



Review Article

PROTECTIVE EFFECT OF PASTEURIZED CAMEL MILK (*USHTRA KSHEERA*) AGAINST
PALMITIC ACID-INDUCED APOPTOSIS IN HEPG2 CELLS: AN ANNEXIN V/PROPIDIUM
IODIDE FLOW CYTOMETRIC STUDY

Drishya Satheesh^{1*}, Shincymol V V², P Y Ansary³, Greeshma K C⁴

¹PG Scholar, ³Professor & Head, ⁴Assistant Professor, Department of Dravyaguna Vigyana, Government Ayurveda College, Tripunithura,

²Professor, Department of Dravyaguna Vigyana, Government Ayurveda College, Thiruvananthapuram, Kerala, India.

Article info

Article History:

Received: 19-08-2026

Accepted: 18-09-2026

Published: 04-10-2026

KEYWORDS:

Pasteurized Camel milk, *Ushtra Ksheera*, HepG2, Palmitic acid, Apoptosis, Flow cytometry.

ABSTRACT

Palmitic acid-induced lipotoxicity is associated with cellular injury and apoptosis in hepatocyte-derived cell models. Annexin V/propidium iodide (PI) flow cytometry permits discrimination of viable, early apoptotic, late apoptotic/dead and debris populations. The present study evaluated the protective effect of pasteurized camel milk (*Ushtra Ksheera*) against palmitic acid-induced apoptosis in HepG2 cells. **Materials and Methods:** HepG2 cells were exposed to palmitic acid (300 μ M) for 1 hour and subsequently treated with pasteurized camel milk at 12.5 μ L/mL for 24 hours. Untreated control and palmitic-acid-induced groups were maintained. Cells were stained with Muse™ Annexin V & Dead Cell Reagent and analysed by flow cytometry. Three observations were available for each experimental group and results were expressed as mean $\pm$ SD. **Results:** The mean viable-cell population was 88.18 $\pm$ 0.15% in control cells and decreased to 69.98 $\pm$ 0.33% following palmitic acid exposure. Treatment with pasteurized camel milk increased the viable population to 79.32 $\pm$ 0.69%. The mean late apoptotic/dead population decreased from 27.50 $\pm$ 0.24% in the palmitic acid group to 16.39 $\pm$ 1.21% after treatment with pasteurized camel milk. The calculated total apoptotic population from the mean early- and late-apoptotic fractions was 11.53% in control, 29.45% in palmitic acid and 20.36% in the camel milk-treated group. **Conclusion:** Under the experimental conditions studied, pasteurized camel milk was associated with improved cell viability and a lower overall apoptotic fraction in palmitic acid-stressed HepG2 cells, with the principal change occurring in the late apoptotic/dead population. These findings support further investigation using molecular apoptotic markers.

INTRODUCTION

Apoptosis is a regulated form of cell death characterized by biochemical and morphological changes that include phosphatidylserine externalization, chromatin condensation and, in later stages, loss of membrane integrity.^[1] Externalization of phosphatidylserine provides the basis for Annexin V-based detection of apoptotic cells, while propidium iodide (PI) identifies cells with compromised membrane integrity.^[2,3]

The combined Annexin V/PI approach therefore permits separation of viable cells from early apoptotic and late apoptotic/dead populations.

HepG2 cells are widely used as an in vitro hepatic model for investigating lipid-induced cellular injury. Palmitic acid, a saturated fatty acid, has been shown to reduce HepG2 cell viability and promote apoptosis. Experimental studies have associated palmitate-induced injury with mitochondrial dysfunction, oxidative stress and apoptotic signalling.^[4-6] Thus, palmitic acid-stressed HepG2 cells provide a relevant experimental system for examining agents that may attenuate cellular injury.

Camel milk is a nutrient-rich milk containing proteins, peptides, vitamins and other bioactive constituents. Reviews of the available literature

Access this article online	
Quick Response Code	
	https://doi.org/10.47070/ijapr.v14i9.4386
Published by Mahadev Publications (Regd.) publication licensed under a Creative Commons Attribution-NonCommercial-ShareAlike 4.0 International (CC BY-NC-SA 4.0)	

describe antioxidant and anti-inflammatory activities associated with camel milk and its components.^[7] Experimental studies have also investigated camel milk and its constituents in HepG2 cells, including effects on cellular survival and apoptotic signalling.^[8] However, the effect of pasteurized camel milk on palmitic acid-induced apoptosis assessed directly by Annexin V/PI flow cytometry remains to be further characterized.

Therefore, the present study was undertaken to evaluate the protective effect of pasteurized camel milk (*Ushtra Ksheera*) against palmitic acid-induced apoptosis in HepG2 cells using Annexin V/PI flow cytometry, with particular emphasis on the distribution of viable, early apoptotic, late apoptotic/dead and dead-cell populations.

MATERIALS AND METHODS

Study design and cell treatment

HepG2 (human hepatic) cells were cultured according to the established cell-culture procedure used for the study. Palmitic acid at a concentration of 300 μ M was added to the cells and incubated for 1 hour. The cells were subsequently treated with Pasteurized Camel milk at 12.5 μ L/mL and incubated for 24 hours at 37°C in a humidified incubator containing 5% CO₂. Untreated control cells and palmitic-acid-induced cells were maintained in parallel.

Annexin V/PI flow-cytometric analysis

Following treatment, cell samples were transferred to 12×75mm polystyrene tubes. The samples were centrifuged at 3000 rpm for 5 minutes and the supernatant was removed without disturbing the cell pellet. A volume of 100 μ L of Muse™ Annexin V & Dead Cell Reagent was added to each tube. Samples were mixed thoroughly by pipetting or vortexing for 3–5 seconds and incubated for 20 minutes at room temperature in the dark. Cells were then analysed using a Muse flow cytometer and Muse FCS 3.0 software. Gating was performed against untreated control cells. The assay distinguishes Annexin V-/PI- viable cells, Annexin V+/PI- early apoptotic cells, Annexin V+/PI+ late apoptotic/dead cells and Annexin V-/PI+ debris/dead populations.^[2,3]

RESULTS AND DISCUSSION

Annexin V/PI flow cytometry demonstrated distinct changes in the distribution of HepG2 cell populations following palmitic acid exposure and subsequent treatment with pasteurized camel milk. The representative control profile showed a predominantly viable population, whereas palmitic acid exposure was associated with a visibly larger late apoptotic/dead population. Following treatment with pasteurized camel milk, the viable population increased and the late apoptotic/dead population decreased. The representative flow-cytometric profiles are presented in Figures 1–3.

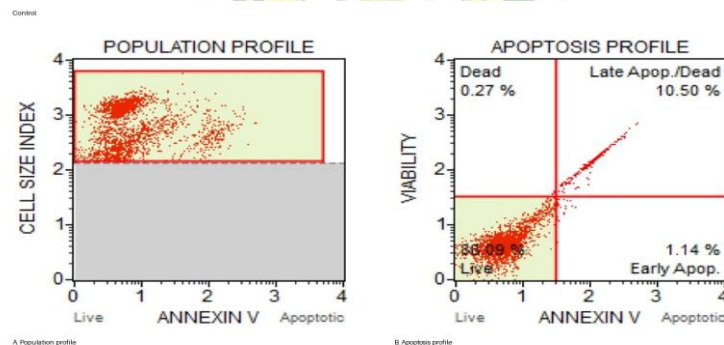


Fig. 1: Annexin V/PI flow-cytometric profile of untreated control HepG2 cells. The representative population profile and apoptosis quadrant distribution show the predominantly viable cell population

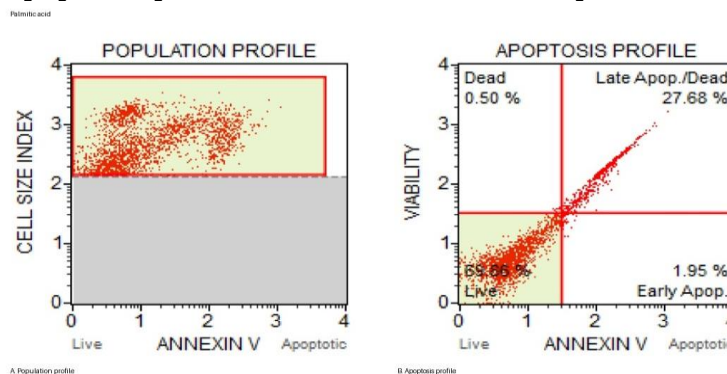


Fig. 2: Annexin V/PI flow-cytometric profile of palmitic acid-treated HepG2 cells. Palmitic acid exposure produced an increased late apoptotic/dead population compared with untreated control

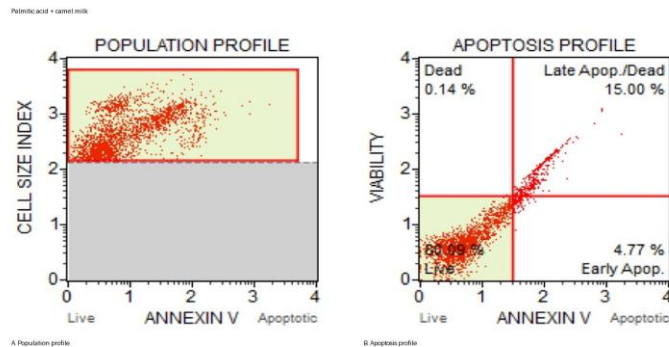


Fig. 3. Annexin V/PI flow-cytometric profile of palmitic acid-exposed HepG2 cells treated with pasteurized camel milk (12.5 µL/mL). The profile shows increased viable cells and reduced late apoptotic/dead cells compared with palmitic acid alone

As shown in Table 1, the mean viable-cell population decreased from 88.18±0.15% in the untreated control to 69.98±0.33% following palmitic acid exposure. Treatment with pasteurized camel milk increased the viable population to 79.32±0.69%. The mean late apoptotic/dead population increased from 10.35±0.26% in the control to 27.50±0.24% after palmitic acid exposure and subsequently decreased to 16.39±1.21% after camel milk treatment.

The early apoptotic population showed a mean value of 1.18±0.06% in control, 1.95±0.14% after palmitic acid exposure and 3.97±0.69% after camel milk treatment. Although the early apoptotic fraction was higher in the treated group than in the palmitic acid group, the substantially lower late apoptotic/dead fraction resulted in a lower calculated overall apoptotic fraction. The mean dead-cell/debris fraction remained low across the groups (0.30±0.05%, 0.56±0.05% and 0.32±0.16%, respectively).

The calculated total apoptotic fraction, obtained by adding the early apoptotic and late apoptotic/dead fractions, was 11.53% in control, 29.45% in palmitic acid-treated cells and 20.36% in the camel milk-treated group. Thus, compared with palmitic acid alone, camel milk treatment was associated with an absolute reduction of approximately 9.09 percentage points in the calculated apoptotic fraction. The corresponding summary is illustrated in Figure 4.

The increase in apoptosis following palmitic acid exposure is consistent with earlier experimental observations that saturated fatty acids can induce hepatocellular lipotoxicity and apoptosis in HepG2 cells.[4-6] The present observations extend this model by demonstrating the population-level effect using Annexin V/PI flow cytometry. The reduction in the late apoptotic/dead population after camel milk treatment is compatible with a protective cellular effect under the present experimental conditions. Previous literature has described antioxidant and anti-inflammatory activities of camel milk and has also reported effects of camel milk on survival and apoptotic signalling in HepG2 cells.[7,8] However, the present experiment does not establish a specific molecular mechanism, and the observed effect should therefore be interpreted as a preliminary protective association.

An important observation is the increase in the early apoptotic fraction after camel milk treatment. Annexin V positivity reflects phosphatidylserine externalization and should be interpreted together with membrane-integrity markers and the overall population distribution.[2,3] The present data therefore suggest that the principal change associated with camel milk treatment was a reduction in the late apoptotic/dead population rather than complete elimination of apoptotic signalling. Further studies incorporating caspase activity, mitochondrial membrane potential, reactive oxygen species and apoptosis-related gene/protein expression would help clarify the mechanism.

Table 1: Flow Cytometric Analysis of Apoptotic Cell Population in Hepg2 Cells: Mean ± SD of Triplicate Observations

Cell population	Control	Palmitic acid	Palmitic acid + Pasteurized camel milk	Interpretation
Live (LL)	88.18 ± 0.15	69.98 ± 0.33	79.32 ± 0.69	Viable cells
Early apoptotic (LR)	1.18 ± 0.06	1.95 ± 0.14	3.97 ± 0.69	Annexin V+/PI-
Late apoptotic/ dead (UR)	10.35 ± 0.26	27.50 ± 0.24	16.39 ± 1.21	Annexin V+/PI+
Dead/debris (UL)	0.30 ± 0.05	0.56 ± 0.05	0.32 ± 0.16	Annexin V-/PI+
Calculated total apoptotic*	11.53	29.45	20.36	Early + late apoptotic/ dead

*Values are mean $\pm$ SD for n=3 observations except calculated total apoptotic fraction, which is the sum of the corresponding mean early- and late-apoptotic/dead fractions. Percentages represent gated populations.

CONCLUSION

Pasteurized camel milk (*Ushtra Ksheera*) at 12.5 μ L/mL was associated with improved viability and attenuation of the late apoptotic/dead cell population in HepG2 cells subjected to 300 μ M palmitic acid. The mean viable population increased from 69.98% with palmitic acid alone to 79.32% after camel milk treatment, while the mean late apoptotic/dead population decreased from 27.50% to 16.39%. The findings indicate a preliminary protective effect under the experimental conditions used. Further investigations with larger replicate numbers, inferential statistical testing and molecular markers of apoptosis are warranted to confirm and elucidate this effect.

Acknowledgement

I sincerely express my gratitude to Dr. P. Y. Ansary, Professor and Head, Department of Dravyaguna Vigyana, Government Ayurveda College, Tripunithura, for his valuable guidance, encouragement, and support throughout the study.

I am deeply grateful to my research guide, Dr. Shincymol V.V, Professor, Department of Dravyagunavigyana, Government Ayurveda college, Thiruvananthapuram for her constant guidance, constructive suggestions, encouragement, and valuable support at every stage of this work.

I extend my sincere thanks to Dr. Reshma P. John, and Dr. Greeshma K. C. Assistant Professors, Department of Dravyaguna Vigyana, Government Ayurveda College, Tripunithura, for their valuable suggestions, encouragement, and support during the course of this study.

I express my heartfelt gratitude to the Director and staffs of the Centre for Research in Molecular and Applied Sciences (CRMAS), Thiruvananthapuram, for providing the necessary facilities, technical assistance, and cooperation for carrying out the experimental investigations.

REFERENCES

1. Elmore S. Apoptosis: a review of programmed cell death. *Toxicol Pathol.* 2007; 35(4): 495-516. doi:10.1080/01926230701320337.
2. Vermes I, Haanen C, Steffens-Nakken H, Reutelingsperger C. A novel assay for apoptosis: flow cytometric detection of phosphatidylserine expression on early apoptotic cells using fluorescein labelled Annexin V. *J Immunol Methods.* 1995; 184(1): 39-51. doi:10.1016/0022-1759(95)00072-I.
3. Koopman G, Reutelingsperger CP, Kuijten GA, Keehnen RMJ, Pals ST, van Oers MHJ. Annexin V for flow cytometric detection of phosphatidylserine expression on B cells undergoing apoptosis. *Blood.* 1994; 84(5): 1415-20.
4. Zhang L, Ji J, Zhu XY, Wu YY, Yu H, Zhang B, Li XL, Sun XZ. Palmitic acid induces apoptosis in human hepatoma cell line, HepG2 cells. *Zhongguo Yi Xue Ke Xue Yuan Xue Bao.* 2004; 26(6): 671-6.
5. Yang X, Chan C. Repression of PKR mediates palmitate-induced apoptosis in HepG2 cells through regulation of Bcl-2. *Cell Res.* 2009; 19(4): 469-486. doi:10.1038/cr.2009.25.
6. Zhu Q, Yang J, Zhu R, Jiang X, Li W, He S, Jin J. Dihydroceramide-desaturase-1-mediated caspase 9 activation through ceramide plays a pivotal role in palmitic acid-induced HepG2 cell apoptosis. *Apoptosis.* 2016; 21(9): 1033-1044. doi:10.1007/s10495-016-1267-9.
7. Behrouz S, Saadat S, Memarzia A, Sarir H, Folkerts G, Boskabady MH. The antioxidant, anti-inflammatory and immunomodulatory effects of camel milk. *Front Immunol.* 2022; 13: 855342. doi:10.3389/fimmu.2022.855342.
8. Korashy HM, Maayah ZH, Abd-Allah AR, El-Kadi AOS, Alhaider AA. Camel milk triggers apoptotic signaling pathways in human hepatoma HepG2 and breast cancer MCF7 cell lines through transcriptional mechanism. *J Biomed Biotechnol.* 2012; 2012: 593195. doi:10.1155/2012/593195.

Cite this article as:

Drishya satheesh, Shincymol V V, P Y Ansary, Greeshma K C. Protective Effect of Pasteurized Camel Milk (*Ushtra Ksheera*) Against Palmitic Acid-Induced Apoptosis in HEPG2 Cells: An Annexin V/Propidium Iodide Flow Cytometric Study. *International Journal of Ayurveda and Pharma Research.* 2026;14(9):167-170.

<https://doi.org/10.47070/ijapr.v14i9.4386>

Source of support: PG Star Session 3, By CCRAS, Conflict of interest: None

*Address for correspondence

Dr. Drishya Satheesh

PG Scholar,
Department of Dravyaguna Vigyana,
Government Ayurveda College,
Tripunithura, Kerala.
Email: drish1692@gmail.com

Disclaimer: IJAPR is solely owned by Mahadev Publications - dedicated to publish quality research, while every effort has been taken to verify the accuracy of the content published in our Journal. IJAPR cannot accept any responsibility or liability for the articles content which are published. The views expressed in articles by our contributing authors are not necessarily those of IJAPR editor or editorial board members.