

ENZYME INHIBITION ASSAYS AND SAFETY PROFILE OF TARAXEROL ACETATE

Ubaid ur Rahman¹, Sadaf Durrani¹, Naheed Khattak¹, Ubaidullah², Hamid Habib³, Safiyya Ubaidullah¹,
Mudassir Ahmad Khan²

¹Department of Biochemistry, Khyber Medical College, Peshawar, Khyber Pakhtunkhwa, Pakistan.

²Department of Biochemistry, Kabir Medical College, Gandhara University, Peshawar, Pakistan.

³Department of Physiology, Institute of Basic Medical Sciences, Khyber Medical University, Peshawar, Pakistan

ABSTRACT

Objective: This study assessed the in vitro enzyme-inhibitory potential of Taraxerol acetate against Lipoxygenase and Cyclooxygenase Isoforms, along with in vivo and in vitro toxicity. It aims to establish the anti-inflammatory properties of Taraxerol Acetate (TA).

Materials & Methods: Lipoxygenase inhibition was assessed by spectrophotometric monitoring of hydroperoxide production from linoleic acid. Cyclooxygenase-1 and Cyclooxygenase-2 inhibition was assessed by radioimmunoassay of prostaglandin E₂ production. Acute in vivo toxicity was evaluated in mice after intraperitoneal administration of TA at doses of 100, 200, and 400 mg/kg body weight, with fourteen days of observation. In vitro cytotoxicity was assessed using the MTT assay in LCKM-2 monkey kidney epithelial cells and mouse hepatocytes.

Results: TA demonstrated significant LOX inhibition with an IC₅₀ of $82.19 \pm 0.01 \mu\text{M}$, compared with the standard inhibitors baicalein (IC₅₀ = $22.0 \pm 0.05 \mu\text{M}$) and tenidap sodium (IC₅₀ = $41.6 \pm 0.02 \mu\text{M}$). TA showed IC₅₀ values of $116.3 \pm 0.03 \mu\text{M}$ for cyclooxygenase 1 and $94.7 \pm 0.02 \mu\text{M}$ for cyclooxygenase 2. The standard ibuprofen showed IC₅₀ values of $14.91 \pm 0.06 \mu\text{M}$ and $27.20 \pm 0.05 \mu\text{M}$ for cyclooxygenase-1 and cyclooxygenase-2, respectively.

Conclusion: TA showed moderate inhibitory activity against lipoxygenase and cyclooxygenase enzymes.

Key Words: Taraxerol acetate, Enzyme inhibition, Safety Profile

This article may be cited as: Rahman UU, Durrani S, Khattak N, Ubaidullah, Habib H, et al. Enzyme Inhibition Assays And Safety Profile Of Taraxerol Acetate. J Med Sci 2026 July - September;34(3):123-126

INTRODUCTION

Inflammation is a complicated pathological response to tissue injury and infection, carried out by an enzymatic cascade that produces pro-inflammatory mediators. The arachidonic acid pathway plays a central role, in which cyclooxygenase (COX) and lipoxygenase (LOX) enzymes produce prostaglandins and leukotrienes, respectively. ¹ The inflammatory processes are governed by these lipid mediators at the stages of initiation, maintenance, and resolution. Consequently, lipoxygenase and cyclooxygenase inhibition has been a therapeutic strategy for managing inflammation, with non-steroidal anti-inflammatory drugs among the most commonly prescribed drugs globally. ²

Historically, natural compounds have been a major source of anti-inflammatory agents, including many plant-derived secondary metabolites with potent enzyme-inhibitory activity. Among these, pentacyclic triterpenes have attracted significant attention for their broad pharmacological properties, including anti-inflammatory, hepatoprotective, and anticancer activities. ^{3, 4} Taraxerol acetate (TA), a pentacyclic triterpenoid acetate ester, has been isolated from various medicinal plants, including species in the Asteraceae family. ⁵ Previous studies have reported that Taraxerol and its derivatives exhibit promising biological activities, including antiviral, anticancer, and gastroprotective effects. However, the specific anti-inflammatory potential of Taraxerol acetate through modulation of LOX and COX enzyme activities, along with its preliminary safety profile, warrants systematic investigation. ⁶

Preliminary studies suggest that Taraxerol possesses antioxidant, anti-inflammatory, and anti-fibrotic effects. ^{7, 8} However, systematic investigations into its enzyme inhibitory profiles and comprehensive safety data remain limited. This project was designed to determine the in vitro enzyme inhibitory potential of Taraxerol acetate (TA) against lipoxygenase and cyclooxygenase isoforms, alongside preliminary in vivo and in vitro toxicity assess-

Correspondence

Dr. Sadaf Durrani

Associate Professor

Department of Biochemistry, Khyber Medical College,
Peshawar, Pakistan

Cell: +92-315-9694973

Email: sadaf.durrani@kmc.edu.pk

Date Received: 01/09/2026

Date Revised: 26/09/2026

Date Accepted: 30/09/2026

ments, to establish a foundation for its development as an anti-inflammatory agent. These assays not only reveal a compound's direct anti-inflammatory efficacy but also help elucidate structure-activity relationships and isoform selectivity, which are critical for predicting therapeutic potential and off-target effects.

MATERIALS AND METHODS

ENZYME INHIBITION ASSAYS

LIPOXYGENASE INHIBITORY ACTIVITY

We used a slightly modified version of the spectrometric method developed by Tappal in 1962 to estimate the inhibitory activity of Taraxerol acetate.⁹ The assay required various concentrations of Taraxerol acetate. The standard (positive control), linoleic acid, and lipoxygenase were obtained from Sigma, St. Louis, MO. A mixture was prepared containing 20 μ L of lipoxygenase solution, 160 μ L of buffer (sodium phosphate 100 mM, pH 7.0), 10 μ L of Taraxerol acetate, and the standard drug. The mixture was incubated for five minutes at 25 °C, then 10 μ L of linoleic acid substrate was added to complete the reaction. The final product was 9Z, 11E-13-13-hydroperoxy-octadeca-9,11-dienoate. Product formation was monitored spectrophotometrically at 234 nm. The test sample and control were dissolved in 50% ethanol. These reactions were performed in triplicate. Baicalein was used as a positive control lipoxygenase inhibitor.¹⁰ Absorbance changes were monitored for 10 minutes. IC₅₀ values were calculated using GraphPad software.

CYCLOOXYGENASE INHIBITORY ACTIVITY

In this assay, the reagents included COX-1 and COX-2 (Cayman Chemical, USA), adrenaline (5 mM), and hematin (1 μ M) as cofactors. Hematin was first dissolved in sodium hydroxide. Cyclooxygenases 1 and 2 were then dissolved in Tris buffer (50 mM, pH 7.5). Variable concentrations of Taraxerol acetate were prepared in the same buffer. We prepared a 190 μ L reaction mixture and performed the procedure in 96-well plates. The mixture contained the test sample, the cofactor, and both isozymes at 10 U/mL, for a total volume of 180 μ L. After incubating the plates for 30 minutes at 37 °C, 20 μ L of arachidonic acid was added as a substrate to initiate the reaction. The reaction was stopped 2 minutes after the addition of 10 μ L of 1 M HCl. At this stage, the pH of the mixture was adjusted to 7.5 using 10 μ L of 1 M sodium hydroxide. PGE₂ was formed as the product. The product was measured by radioimmunoassay (RIA), which helped assess the activity of both isozymes and the inhibitory activity of Taraxerol acetate at various concentrations. Results were shown as the percentage of PGE₂ production. The procedure was repeated two more times to ensure accuracy. IC₅₀ values were determined by nonlinear regression analysis using

GraphPad Prism software. Experimental results were expressed as mean $\pm$ SEM.

IN VIVO ACUTE TOXICITY STUDY

ANIMALS

For this in vivo study, we used adult Wistar rats. They were of either sex and weighed 180 $\pm$ 10 g. We housed 10 animals per cage under a 12-hour light-dark cycle in standard laboratory conditions and provided water and rat feed. All established ethical principles for laboratory animals were observed. Before the experiments began, all rats were acclimatized to the laboratory environment for 7 days.

These mice were assigned to groups 1, 2, 3, and 4. Each group contained four mice. Group 1 (control) received distilled water. Group 2 received intraperitoneal Taraxerol acetate at 100 mg/kg body weight. Similarly, groups 3 and 4 received intraperitoneal TA at higher concentrations (200 and 400 mg/kg body weight, respectively). Mice were continuously observed for one hour to assess food intake, water consumption, general behavior, and toxic symptoms. They were then observed intermittently at 5 hours, 24 hours, and 14 days after treatment.^{12, 13}

IN VITRO CYTOTOXICITY (MTT ASSAY) (14)

The in vitro cytotoxicity of Taraxerol acetate was investigated using Mice Hepatic Cells and Rhesus Kidney Epithelial Cells (LLC-MK2). These cells were seeded in 96-well plates for 24 hours. After this, the cells were treated with various concentrations (i.e., 0, 5, 10, 25, 50, 100, and 150 μ M) of Taraxerol acetate, along with the vehicle (i.e., dimethylsulphoxide). Doxorubicin hydrochloride was used as the positive control. The cells were incubated one more time for 48 hours. Finally, for the MTT assay, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide was used at 0.5 mg/mL, and the cells were incubated for 4 h. After removing the medium, the resulting formazan crystals were dissolved in 200 μ L dimethylsulphoxide. The ELISA microplate reader measured the optical density at 540 nm.

Cell viability was expressed as a percentage of the control and calculated using the following equation:

$$\text{Cell viability (\%)} = [\text{OD of treated cells} / \text{OD of control cells}] \times 100.$$

The control group was considered to represent 100% cell viability.

RESULTS

ENZYME INHIBITION ASSAYS

LIPOXYGENASE INHIBITORY ACTIVITY OF

TARAXEROL ACETATE

Taraxerol acetate showed inhibitory activity against the lipoxygenase enzyme, with an IC_{50} of $82.19 \pm 0.01 \mu M$. Baicalein (standard) had an IC_{50} of $22.0 \pm 0.05 \mu M$, and Tenidap sodium had an IC_{50} of $41.6 \pm 0.02 \mu M$.

CYCLOOXYGENASE INHIBITORY ACTIVITY OF TARAXEROL ACETATE

TA showed significant inhibitory activity against cyclooxygenase-1 ($IC_{50} = 116.3 \pm 0.03 \mu M$) and cyclooxygenase-2 ($IC_{50} = 94.7 \pm 0.02 \mu M$). The standard compound Ibuprofen showed IC_{50} values of $14.91 \pm 0.06 \mu M$ and $27.20 \pm 0.05 \mu M$ for cyclooxygenase-1 and -2, respectively.

IN VIVO TOXICITY

Taraxerol acetate was administered at doses of 100, 200, and 400 mg/kg body weight. During the observation period, we monitored mortality and overt signs of toxicity. No mortality was observed at any tested dose. No apparent treatment-related behavioral or clinical abnormalities were observed, and the animals remained generally active and responsive. Food and water intake were not noticeably altered compared with control animals. Thus, under the conditions of the present study, administration of Taraxerol acetate at 100, 200, and 400 mg/kg body weight did not produce any apparent acute toxic effects in Wistar rats.

IN VITRO CYTOTOXICITY

Taraxerol acetate (TA) showed no appreciable cytotoxicity against Rhesus monkey kidney epithelial cells (LLC-MK2) or mouse hepatic cells across the tested concentration range of 5–150 μM . In LLC-MK2 cells, cell viability was $98.7 \pm 3.1\%$, $97.9 \pm 2.8\%$, $97.2 \pm 3.0\%$, $96.4 \pm 3.2\%$, $95.3 \pm 3.5\%$, and $94.6 \pm 3.7\%$ at 5, 10, 25, 50, 100, and 150 μM , respectively. In mouse hepatic cells, the corresponding cell viability values were $99.1 \pm 3.4\%$, $98.5 \pm 3.0\%$, $97.8 \pm 2.9\%$, $96.9 \pm 3.1\%$, $96.0 \pm 3.3\%$, and $95.2 \pm 3.5\%$, respectively. Thus, the data indicate that TA caused only a modest reduction in cell viability, even at the highest tested concentration.

DISCUSSION

LIPOXYGENASE INHIBITORY ACTIVITY OF TARAXEROL ACETATE

Lipoxygenase can accommodate flat, flexible compounds in its active site, such as Taraxerol acetate; however, its methylated E and D rings make unfavorable contacts with LOX, making TA a less potent inhibitor than standard inhibitors such as ibuprofen. By inhibiting the lipoxygenase pathway, Taraxerol acetate may interfere with leukotriene biosynthesis, which is involved in inflammatory

processes. This interpretation is based on the lipoxygenase-inhibitory activity of Taraxerol acetate observed in the present study and the established role of 5-lipoxygenase in leukotriene biosynthesis.^{15, 16}

CYCLOOXYGENASE INHIBITORY ACTIVITY OF TARAXEROL ACETATE

Taraxerol acetate (TA) showed inhibitory activity against both cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2), with greater inhibition of COX-2 than COX-1. The observed COX-2 inhibitory activity is consistent with previously reported findings for Taraxerol acetate. Previous molecular docking studies have reported hydrophobic and electrostatic interactions of Taraxerol acetate within the COX-2 binding site; these findings may provide a structural explanation for its COX-inhibitory activity (17).

IN-VIVO TOXICITY

Preliminary in vivo toxicity results from oral administration in rats showed that TA was safe up to 400 mg/kg. This preliminary safety profile indicates the strong potential of TA to be further developed as a new lead compound.

IN-VITRO CYTOTOXICITY

Preliminary in vitro cytotoxicity assay results showed that Taraxerol acetate was safe in LLC-MK-2 (kidney epithelial cells) and mouse hepatocytes. These data encourage future extensive studies regarding the safety of Taraxerol acetate.

CONCLUSION

Taraxerol acetate showed moderate inhibitory activity against lipoxygenase and cyclooxygenase enzymes, suggesting its potential to modulate pathways involved in arachidonic acid metabolism and inflammation. Taraxerol acetate also demonstrated a favorable safety profile, with no apparent toxicity at doses up to 400 mg/kg in vivo and no appreciable cytotoxicity against the tested cell lines in vitro. These findings suggest that Taraxerol acetate may be a bioactive compound with anti-inflammatory activity; however, further studies are needed to establish its pharmacological effects and therapeutic potential. Future studies should investigate the long-term effects of Taraxerol acetate on liver health to fully elucidate its therapeutic potential.

REFERENCES

1. Jyoti U, Mittal N, Gurjeet TS, Singh R, Devi S. Taraxerol: A Promising Natural Product in the Management of Inflammation. *Nat. Prod. Journal*. 2025;15(1);13.
2. Jiao F, Tan Z, Yu Z, Zhou B, Meng L, Shi X. The phytochemical and pharmacological profile of taraxaxerol. *Front. Pharmacol*. 13:927365. doi: 10.3389/fphar.2022.927365.

3. Samuelsson B. Role of basic science in the development of new medicines: examples from the eicosanoid field. *J Biol Chem.* 2012;287(13):10070-80.
4. Newman DJ, Cragg GM. Natural products as sources of new drugs over the nearly four decades from 01/1981 to 09/2019. *J Nat Prod.* 2020;83(3):770-803.
5. Ghosh S, Nag S, Bhattacharjee S, Saha S. Taraxerol acetate: a pentacyclic triterpenoid with diverse pharmacological activities. *Phytochem Rev.* 2020;19(4):873-93.
6. Raza ML, Hassan M. Acute toxicity studies of natural products: a review. *J Pharm Sci Res.* 2016;8(8):768-73.
7. Aodah AH, Devi S, Alkholifi FK, Yusufoglu HS, Foudah AI, Alam A. Effects of Taraxerol on oxidative and inflammatory mediators in isoproterenol-induced cytotoxicity in an animal model. *Molecules.* 2023;28(10):4089.
8. Bag A, Byahut A, Khandelwal B. Medicinal plants with kidney-protecting effect in diabetic nephropathy. *Curr Sci (00113891).* 2022 Aug 25;123(4). Page 2200.
9. Tappel AL. Lipoxidase. In: Colowick SP, Kaplan NO, editors. *Methods in enzymology.* Vol. 5. New York: Academic Press; 1962. p. 539-542.
10. Khan I, Nisar M, Ebad, F, Nadeem S, Saeed M, Khan H, et al. Anti-inflammatory activities of Sieboldogenin from *Smilax china* Linn.: Experimental and computational studies. *J. Ethnopharmacol.* 2009; 121: 175-177.
11. Motulsky H, Christopoulos A. Fitting models to biological data using linear and nonlinear regression: a practical guide to curve fitting. San Diego: GraphPad Software Inc.; 2004.
12. Hajam YA, Kumar R, Reshi MS, et al. Administration of *Costus igneus* Nak leaf extract improves diabetic-induced impairment in hepatorenal functions in male albino rats. *J King Saud Univ Sci.* 2022;34:101911.
13. Bruce RD. An up – and – down procedure for acute toxicity testing. *Fund App Toxicol* 1985; 5: 151–157.
14. Wang RR, Gu Q, Wang YH, Zhang XM, Yang LM, et al. Anti-HIV-1 activities of compounds isolated from the medicinal plant *Rhus chinensis*. *J. Ethnopharmacol.* 2008; 117: 249–256.
15. Silverman ES, Drazen JM. The biology of 5-lipoxygenase: function, structure, and regulatory mechanisms. *Proc Assoc Am Physicians.* 1999;111(6):525-536. doi:10.1046/j.1525-1381.1999.t01-1-99231.x.
16. Samuelsson B, Dahlén SE, Lindgren JA, Rouzer CA, Serhan CN. Leukotrienes and lipoxins: structures, biosynthesis, and biological effects. *Science.* 1987;237(4819):1171-1176. doi:10.1126/science.2820055.

CONFLICT OF INTEREST: Authors declare no conflict of interest

GRANT SUPPORT AND FINANCIAL DISCLOSURE: NIL

Authors Contribution:

Following authors have made substantial contributions to the manuscript as under

Authors	Conceived & designed the analysis	Collected the data	Contributed data or analysis tools	Performed the analysis	Wrote the paper	Other contribution
Rahman UU	✓	✓	✗	✗	✓	✗
Durrani S	✓	✗	✓	✓	✓	✗
Khattak N	✓	✓	✗	✗	✗	✓
Ubaidullah	✓	✗	✓	✓	✓	✗
Habib H	✓	✓	✗	✗	✗	✓
Ubaidullah S	✓	✗	✓	✓	✓	✗
Khan MA	✓	✗	✓	✓	✓	✗

Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Ethical Approval:

This Manuscript was approved by the Ethical Review Board of IREB of Khyber Medical College, Peshawar. Vide No. 533/DME/KMC. Dated: 29 07 2026



This work is Licensed under a Creative Commons Attribution-(CC BY 4.0)