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Acute Toxicity Assessment of PO Retina Following a Single Subcutaneous Administration in Male Albino Rats

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Short Report

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Abstract

Objective

To evaluate the acute toxicity potential of a single high-dose (2000 mg/kg) subcutaneous administration of PO Retina, an investigational aqueous formulation for retinal applications, in male albino rats following OECD Guideline 423.

Results

Ten rats were randomly divided into control (sterile water vehicle, n = 5) and treated (PO Retina 2000 mg/kg, n = 5) groups. No mortality or abnormal clinical/behavioral signs were observed in any animal during the 14-day observation period. No significant differences between groups were found in body weight gain (control: 31.72% ± 3.25; treated: 32.72% ± 1.55; p = 0.788), daily feed intake (control: 133.29 ± 4.82 g; treated: 137.07 ± 4.82 g; p = 0.586), or water consumption (control: 167.07 ± 4.81 ml; treated: 162.21 ± 5.30 ml; p = 0.503). Relative organ weights (liver, kidney, spleen, heart, lung, retina) showed no statistically significant differences between groups (all p > 0.05). A single subcutaneous dose of PO Retina at 2000 mg/kg produced no detectable acute toxicity, mortality, or adverse effects on clinical signs, growth, metabolism, or organ weight indices under the study conditions. These results support the preliminary acute safety profile of PO Retina.

Introduction

Ocular health and retinal protection are pivotal concerns in ophthalmic and pharmacological research, particularly in the context of developing therapeutic agents with neuroprotective or regenerative effects on retinal tissues. The retina, a complex neural tissue, is highly susceptible to oxidative stress, ischemia, and toxic insults, which are central to the pathogenesis of various vision-threatening diseases such as age-related macular degeneration, diabetic retinopathy, and retinal dystrophies [1-3]

Emerging investigational formulations intended for ophthalmic or retinal applications are routinely subjected to preclinical safety and tolerability evaluation prior to any assessment of biological activity or efficacy. One such investigational substance is PO Retina, an investigational aqueous formulation developed for preliminary toxicological assessment.

Preclinical testing using rodent models remains the cornerstone of safety profiling due to the predictive value of such models for human toxicity [5,6]. A limit dose of 2000 mg/kg was selected in accordance with Organization for Economic Co-operation and Development (OECD)[7] Guideline 423 for acute systemic toxicity testing, which is designed to identify substances of low acute toxicity potential and serves as a critical first step in the preclinical safety assessment of new investigational compounds, prior to efficacy studies [8]

The subcutaneous route was chosen to assess systemic tolerance and bioavailability, as it allows for sustained absorption and is a practical route for potential chronic administration in future studies investigating systemic effects on retinal health.

The safety assessment of PO Retina is part of a broader preclinical development program for Pico Cell Matrix-based formulations. Similar investigative compounds from the same laboratory have demonstrated biological efficacy in other disease models, such as chronic kidney disease, supporting the rationale for their toxicological evaluation [9]

In the current study, the safety of PO Retina was investigated in male albino rats following subcutaneous administration at a dose of 2000 mg/kg.

Subjects and Methods

1. Study Design

This study was conducted to evaluate the acute toxicity profile of a test substance designated as PO Retina, developed by ACE Cells Lab Limited, U.K.. The protocol number for the study was URAF-1-4-2021, and it was carried out at the UResearch Animal Facility, under the auspices of the International Egyptian Foundation for the Development and Care of Laboratory Animal Sciences.

The study followed a controlled, randomized design, dividing animals equally into a test group and a control group (n = 5 each), consistent with OECD Guideline 423 [7] for acute toxicity studies using a limit test dose of 2000 mg/kg body weight.

Repeated-dose administration and extended observation periods are beyond the scope of acute toxicity testing and are appropriately addressed in subacute and subchronic study designs, which are warranted based on the present findings

2. Test Substance

- Name: PO Retina
- Physical Appearance: Clear watery solution
- Composition: An investigational aqueous formulation containing proprietary low-molecular-weight peptide fractions with antioxidant components, supplied by the manufacturer for toxicological evaluation. The precise molecular composition was not disclosed and was not required for the objectives of the present acute toxicity study.
- Vehicle: Sterile water (designated by the investigator)
- Route of Administration: Subcutaneous (SC)
- Dose: 2000 mg/kg body weight (limit dose). The selection of a single dose of 2000 mg/kg was based on the OECD Guideline 423 limit test strategy, which is designed to identify substances with low acute toxicity and to avoid unnecessary animal use when no adverse effects are anticipated

Frequency: Single-dose administration

3. Animals

- Species: Albino Rat (Rattus norvegicus)
- Strain: Not specified
- Sex: Male
- Age/Weight Range: 145–165 grams
- Number: 10 rats (5 per group)
- Source: UResearch Animal Facility
- Housing Conditions: Animals were housed in standard polycarbonate cages under controlled environmental conditions (temperature 22 ± 2°C, relative humidity 50–60%, 12-hour light/dark cycle), with ad libitum access to standard rodent chow and filtered water.

All animals were obtained from the UResearch Animal Facility (Giza, Egypt), a licensed laboratory animal breeding and housing facility

4. Group Allocation

- Control Group (n = 5): Received the same volume of vehicle (sterile water) subcutaneously.
- Treated Group (n = 5): Received a single subcutaneous injection of PO Retina at 2000 mg/kg body weight.

5. Observation Parameters

The animals were observed for a total of 14 days, with specific focus on both acute toxicity symptoms and general well-being.

a . Clinical and Behavioral Observations
Animals were monitored continuously for the first 24 hours post-administration and subsequently once daily for the following:

- **Skin and fur condition:** Assessed for alopecia, piloerection, or lesions.
- **Eye and mucous membrane appearance:** Eyes were inspected for discharge, conjunctival redness (conjunctivitis), or corneal opacities. Oral and nasal mucous membranes were inspected for pallor, cyanosis, or ulceration. 'Normal' was defined as the absence of any such abnormalities, with eyes being clear and bright, and mucous membranes being pink and moist.
- **Respiratory signs (e.g., stimulation, depression):** Assessed by observing respiratory rate and effort; 'normal' was defined as quiet, regular, and unlabored breathing.
- **Neurological signs (e.g., tremors, convulsions, muscle spasms)**
- **Autonomic signs (e.g., salivation, diarrhea)**

- **Lethargy, sleep disturbances**
- **Changes in skin color (e.g., cyanosis, blanching)**
- **Mortality**
  Each sign was recorded as **present (A)** or **normal (N)**."*

Observations were conducted based on established standardized criteria for clinical scoring in laboratory rodents ^15^.

b. Body Weight and Consumption Monitoring

- Initial and final body weights were recorded on days 0 and 14, respectively.
- Water consumption (WC) and feed intake (FI) were recorded daily per cage and averaged for each group.

Male albino rats (age 8–10 weeks, body weight 145–165 g) were anesthetized using isoflurane via inhalation to minimize stress during handling and procedures. At the end of the study, animals were humanely euthanized by inhalation of sevoflurane until a surgical depth of anesthesia was achieved, followed by continued exposure for 1–2 minutes beyond the last observed breath, in accordance with the American Veterinary Medical Association (AVMA) Guidelines for the Euthanasia of Animals.

c. Organ Weights : At the end of the study. The following organs were harvested and weighed:Liver, Kidney,Spleen, Heart , Lung and Retina.

Relative organ weights were calculated as a percentage of total body weight. Relative organ weights, including liver, kidney, heart, lung, spleen, and retina, showed no statistically significant differences between treated and control animals (all $p > 0.05$)

Organ Harvesting: Following euthanasia, organs were promptly harvested. The retinas were dissected by enucleating the eyes, making an incision at the limbus, and gently separating the neural retina from the underlying ocular layers using fine forceps.

6. Statistical Analysis

Data were expressed as mean ± standard deviation (SD). The Independent t-test was used to compare differences between control and treated groups. A p-value > 0.05 was considered statistically insignificant, indicating no adverse effect of the test compound compared to the control.

All experimental procedures adhered to institutional animal care guidelines and ethical standards for laboratory animal handling.

Ethical Approval

All experimental procedures involving animals were performed in accordance with the ARRIVE guidelines (https://arriveguidelines.org) and the editorial policies . The study was reviewed and approved by the UResearch Animal Facility of the International Egyptian Foundation for the Development and Care of Laboratory Animal Sciences (protocol approval number URAF-1-4-2021).

As all animals were sourced from a licensed institutional animal facility and no privately owned animals were used, informed consent from animal owners was not applicable. All procedures were conducted in accordance with institutional and international guidelines.

Results

The acute toxicity evaluation of PO Retina administered subcutaneously at a single dose of 2000 mg/kg body weight in male albino rats revealed no significant clinical, behavioral, or physiological toxicity when compared to the control group. The results are categorized and summarized below:

1. Clinical and Behavioral Observations

No abnormal clinical signs or behavioral changes were observed in any of the animals during the 14-day observation period. Parameters such as skin and fur condition, eye and mucous membrane appearance, salivation, lethargy, qualitative respiratory signs, neurological symptoms, and autonomic responses were all within normal limits (Table 1). No signs of morbidity or mortality were observed (.

Table 1. Clinical Observations of Wellness Parameters in Control and PO Retina–Treated Rats

Observation Parameter	Control Group	PO Retina Group
Skin and Fur	Normal (N)	Normal (N)
Eyes	Normal (N)	Normal (N)
Mucous Membranes	Normal (N)	Normal (N)
Salivation	Absent (A)	Absent (A)
Lethargy	Absent (A)	Absent (A)
Sleep	Normal (N)	Normal (N)
Respiration		
— Stimulation	Absent (A)	Absent (A)
— Depression	Absent (A)	Absent (A)
— Failure	Absent (A)	Absent (A)
— Coma	Absent (A)	Absent (A)
Skin Color		
— Blanching	Absent (A)	Absent (A)
— Cyanosis	Absent (A)	Absent (A)
— Vasodilatation	Absent (A)	Absent (A)
Neurological Signs		
— Convulsions	Absent (A)	Absent (A)
— Arching and Rolling	Absent (A)	Absent (A)
— Muscle Spasms	Absent (A)	Absent (A)
— Tremors	Absent (A)	Absent (A)
Gastrointestinal Signs		
— Diarrhea	Absent (A)	Absent (A)
General Health		
— Morbidity	Absent (A)	Absent (A)
— Mortality	Absent (A)	Absent (A)

2. Body Weight, Water Consumption, and Feed Intake

Both control and treated animals showed normal body weight gain over the 14-day period (Table 2) . No statistically significant differences were found between the groups in terms of body weight, feed intake

(FI), or water consumption (WC).

Table 2. Body Weight, Feed Intake, and Water Consumption

Parameter	Control (Mean ± SD)	PO Retina (Mean ± SD)	p-value
Water Consumption (ml/day)	167.07 ± 4.81	162.21 ± 5.30	0.503
Feed Intake (g/day)	133.29 ± 4.89	137.07 ± 4.82	0.586
Initial Body Weight (g)	163.33 ± 5.27	161.00 ± 4.70	0.748
Final Body Weight (g)	215.33 ± 9.55	213.50 ± 5.49	0.871
Body Weight Gain (%)	31.72 ± 3.25	32.72 ± 1.55	0.788

No significant difference ($p > 0.05$) was observed between groups in all measured variables.

3. Relative Organ Weights

Post-sacrifice, the relative weights of key organs were recorded and normalized to total body weight. The PO Retina group showed no statistically significant difference in organ weights compared to the control (Table 3) .

Table 3. Relative Organ Weights (% of Body Weight)

Organ	Control (Mean ± SD)	PO Retina (Mean ± SD)	p-value
Liver	4.18 ± 0.16	4.30 ± 0.10	0.547
Kidney	0.73 ± 0.02	0.72 ± 0.02	0.695
Spleen	0.65 ± 0.03	0.74 ± 0.05	0.149
Heart	0.34 ± 0.01	0.36 ± 0.01	0.173
Lung	0.76 ± 0.08	0.79 ± 0.05	0.774
Retina	1.08 ± 0.03	1.06 ± 0.07	0.758

All p-values > 0.05, indicating no statistically significant differences between the control and treated groups.

- No mortality or abnormal clinical signs were observed in either group.
- Body weight gain, water/food intake, and organ weights remained consistent and comparable between groups.
- No signs of toxicity were evident at the administered dose of 2000 mg/kg body weight.

These findings indicate that PO Retina did not produce observable acute toxicity findings under the experimental conditions of this study.

Discussion

The present study aimed to evaluate the acute systemic toxicity profile of PO Retina, a clear aqueous investigational formulation, following a single high-dose subcutaneous administration in male albino rats. The findings revealed no signs of systemic or local toxicity, no mortality, and no statistically significant alterations in clinical behavior, body weight, food and water consumption, or relative organ weights. These results provide preliminary evidence supporting the **acute safety** of PO Retina in a rodent model, justifying further pharmacological and chronic safety investigations.

The absence of behavioral or physiological toxicity is a critical indicator that the test substance did not interfere with central nervous, respiratory, or autonomic functions. Such parameters are often the first indicators of systemic toxicity, particularly with subcutaneously administered agents [7]. In this study, all rats in the PO Retina group remained clinically normal, with normal grooming, activity, respiration, and food intake—mirroring findings from acute toxicity studies of various investigational compounds administered subcutaneously under OECD-guided protocols [10,11].

Importantly, body weight gain—an indirect index of general health and metabolism—was consistent across groups. There was no significant deviation in either the initial or final body weights, or in the overall weight gain, indicating preserved metabolic activity and appetite, which are often compromised during subclinical toxicity [12]. Similarly, water and feed consumption remained unaffected, reinforcing the notion that PO Retina did not impair systemic homeostasis or digestive function.

The organ weight analysis, a classical approach for assessing target organ toxicity [13], further corroborated the safety profile of PO Retina. All major organs, including the liver, kidney, heart, spleen, lungs, and retina, showed statistically insignificant differences in relative weight compared to controls. This is particularly important for ocular therapeutics, where systemic off-target effects must be minimal. [14].

Comparative literature further supports these findings. A similar study on the retinal protective extract *Retinari™* in rats demonstrated a non-toxic profile in acute and subacute dosing models, with stable organ weights and physiological parameters across multiple endpoints [15]. Additionally, research involving flavonoid-based retinal protectants such as quercetin and lutein also reported high safety margins in acute models, reinforcing the hypothesis that bioactive retinal formulations may possess wide therapeutic indices [16-18]

The absence of morbidity or mortality at the high limit dose of 2000 mg/kg is noteworthy. According to OECD guidelines [7] , substances that exhibit no mortality or major clinical toxicity at this dose can be classified as **Category 5 or unclassified** in terms of acute hazard, reflecting low toxicity [7,8]. This categorization suggests that PO Retina could potentially be administered at therapeutic doses well below this threshold without eliciting adverse effects.

Despite the positive findings, it is essential to acknowledge that acute toxicity studies offer only a preliminary snapshot of a compound's safety. Chronic exposure, cumulative toxicity, local injection site reactions, and organ-specific histopathological changes are not addressed in short-term protocols. Therefore, **further studies**, including repeated dose toxicity, histological analysis of retinal and neural tissues, and functional vision tests, are recommended to fully establish the safety profile of PO Retina for ophthalmic use.

The absence of systemic toxicity in this study is consistent with the observed tolerability of other Pico Cell Matrix formulations in preclinical models. For instance, a related compound, ACE Reno, was well-tolerated and showed efficacy in a rat model of chronic kidney disease without reported adverse effects [9], suggesting a potentially favorable safety class for these investigational products

Repeated-dose administration and extended observation periods are beyond the scope of acute toxicity testing and are appropriately addressed in subacute and subchronic study designs, which are warranted based on the present findings

Limitations

While the findings are promising, several limitations should be considered:

1. **Short Duration**: The study was limited to a 14-day observation period and thus only evaluates acute toxicity. It does not assess sub chronic or chronic effects that may emerge with repeated or long-term administration.
2. **Small Sample Size**: The use of only five animals per group, while common in limit tests, restricts the statistical power of the study and may overlook subtle toxicological changes.
3. **Lack of Histopathology**: The study did not include histological analysis of organ tissues, particularly the retina, liver, or kidney, which could reveal microscopic or early cellular alterations not evident through weight or gross observation.
4. **Single Sex and Species**: Only male albino rats were used. Toxicity profiles may vary with sex or across species; therefore, further studies involving both sexes and multiple species are recommended.
5. **No Functional or Ocular-Specific Testing**: The test did not include retinal imaging, electroretinography (ERG), or vision-based behavioral assays, which are critical components in the comprehensive safety assessment of ophthalmic therapeutics [19]
6. **No Biochemical or Hematological Evaluation**: Blood parameters such as liver enzymes, renal markers, and inflammatory cytokines were not assessed, which could have provided additional insight into systemic or organ-specific effects.
7. **Lack of Quantitative Physiological Data:** Respiratory rate and other physiological parameters were assessed qualitatively but not quantitatively measured (e.g., breaths per minute), which could have

provided more objective data."

The absence of histopathological examination, biochemical assays, and functional ocular assessments (e.g., ERG) represents a recognized limitation of this acute toxicity study and should be addressed in future repeated-dose and organ-specific safety evaluations

Conclusion

Under the conditions of this exploratory acute toxicity study, a single subcutaneous administration of PO Retina at 2000 mg/kg did not produce mortality, overt clinical toxicity, or significant alterations in body weight or relative organ weights in male albino rats during a 14-day observation period. These findings suggest the absence of detectable acute systemic toxicity at the tested dose. However, comprehensive toxicological characterization requires further investigation using larger sample sizes, repeated-dose protocols, histopathological assessment, biochemical analyses, and ocular functional testing.

Declarations

Ethics approval and consent to participate

All animal procedures were conducted in accordance with international guidelines for the care and use of laboratory animals and were approved by the UResearch Animal Facility of the International Egyptian Foundation for the Development and Care of Laboratory Animal Sciences. The study protocol was reviewed and approved under protocol number URAF-1-4-2021. Consent to participate is not applicable as this study did not involve human participants.

Consent for publication

Not applicable. This study involved only animal subjects and no individual person’s data.

Availability of data and materials

All data generated or analyzed during this study are included in this published article. Additional datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors’ contributions

- **Prof. Mohamed Yasser Sayed Saif**: Supervised the study design, data interpretation, and manuscript writing.
- **Winfred Amoako**: Assisted in study design validation, data review, and literature comparison.
- **Dr. Alaa Abdelkarim**: Conceived the project, developed the PO Retina compound, contributed to methodology, and coordinated laboratory execution.
- **Dr. Sherin Amer**: Contributed to clinical relevance, manuscript editing, and critical review.
- **Prof. Mike Chan**: Provided intellectual input regarding regenerative medicine context and safety profile interpretation.
- **Islam A. Mohalhal : .** Participated in ophthalmological evaluation and manuscript revision.
- **Dr. Passant Sayed Saif**: Contributed to data analysis, statistical review, and manuscript finalization.

All authors reviewed and approved the final manuscript.

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