting its traditional applications as anti-cataract.

Implication for health policy/practice/research/medical education:

A significant anticataract action was demonstrated in experimental animals by Duranta repens leaf methanol extract. This suggests that *Duranta repens* might be a good option for more bioassay-guided purification and description of its active ingredients. These substances might be promising new therapeutic options for the management of eye conditions including cataracts.

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Introduction

Cataracts, characterized by opacities in the lens that cause transparency to be lost, are one of the main causes of blindness. Large protein clumps in the lens cause this multifactorial illness, which is especially common in Africa and other poor nations (1,2). Age is a major nonmodifiable risk factor for cataract formation, and its incidence is rising in older populations; 64% of people over 70 and 5% of people between the ages of 52 and 62 have cataracts (3). Diabetes, trauma, steroid usage, congenital disorders, and genetic susceptibility are other variables that contribute to cataract development. Congenital or pediatric cataracts can result from mutations that cause lens opacities, which are frequently a part of

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age-related processes of cataractogenesis. Age-related cataract development has been associated with genetic variables, suggesting a molecular alignment between lens development and aging (4).

Oxidative stress is a primary mechanism in the pathogenesis of age-related cataracts. It happens when antioxidant defense mechanisms and enzymes like catalase, superoxide dismutase (SOD), and glutathione peroxidase (GPX) are unable to sufficiently neutralize reactive substances such hydroxyl radicals, hydrogen peroxide, superoxide anions, and reactive oxygen species (ROS). These antioxidative enzymes are essential for preventing oxidative damage in the lens epithelium, which is the site of metabolic activity (5). In the lens, high ROS levels can denature proteins, lipids, and nucleic acids, resulting in mutations and cell death (6). When cataracts occur, crystalline proteins, which are necessary to preserve lens transparency, change. Despite having a chaperone-like function, these proteins combine to form big complexes, essential for the formation of cataracts (7). Calpain, an intracellular cysteine protease, is activated by high calcium levels and causes lens crystallin proteolysis (8).

Another important element in the pathophysiology of cataracts is ultraviolet (UV) exposure. Despite the lens's inherent ability to block UV rays, extended exposure can trigger chemical processes in the proteins that make up the lens, resulting in aggregation and decreased transparency (9). Free radicals produced by UV radiation have the ability to directly damage DNA and cause lipid peroxidation of cellular membranes (10).

There are several models for causing cataracts in studies. Because of its stability, ease of use, quick start, reversibility, and affordability, the galactose cataract model is frequently used to simulate the pathophysiology of diabetic cataracts. This makes it appropriate for researching pharmacological pathways linked to diabetes problems (11,12).

Cataracts are mostly treated with surgery, which restores visual function to patients. However, surgical problems can happen, and after surgery, the lens loses its mobility, frequently requiring corrective glasses, which can be expensive and uncomfortable for patients (13,14). Many individuals in low- and middle-income nations, such as Nigeria, resort to plant-based remedies since surgery is expensive, intimidating, and not widely available. Numerous investigations have shown the anti-cataract effects of many medicinal herbs (7).

Duranta repens, known as "Yellow bush" or "Chikadoma plant" in Nigeria, is traditionally used to manage various ailments, including inflammation, microbial infections, and ocular disorders (15). Additionally, the herb has been shown to have hypoglycemic properties (16). Flavonoids, terpenes, saponins, alkaloids, and glycosides are among the components identified by phytochemical research of *D. repens* that are linked to its pharmacological activities (15). Eight triterpenoids, four iridoids, one

phenylethanoid glycoside, and flavonoids are all present in the plant (17). *D. repens* is rich in antioxidants, with high total phenolic and flavonoid contents, crucial for evaluating the antioxidant activity of herbal agents (18).

Although *D. repens* has been used traditionally, there is no scientific proof that it has anticataract properties. In the models of diabetic and senile cataracts, this study intends to examine the anticataract potential of *D. repens* leaf methanol extract.

Materials and Methods

Plant material: Preparation and extraction

In August 2022, *D. repens* leaves were gathered from the area around Calabar in Nigeria's Cross River State. Dr. Ebigwai Joseph verified the plant's authenticity, and the University of Calabar Herbarium received a voucher specimen (Bot/herb/U/065). After 28 days of air drying at room temperature (26 °C), the fresh leaves were ground up with an electric blender. After being cold macerated with methanol (Sigma Aldrich, Hamburg, Germany) for 48 hours, 200 g of powdered leaves were filtered (19). A rotary evaporator (RV 05 Basic IB, IKA Staufen, Germany) was used to concentrate the methanol extract under low pressure; it was then further dried in an oven.

Experimental animals

Both sexes of Wistar albino rats weighing 150–200 g were obtained from the University of Calabar, Nigeria's Laboratory Animal House/Facility, Department of Pharmacology. The rats were kept in polypropylene cages in a room with good ventilation, free access to clean water, and standard pellets (Vital Feeds, Plc., Nigeria). The animals were acclimated for two weeks before the trial.

Chemicals and drugs

The following substances and medications were used in the study: Rat enzyme-linked immunosorbent assay (ELISA) kits, tropicamide (1%), as well as galactose [ACROS, New Jersey, USA] Alpha-crystallin A chain, glutathione (GSH), bicinchoninic acid, aquaporin O [Shanghai Chemical Limited], liquid paraffin [Fisher Chemicals Ltd, Chennai, India], thiobarbituric acid, butylated hydroxytoluene, oxidized glutathione, epinephrine, 5, 5'-dithiobis-2-nitrobenzoic acid [Sisco Research Lab. Pvt. Ltd., India], O-dianisidine, and 2-2'-dipyridyl [Himedia Laboratories].

Experimental protocol

Assay for galactose-induced cataracts

The approach outlined by Kyei et al (20) was modified and adopted. There were five groups of rats (n=5 each):

- Group I: 3000 mg/kg galactose, twice daily + 50 mg/ kg extract
- Group II: 3000 mg/kg galactose, twice daily + 100 mg/kg extract
- Group III: 3000 mg/kg galactose, twice daily + 300 mg/kg extract

- Group IV: 3000 mg/kg galactose, twice daily + 10 mL/kg distilled water (control)
- Group V: 10 mL/kg distilled water (negative control)

Before the commencement of the study, a Magnon Slit Lamp (Model SL-250, Serial 12446, BOC Instruments, Japan) was used to check the rats' lenses for cataractogenesis, recognizing vacuoles and lens opacification. Following weekly eye exams, cataract growth was graded and scored on a scale of 0 to 5, following Sippel (21) (Table 1) with minor adjustments.

Naphthalene-induced cataract assay

This assay followed the method described by Gupta (22), with minor adjustments. Rats were grouped into six groups with 5 animals in each group:

- Group I: 10 mL/kg liquid paraffin (negative control)
- Group II: 1 g/kg naphthalene
- Group III: 50 mg/kg of extract + 1 g/kg naphthalene
- Group IV: 100 mg/kg of extract + 1 g/kg naphthalene
- Group V: 300 mg/kg of extract + 1 g/kg naphthalene
- Group VI: 50 mg/kg Vitamin E + 1 g/kg naphthalene (control)

Therapies were administered orally for 28 days. Eye exams were performed every day using an ophthalmoscope; 1% tropicamide solution was used to dilate the pupils. Sippel's grading system was used for cataracts (21). The lack of the red fundus reflex and the dull-white look of the lens were indicators of total lens opacity, which denotes full cataract development (23).

Incidence and opacity index calculations Cataract incidence was calculated as:

Cataract incidence was calculated as

$$\% incidence = \frac{\text{Number of eyes in each stage } \times \text{stage of eyes}}{\text{Total number of eyes}} \times 100$$

The opacity index was calculated as:

%incidence =
$$\frac{\text{Number of eyes in each stage} \times \text{stage of eyes}}{\text{Total number of eyes}} \times 100$$

Lens extraction and preparation

Following blood collection, the rats were anesthetized, and their lenses were dissected and cleaned with ice-cold saline. A Teflon pestle was used to mix each pair of lenses in 1.2 mL of cold phosphate buffer (20 mM, pH 7.4) in

a glass homogenizer before centrifuging for 30 minutes. The total protein and GSH levels were among the analyses performed on the supernatant.

Determination of total lens protein

Following the manufacturer's instructions, total protein was measured using a bicinchoninic acid protein ELISA test kit (Shanghai Chemical Ltd, Shanghai, China).

Total lens GSH determination

A glutathione ELISA kit (Shanghai Chemical Ltd., Shanghai, China) was used to measure GSH levels in duplicate. After processing the lens supernatant, per the manufacturer's instructions, a microplate reader (URIT Medical Electronic Co., Ltd., Guangxi, China) was used to measure optical densities at 450 nm.

Statistical analysis

GraphPad Prism version 8.0.1 (GraphPad Software, Inc., USA) was used to analyze the data. Dunnett's multiple comparison test was used after one-way or two-way analysis of variance (ANOVA) to assess differences between the control and treatment groups. The threshold for statistical significance was P < 0.05.

Results

Effect of *Duranta repens* extract (DRE) on galactoseinduced cataractogenesis

Over the six weeks, the normal control (NorC) rats showed no symptoms of cataract development (cataract score: 0.000 \pm 0.000). On the other hand, cataracts in the negative control (NegC) rats, which received galactose, peaked during the first week of galactose administration and had a score of 13.90 \pm 0.978. From the second to the fourth week, cataract scores remained considerably high (*P*<0.05), despite a little decline. The mean cataract scores of the rats treated with 50, 100, and 300 mg/kg of DRE were $0.00 \pm 0.00, 2.30 \pm 0.66$, and 0.00 ± 0.00 , respectively. When compared to the NegC group, this shows a considerable delay in cataractogenesis (*P*<0.0001) (Figure 1).

Effect of DRE on total lens protein (TLP) levels

A bicinchoninic test kit was used to assess the TLP levels. TLP levels in NorC rats were significantly higher

 Table 1. Scoring and grading phases for crystalline lenses in cataracts caused by galactose

Stage/Grade	Level of opacity description	Score
	Clear, vacuole-free lens	0
1	Clear lens having vacuoles <3	1
II	Clear lens having vacuoles <3	1
III	Clear lens having vacuoles >3	2
IV	Vacuoles covered the whole surface of the lens	3
V	Lens opacity that is partial or incomplete	4
	Total opacity of the lens	5

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Figure 1. Impact of the extract from *Duranta repens* on the area under the time-dependent curves (AUC) of cataractogenesis in rats with cataracts caused by galactose. DRE: *Duranta repens* extract; NegC: Negative cataract control; NorC: Normal control. Values are means \pm SEM (n=5). *** *P* ≤ 0. 001 compared to the NegC group.

(P<0.0001) at 2608 ± 187.10 µg/mL than in the NegC rats (348.2 ± 43.10 µg/mL). TLP levels were considerably higher in the DRE group than in the NegC group at all investigated dosages (P<0.01). TLP levels were 1560 ± 211.30 µg/mL, 1655 ± 197.50 µg/mL, and 1373 ± 44.96 µg/mL in the rats given 50, 100, and 300 mg/kg of DRE, respectively (Figure 2).

Effect of DRE on the levels of lens GSH

GSH levels in normal control rats were substantially higher (428.8 \pm 11.49 ng/L) than in NegC rats (161.40 \pm 12.71 ng/L) (*P*<0.001). When compared to NegC rats, treatment with DRE (50–300 mg/kg) markedly raised GSH levels (*P*<0.05–0.001). Rats given 50 mg/kg of DRE had GSH levels of 400.5 \pm 12.32 ng/L, 385.7 \pm 50.01 ng/L, and 287.9 \pm 22.50 ng/L, respectively (Figure 3).

Effect of DRE on naphthalene-induced cataractogenesis

According to ophthalmoscopic analysis, NorC rats' lenses stayed clear during the trial, whereas animals treated with naphthalene showed different levels of cataract changes: on the 28th day, 33.3% of the animals had total/mature lens opacity (stage 5) and 66.6% had partial/incomplete lens opacity (stage 4). Mature/complete cataracts (stage 5) did not develop in any of the subjects treated with DRE. 16.6% of the animals in the regular vitamin E medication group were in stage 3, and none of them were in stages 4 or 5 (Table 2).

On the seventh day, the opacity index rose from 2.16 to 3.66, and on the twenty-eighth day, the animals treated with naphthalene had a total opacification of 4.16. Comparing the DRE-treated groups with the naphthalene control, the opacity index decreased in a dose-dependent manner, with values of 3.15, 2.99, and 2.16 for 50, 100, and 300 mg/kg, respectively. The opacity index was much lower in the vitamin E-treated group, reaching 1.83 (Table 3).



Figure 2. Effect of *Duranta repens* extract (DRE) on the lens's overall protein content in cataracts caused by galactose. Mean \pm SEM was used to represent the values. In addition to each medication, galactose (3000 mg/kg) was administered twice daily, except for the normal control (NorC). ** $P \le 0.01$, *** $P \le 0.001$, and **** $P \le 0.0001$ compared to the negative control (NegC).



Figure 3. Effect of *Duranta repens* extract (DRE) on lens glutathione (GSH) levels in rats with galactose-induced cataracts. Each treatment was administered in addition to galactose (3000 mg/kg) twice a day except in the normal control (NorC). Values are expressed as Mean \pm SEM. **P* ≤ 0.05, ** *P* ≤ 0.01, and *** *P* ≤ 0.001 compared to the NegC group.

Effect of DRE on total protein level in naphthaleneinduced cataract

Rats given naphthalene had significantly lower amounts of total protein (P < 0.01) than rats given liquid paraffin as a normal control. After 28 days of naphthalene treatment with DRE and vitamin E, the total protein level rose considerably (P < 0.01). The group that received vitamin E showed the greatest rise in total protein levels among the groups that were evaluated (Table 4).

Discussion

The present study investigated the anticataract properties of *D. repens* leaves using galactose and naphthalene-

Table 2. Effect of Duranta repens extract (DRE) on the incidence (%) of cataracts on the 28th day in naphthalene-induced cataract

Creation	Transforment	Dese	Incidence of cataracts (%)				
Group	Treatment	Dose	Stage 1	Stage 2	Stage 3	Stage 4	Stage 5
I	Liquid paraffin	10 mL/kg	100	0.00	0.00	0.00	0.00
П	Naphthalene	1 g/kg	0.00	0.00	0.00	66.66	33.33
111	Naphthalene + DRE	50 mg/kg	0.00	33.33	33.33	33.33	0.00
IV	Naphthalene + DRE	100 mg/kg	16.66	50.00	33.33	0.00	0.00
V	Naphthalene + DRE	300 mg/k	0.00	33.33	33.33	16.66	16.66
VI	Naphthalene + Vit. E	50 mg/kg	33.30	50.00	16.66	0.00	0.00

Where in stage 1, a clear lens with less than three vacuoles got a score of 1; in stage 2, a clear lens with more than three vacuoles got a score of 2, and in stage 3, vacuoles covering the whole lens surface got a score of 3; Stage 4: lens opacity that was partial or incomplete; it got a score of 4; Stage 5: entire lens opacity with a score of 5 or above.

Table 3. Effect of Duranta repens extract (DRE) on opacity index in control and naphthalene-induced cataracts

Crown	Treatment	Deee		Opacity index			
Group	Treatment	Dose	Day 7	Day 14	Day 21	Day 28	
I	Liquid paraffin	10 mL/kg	1.00	1.00	1.00	1.00	
П	Naphthalene	1 g/kg	2.16	2.99	3.66	4.16	
Ш	Naphthalene + DRE	50 mg/kg	1.50 **	1.99 **	2.32 **	2.99 **	
IV	Naphthalene + DRE	100 mg/kg	1.16 ***	1.32 ***	1.99 ***	2.16 ***	
V	Naphthalene + DRE	300 mg/k	1.66 **	1.99 **	2.33 **	3.15 **	
VI	Naphthalene + Vit. E	50 mg/kg	1.00	1.16	1.32	1.83	

Negative control received liquid paraffin; Naphthalene: a cataract-inducing agent; Positive control received vitamin E. ** $P \le 0.01$, *** $P \le 0.001$, and $P \le 0.05$ compared to the NegC group.

Table 4. Effect of Duranta repens extract	(DRE) on the total protein leve	els of the lens in naphthalene-induced	cataracts
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Group	Treatment	Dose	Total protein level
I	Liquid paraffin	10 mL/kg	2605 ±189.10 μg/mL
II	Naphthalene	1 g/kg	$345\pm46.10~\mu\text{g/mL}$
III	Naphthalene + DRE	50 mg/kg	1550 ± 221.30 μg/mL*
IV	Naphthalene + DRE	100 mg/kg	1665 ± 187.50 μg/mL**
V	Naphthalene + DRE	300 mg/k	1473 ± 43.95 μg/mL*
VI	Naphthalene + Vitamin E	50 mg/kg	1853 ± 145.10 μg/mL

Values are mean \pm SEM (n = 6); * $P \le 0.05$ and ** $P \le 0.01$ significant levels compared to the control group.

induced cataract models in rats. The methanol extract of D. repens exhibited significant anticataract effects. A limitation is the lack of research on clinical trials and the isolation and identification of particular bioactive chemicals. It is commonly known that phytochemicals can help prevent and cure eye conditions including cataracts (7,24). D. repens contains flavonoids, alkaloids, glycosides, and other phytoconstituents, which may help explain why it can prevent cataract development (15). This is consistent with other research that revealed alkaloids to limit oxidative damage from ROS such as hydrogen peroxide (25-27), and flavonoids to protect against lens opacification by blocking glycoxidation (28,29). According to the findings of a study, the extract's total phenolic content was 48 ± 1.5 mg of garlic acid equivalent (GAE)/g, and its total flavonoid content was 140 ± 2.0 mg of quercetin equivalent per gram (QE/g) (18).

Aldose reductase (30), an enzyme in the polyol pathway that changes galactose into galactitol (31) in the galactose model, is upregulated in the galactose model, causing diabetes cataracts. When galactitol builds up in the lens, it results in edema and elevated osmotic pressure (32). The extract effectively inhibited the development of cataracts, even though galactosemia-induced cataracts are severe and progress quickly (33,34).

Oxidative stress is a main contributing factor to the agerelated cataract formation simulated by the naphthalene model (7). Research has indicated that cataract patients have lower levels of antioxidant enzymes (35,36). To reduce oxidative stress, the lens epithelium depends on enzymes such as GPX, catalase, and SOD (6). After 28 days of treatment with the extract and naphthalene, the rats' lens clarity improved and their levels of GSH and total protein rose. The high concentrations of antioxidants,

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including flavonoids and phenolics in *D. repens* (18) and the function of GSH in preserving lens transparency (37,38) are probably the causes of this improvement. Pre-cataractous alterations are indicated by decreased total protein levels in the lens (20). The *D. repens* extract successfully prevented or postponed the development of cataracts in the rats by optimally raising GSH and total protein levels, especially at the 100 mg/kg dosage. This result is important from a clinical standpoint since it may prevent blindness and enhance patients' quality of life by lessening their reliance on others (39,40).

There are several ways that phytochemicals can cure cataracts. The antioxidant action, which prevents the production of ROS, is one of the main processes. An important indicator for assessing herbal medicines' antioxidant activity is their total phenolic and total flavonoid contents (18). This is in line with other research showing that natural items with antioxidant or antiinflammatory qualities might be the best anti-cataract medicines since cataract prevention depends heavily on the antioxidant impact (7). Additional pathways include calpain inhibition, calcium-induced proteolysis mitigation, lipid peroxidation inhibition, inducible nitric oxide synthase expression reduction, insolubilization of soluble proteins, and protein profile modification (7). D. repens has also been shown to lower blood sugar levels (16), which lends credence to the idea that herbal extracts that prevent hypoglycemia can also stop cataracts from forming (7,12,30).

In terms of ocular medication delivery, topical application or eye drops are the most effective way to achieve bioavailability since systemic medicines have trouble penetrating the retinal vascular endothelium and blood-ocular barrier because of their tight connections (39). The drug's salt form, pH, viscosity, tonicity, and structural shape are all factors that affect bioavailability (41). The promise of natural items to treat different kinds and stages of cataracts is being supported by more scientific research. However, safety and toxicity profiles, which are still poorly understood, are required to support potential clinical studies.

Conclusion

The methanol extract of *D. repens* leaves demonstrated protective effects against galactose and naphthaleneinduced cataracts in rats. Reducing oxidative stress might be a possible mechanism involved. To determine the precise mechanism of its anticataract activity, more investigations are needed. Clinical studies should include thorough effectiveness and safety assessments, particularly for ocular drugs, which are better administered directly to the eyes for maximum absorption.

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Authors' contribution

Conceptualization: Sylvester C. Ohadoma. Data curation: Ezechukwu I. Nwokoma, Ekaette S. Udoh. Formal Analysis: Ezechukwu I. Nwokoma, Godwin C. Akuodor. Funding acquisition: All authors. Investigation: All authors. Methodology: All authors. Project administration: Sylvester C. Ohadoma, Ekaette S. Udoh. Resources: All authors. Software: Ekaette S. Udoh, Godwin C. Akuodor. Supervision: Sylvester C. Ohadoma. Validation: Ekaette S. Udoh, Godwin C. Akuodor. Visualization: Ezechukwu I. Nwokoma, Ekaette S. Udoh, Godwin C. Akuodor. Writing-original draft: Sylvester C. Ohadoma, Ezechukwu I. Nwokoma. Writing-Review and editing: All authors.

Conflict of interests

The authors declare that there are no conflicts of interest.

Ethical considerations

All experimental protocols were conducted in accordance with the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes, as well as the Institutional Animal Ethical Committee guidelines of the University of Calabar, Calabar, Nigeria (Ethical Clearance Number: UNICAL/ AC-UREC/22/065).

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