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Effect of the Bioactive Nutritional Component Piperlongumine on Histone Deacetylase Enzyme Activity in Human Cancer and Normal Cells

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ABSTRACT

Currently, the effects of various bioactive compounds on Histone Deacetylase (HDAC) enzyme activity are being investigated. In this context, the aim of this study was to examine the effect of Piperlongumine (PL) on HDAC enzyme activity in HeLa and HEK293T cells *in vitro*. The IC_{50} doses of PL for HeLa and HEK293T cell lines were determined using MTT and AO/PI staining methods. HDAC activity was measured using a commercial kit in lysates obtained via the NETN lysis method from cells treated with IC_{50} doses of PL for 24 and 48 hours *in vitro*. Additionally, HDAC activity was assessed in lysates from cells cultured under normal conditions and directly treated with IC_{50} doses of PL immediately before the HDAC assay. After 24- and 48-hour PL treatments, a statistically significant increase in HDAC activity was observed in HEK293T cells, whereas a non-significant increase was found in HeLa cells. In lysates directly treated with PL, HDAC activity significantly increased in HeLa cells but showed no significant change in HEK293T cells. These findings suggest that PL may affect HDACs via distinct mechanisms depending on cell type. The significant increase in HDAC activity upon direct treatment of HeLa lysates suggests a possible physical interaction between PL and HDACs in cancer cells. Conversely, the increased HDAC activity observed in HEK293T cells following 24- and 48-hour *in vitro* PL treatment implies that PL may act in normal cells through indirect and delayed pathways.

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Biyoaktif Besin Bileşeni Piperlongumin'in İnsan Kanser ve Normal Hücrelerinde Histon Deasetilaz Enzim Aktivitesi Üzerine Etkisi

ÖZET

Günümüzde çeşitli biyoaktif bileşiklerin Histon Deasetilaz (HDAC) enzim aktivitesi üzerindeki etkileri araştırılmaktadır. Bu bağlamda, bu çalışmanın amacı Piperlongumine (PL)'in HeLa ve HEK293T hücrelerinde *in vitro* HDAC enzim aktivitesi üzerindeki etkisini incelemektir. HeLa ve HEK293T hücre hatları için PL'in IC_{50} dozları MTT ve AO/PI boyama yöntemleri kullanılarak belirlenmiştir. HDAC aktivitesi, 24 ve 48 saat boyunca *in vitro* olarak IC_{50} dozunda PL ile muamele edilen hücrelerden NETN lizis yöntemiyle elde edilen lizatlarda ticari bir kit kullanılarak ölçülmüştür. Ayrıca, normal koşullarda kültüre edilen ve HDAC aktivite analizinden hemen önce IC_{50} dozunda PL ile doğrudan muamele edilen hücrelerden elde edilen lizatlarda da HDAC aktivitesi değerlendirilmiştir. 24 ve 48 saatlik PL uygulamaları sonrasında, HEK293T hücrelerinde HDAC aktivitesinde istatistiksel olarak anlamlı bir artış gözlenmiş, HeLa hücrelerinde ise anlamlı olmayan bir artış saptanmıştır. PL ile doğrudan muamele edilen lizatlarda ise, HeLa hücrelerinde HDAC aktivitesi anlamlı düzeyde artarken, HEK293T hücrelerinde anlamlı

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bir değişiklik gözlenmemiştir. Bu bulgular, PL'in hücre tipine bağlı olarak HDAC'leri farklı mekanizmalarla etkileyebileceğini düşündürmektedir. HeLa hücre lizatlarında gözlenen HDAC aktivite artışı, PL'in kanser hücrelerinde HDAC'lerle fiziksel etkileşim yoluyla etkili olabileceğini göstermektedir. Buna karşılık, kültür ortamında24 ve 48 saat süreyle PL ile muamele edilen HEK293T hücrelerinde gözlenen aktivite artışı, PL'in normal hücrelerde dolaylı ve gecikmeli yollarla etkili olabileceğini düşündürmektedir.

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INTRODUCTION

The Human Genome Project has enabled the identification of the interrelationships between nutrients, genes, and diseases (Prasad et al., 2011; Nasir et al., 2020). On the other hand, it has been determined that nutrition and lifestyle can affect physiological and pathological processes by causing various epigenetic changes (Hardy & Tollefsbol, 2011; Zam & Khadour, 2017; Singh et al., 2018). Histone protein acetylation, one of the epigenetic changes, is regulated by histone acetyltransferases (HATs) and histone deacetylases (HDACs) (Orlikova et al., 2012; West & Johnstone, 2014; Vahid et al., 2015). HAT and HDAC enzymes play important roles in regulating gene expression through chromatin remodeling, a process linked to various medical conditions, including cancers, lung diseases, neurodegenerative diseases, and immune disorders. There are 18 HDAC enzymes within 4 classes in mammals: Class I (HDAC1-3, HDAC8), Class II (IIa: HDAC4-5, HDAC7, HDAC9, and IIb: HDAC6, HDAC10), Class III, Sirtuins (SIRT1-7), and Class IV (HDAC11) (Khatun et al., 2024). HDACs catalyze the deacetylation of both histone and non-histone proteins. Hyperacetylation of histones has been linked to transcriptional activation, while removal of HDAC-bound acetyl groups is associated with chromatin condensation and transcriptional repression (Levidou et al., 2024). In recent years, HDAC enzyme inhibition has been a new approach to the treatment of various diseases. Histone Deacetylase Enzyme Inhibitors (HDACi), which act on HDAC enzymes, are naturally derived or synthetic compounds widely used therapeutically for various diseases associated with abnormal HDAC function (Falkenberg & Johnstone, 2014). Today, HDAC inhibitors such as Vorinostat, Romidepsin, Panobinostat, and Belinostat are usetont treat lymphoma and multiple myeloma. In addition, HDAC inhibition has beneficial effects in various neurodegenerative diseases, inflammation-induced diseases, and cardiovascular diseases (Yoon & Eom, 2016). In addition to HDACi drugs with therapeutic effects on cancer, nutrients with HDACi activity have also been identified (Rajendran et al., 2011). Some of these include allyl mercaptan, allicin, and diallyl disulfide in garlic; sodium butyrate and sodium propionate formed as a result of fermentation of dietary fiber; sulforaphane in broccoli; catechins in green tea; hydroxycinnamic acid in cinnamon; and curcumin in turmeric (Bassett & Barnett, 2014; Kim et al., 2016; Singhal et al., 2020). Therefore, dietary compounds that can alter histone acetylation may offer new strategies to prevent, delayngor treat various diseases (Bassett & Barnett, 2014). Piperlongumine (PL) is an amide alkaloid obtained from the plant Piper longum L., which grows mainly in Southeast Asia and South India, and is also known as piplartin (Bezerra et al., 2013; Afolabi et al., 2021; Ye et al., 2022). In addition, various PL derivatives have been synthesized, and their effects have been investigated (Zhu et al., 2021). PL has been described as an anticancer molecule that modulates cell proliferation and migration, apoptosis, and reactive oxygen species (ROS) production, and exerts selective cytotoxic effects on various cancer cell types, including breast, prostate, renal, and pancreatic cancers (Allaman-Pillet & Schorderet, 2021). PL has been reported to exhibit various biological activities, including anti-inflammatory, antiatherosclerotic, antinociceptive, anxiolytic, antidepressant, neuroprotective, antiarteriosclerotic, and antimicrobial properties, in addition to its anticancer effect (Piska et al., 2018; Henrique et al., 2020; Huang et al., 2020). Recent studies have demonstrated that piperlongumine exhibits a wide range of pharmacological activities; however, the molecular pathways mediating PL-associated cellular events remain poorly understood (Thatikonda et al., 2020; Xu et al., 2021). For example, PL has been shown to inhibit glutathione S-transferase (GST) enzymes, thereby suppressing cellular antioxidant defenses, and this effect has been associated with reduced tumor growth in preclinical cancer models (Alnasser, 2025). In another study, piperlongumine was shown to attenuate hyperglycemia by protecting pancreatic ß cells and to suppress inflammation and apoptosis in diabetic rats via modulation of the GLUT-2/4 and AKT signaling pathways (Xu et al., 2021). In addition, piperlongumine has been shown to protect brain tissue in rats with cerebral ischemia by suppressing the NF-κB and MAPK signaling pathways (Yang et al., 2014; Dong et al., 2023). Piperlongumine has also been reported to attenuate angiotensin

II–induced cardiac hypertrophy *in vitro* and *in vivo* via the AKT–Fox1 signaling pathway. Modulation of HDAC activity could be a potential mechanism of action of PL. Research on the effect of PL on HDAC enzymes is limited (Thatikonda et al., 2020). For these reasons, in this study, the effect of the PL bioactive nutrient component, which is reported to have positive effects on various diseases, on HDAC activity in HEK293T and HeLa cells was experimentally investigated *in vitro*.

MATERIAL and METHOD

Human cervical cancer cell line HeLa and human embryonic kidney cell line HEK293T, previously purchased from the American Type Culture Collection (ATCC) cell culture bank, were used in this study. The thawed cells were transferred to medium containing 10% fetal bovine serum and incubated at 37°C and 5% CO_2. Afterward, the cells were routinely monitored 2-3 times a week in Dulbecco's Modified Eagle's Medium (DMEM) with an inverted microscope until the cell density reached 80-90%, and the cell culture was maintained. The approximate IC_{50} values of PL on HeLa and HEK293T cells after 24 hours were determined using the MTT method. The validity of the determined IC_{50} values was supported by phase-contrast and fluorescence microscopy images after AO/PI staining. The effects of the determined IC_{50} doses on HDAC activity in HeLa and HEK293T cells were assessed using a colorimetric HDAC activity assay. In this study, protein quantification was performed by the Bradford method in cellular lysates prepared by the NETN lysis method from HeLa and HEK293T cells treated with IC_{50} doses of PL for 24 and 48 hours under *in vitro* conditions and also in cellular lysates prepared by NETN lysis method from HeLa and HEK293T cells grown under normal conditions and directly treated with IC_{50} doses of PL just before the HDAC activity assay. Subsequently, total cellular HDAC activity in cellular lysates was determined using a commercial colorimetric kit. The data obtained in the study were analyzed using SPSS 20.0 at a 95% confidence level. One-way analysis of variance (ANOVA) was used to determine whether there was a difference between the experimental groups. In cases of differences, the post hoc Dunnett's test was used to determine the significance of group differences relative to the control group. $p < .05$ values were considered statistically significant.

RESULTS

Determination of PL IC_{50} Doses

The IC_{50} of PL for 24 hours in HeLa cells was determined to be 171 µM in a previous study by us (Seber et al., 2020). To determine the IC_{50} doses of PL for 24 hours in HEK293T cells by the MTT method, PL was applied to HEK293T cells for 24 hours with final concentrations of 5 µM, 10 µM, 20 µM, 40 µM, 80 µM, 120 µM, 160 µM, 200 µM, 240 µM, 360 µM, and DMSO as a control. The data obtained from the experiment were analyzed using SigmaPlot, and the 24 h IC_{50} dose of PL, which killed 50% of HEK293T cells, was 18 µM. In addition, the IC_{50} of PL in HEK293T cells was confirmed by fluorescence microscopy using the AO/PI dye (Data not shown). The results of the MTT assay investigating the effects of different PL concentrations on HEK293T cells are shown in Figure 1.

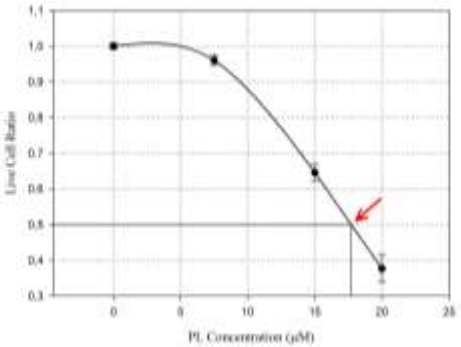

Figure 1. Determination of the IC_{50} dose of PL in HEK293T cells for 24 hours by the MTT method. The concentration that reduces the proportion of viable cells by half is defined as the IC_{50}.

Şekil 1. HEK293T hücrelerinde 24 saat boyunca MTT yöntemiyle PL'nin IC_{50} dozunun belirlenmesi. Canlı hücre oranını yarıya düşüren konsantrasyon, IC_{50} dozu olarak belirlenmiştir.

Measurements of HDAC Activities

When mean OD values reflecting HDAC activity in cellular lysates from HeLa cells were compared with controls, decreases of 21% *($p = .249$)* and 15% *($p = .544$)* in HDAC enzyme activity were observed with 24 h and 48 h PL IC_{50} treatments, respectively. There was no statistically significant difference. When the cellular lysates obtained from

HeLa cells were directly treated with PL during the HDAC activity determination experiment without keeping in culture medium, and the change in mean OD values was compared to the controls, a statistically significant increase of 32% was found in HDAC enzyme activity with PL IC_{50} doses *(p = .003)* (Figure 2).

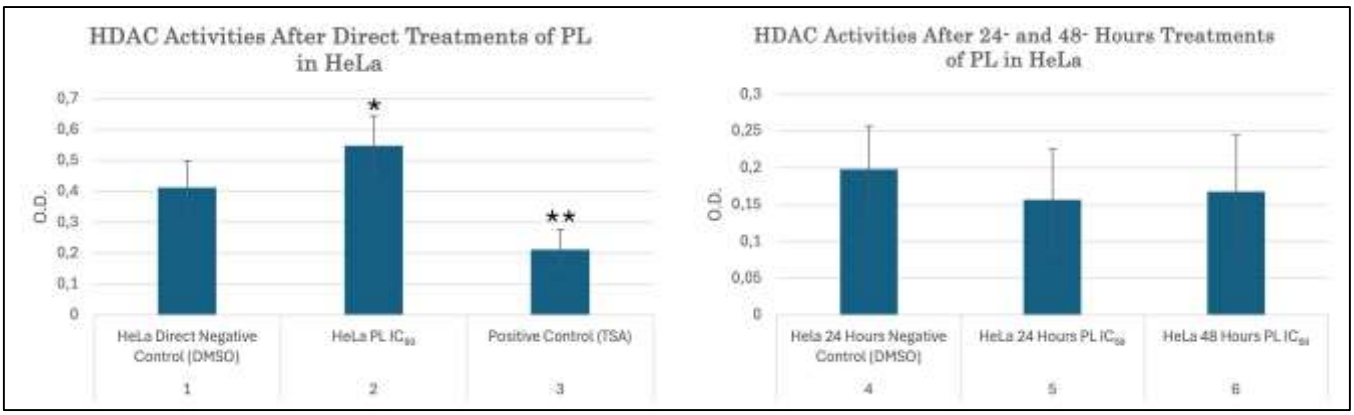

Figure 2. Comparison of OD Values Indicating HDAC Activity in HeLa Cells. 1) HeLa Control (DMSO), 2) HeLa PL IC_{50}, 3) Positive Control (TSA), 4) HeLa 24 Hours Control (DMSO), 5) HeLa 24 Hours PL IC_{50}, 6) HeLa 48 Hours PL IC_{50}. (Sample no. 1,2,3 and sample no. 4,5,6 are 2 different experimental setups and evaluated in their own right). *; there was a statistically significant difference in sample number 2 compared to control number 1 (p = .003); **; there was a statistically significant difference in sample number 3 compared to control number 1 (p < .001).

Şekil 2. HeLa hücrelerinde HDAC aktivitesini gösteren OD değerlerinin karşılaştırılması. 1) HeLa Kontrol (DMSO), 2) HeLa PL IC_{50}, 3) Pozitif Kontrol (TSA), 4) HeLa 24 Saat Kontrol (DMSO), 5) HeLa 24 Saat PL IC_{50}, 6) HeLa 48 Saat PL IC_{50}.(Numune no. 1, 2, 3 ile numune no. 4, 5, 6 iki farklı deney düzeneğidir ve kendi içinde değerlendirilmiştir).; Numune 2'nin, kontrol 1'e göre istatistiksel olarak anlamlı fark gösterdiği bulunmuştur (p = .003),**; Numune 3'nın, kontrol 1'e göre istatistiksel olarak anlamlı fark gösterdiği bulunmuştur (p < .001).*

When the OD values reflecting HDAC activity in cellular lysates obtained from HEK293T cells were compared to the controls, a statistically insignificant increase of 151% in HDAC enzyme activity was observed with 24-hour PL IC_{50} treatment *(p = .096)*, whereas a statistically significant increase of 312% was found following 48-hour PL IC_{50} treatment *(p < .001)*. Additionally, when the OD values of cellular lysates obtained from HEK293T cells, proliferated under normal conditions and directly treated with PL IC_{50} concentrations just before the HDAC activity assay, were compared to the controls, a statistically insignificant decrease of 10% in HDAC enzyme activity was observed *(p = .124)* (Figure 3).

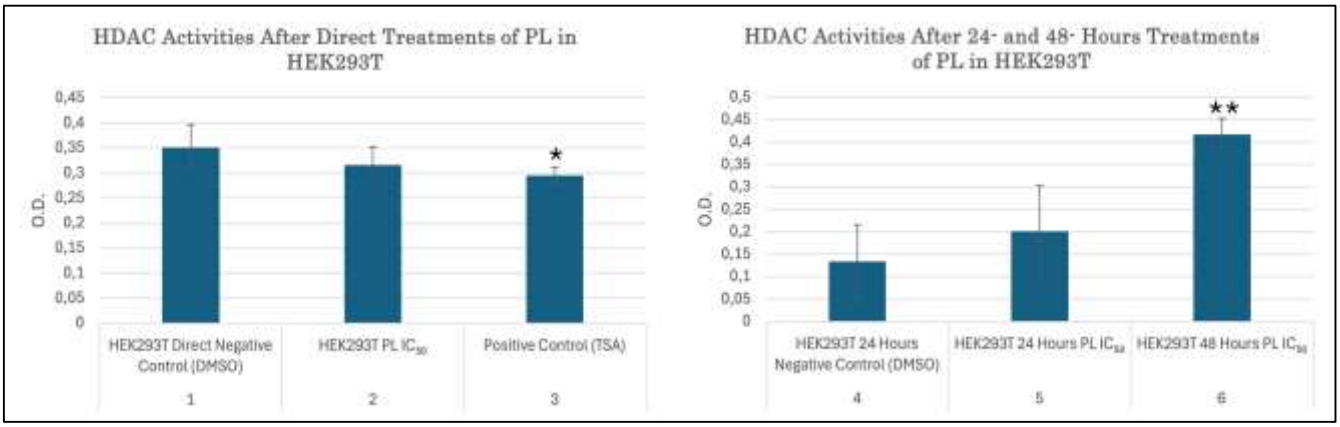

Figure 3. Comparison of OD values showing HDAC activity in HEK293T cells.*; A statistically significant difference was observed between the negative control group (DMSO) and the positive control group (TSA) (p = .034). **; A statistically significant difference was observed between HEK293T cells treated with PL IC_{50} for 48 hours and the 24-hour control group (DMSO) (p < .001). (Sample no. 1,2,3 and sample no. 4,5,6 are 2 different experimental setups and evaluated in their own right).

*Şekil 3. HEK293T hücrelerinde HDAC aktivitesini gösteren OD değerlerinin karşılaştırılması. *; Negatif kontrol grubu (DMSO) ile pozitif kontrol grubu (TSA) arasında istatistiksel olarak anlamlı bir fark bulunmuştur (p = .034). **; HEK293T hücrelerinde 48 saat PL IC_{50} dozu uygulanan grup ile 24 saatlik kontrol grubu (DMSO) arasında istatistiksel olarak anlamlı fark bulunmuştur (p < .001), (Numune no. 1, 2, 3 ile numune no. 4, 5, 6 iki farklı deney düzeneğidir ve kendi içinde değerlendirilmiştir).*

DISCUSSION

In this study, *in vitro* experiments were conducted to determine the effect of PL on total cellular HDAC enzyme activity in HEK293T cells, a normal human embryonic cell line, and HeLa cells, a cancer cell line. HDAC activities were measured under two different experimental settings: (1) direct treatment of cell lysates with PL at the IC_{50} dose immediately before the HDAC activity assay, and (2) exposure of cells to PL at the IC_{50} dose in culture medium for 24 and 48 hours. According to our experimental findings, PL affected HDAC activity.

When HeLa cell lysates were treated with the IC_{50} dose of PL for 24 hours, HDAC enzyme activity decreased by 21% compared to the control *($p = .249$)*. Extending the treatment to 48 hours resulted in a 15% decrease in HDAC activity *($p = .524$)*. However, neither of these decreases reached statistical significance. Based on these results, PL exposure for 24 and 48 hours appears to cause a non-significant reduction in HDAC activity in HeLa cells. In contrast, when HeLa cell lysates were treated directly with the PL IC_{50} dose immediately prior to the HDAC activity assay, a significant 32% increase in enzyme activity was observed compared to the control *($p = .003$)*. This increase suggests a potential direct interaction between PL and HDAC enzymes, leading to elevated total cellular HDAC activity. While direct PL treatment increased HDAC activity, prolonged exposure for 24 and 48 hours decreased it, suggesting that indirect mechanisms may contribute to the observed reduction in HDAC activity following PL treatment in HeLa cells.

Cellular lysates obtained from HEK293T cells showed a 151% increase *($p = .096$)* in HDAC enzyme activity compared to the control when the PL IC_{50} dose was applied for 24 hours. When the PL IC_{50} dose was applied for 48 hours, a statistically significant 312% increase *($p < .001$)* in HDAC enzyme activity was observed compared to the control. Based on these findingst prolonged PL treatment increased HDAC activity in normal, healthy HEK293T cells. When the IC_{50} dose of PL was administered just before HDAC activity determination, a statistically insignificant 10% decrease in HDAC enzyme activity was observed compared to the control *($p = .124$)*. When the different experimental conditions are collectively evaluated, it is thought that PL treatment in cell culture conditions increases HDAC enzyme activity in HEK293T cells through indirect mechanisms rather than direct physical interactions. PL treatments of cell lysates grown under normal conditions and PL treatments of living cells for 24 and 48 hours under *in vitro* conditions yielded completely opposite findings.

Piperlongumine is one of the major bioactive compounds isolated from *Piper longum* and exerts its effects through multiple cellular and molecular targets. Studies have demonstrated that piperlongumine can induce cell cycle arrest at the G1 or G2/M phase, promote apoptosis, and reduce the expression of vascular endothelial growth factor (VEGF). In addition, it has been reported to inhibit atherosclerotic plaque formation and suppress the activity of recombinant human aldose reductase. Piperlongumine has also been shown to increase oxidative stress in cancer cells by targeting mitochondria and to trigger caspase-independent apoptosis, thereby contributing to its anticancer activity PL exhibits antitumor, anticancer, antidepressant, and anxiolytic properties and can selectively target cancer cells while minimally affecting normal cells (Ntagwabira et al., 2022). It has been reported to induce apoptosis in various cancer cell types through enhanced production of reactive oxygen species (ROS) and, owing to its low toxicity profile, represents one of the few molecules capable of selectively eliminating senescent cells(Chen et al., 2022). Accumulating preclinical and experimental evidence suggests that piperlongumine exerts potential therapeutic effects in diverse diseases by modulating multiple molecular pathways, including MAPK, PI3K/Akt, NF-κB, glutathione S-transferase P1, thioredoxin reductase-1, and cyclooxygenase-2 (COX-2) (Chen et al., 2022; Ntagwabira et al., 2022). Nevertheless, despite these promising findings, comprehensive mechanistic studies and clinical validation remain insufficient.

As a result of the literature review, it was observed that the effect of PL on HDAC enzyme activity was investigated in only one publication. In the study by Thatikonda et al. 2020, which examined the epigenetic changes induced by piperlongumine in psoriasis and the associated signaling mechanisms, PL was shown to modulate epigenetic pathways and alleviate psoriasis-like skin lesions by inhibiting hyperproliferation and inflammation. They also analyzed the effect of PL on HDACs in lipopolysaccharide-exposed macrophage cells using the Fluorometric HDAC Assay Kit. In this study, HeLa cell lysates were treated with four concentrations of PL (0.05, 0.1, 1, and 2.5 μM), and HDAC inhibitory activity was compared with that of TSA at 2.5 μM. The results showed that HDAC levels were 0.52 ± 0.02 μM in the TSA-treated group, 0.44 ± 0.06 μM in the 0.05 μM PL-treated group, 0.49 ± 0.13 μM in the 0.1 μM PL-treated group, 0.26 ± 0.03 μM in the 1 μM PL-treated group, and 0.24 ± 0.01 μM in the 2.5 μM PL-treated group. PL treatment significantly decreased (inhibited) HDAC levels in a dose-dependent manner. This study differs from this one in that the method used to determine HDAC activity was fluorometric, and much lower doses of PL were used in this study. In this study, total HDAC activity in cellular lysates was determined using a colorimetric HDAC Activity Assay Kit. The fluorometric and colorimetric methods used to determine HDAC activity in both studies are methodologically quite similar.

In this study, HDAC activity was investigated in HeLa cells exposed to the IC_{50} dose of PL (171 μM) for 24 and 48 hours under cell culture conditions, as well as in HeLa cell lysates directly treated with PL. In the comparative

study in the literature, only HeLa cellular lysates were treated with PL concentrations of 0.05, 0.1, 1, and 2.5 µM. In this respect, this study partially overlaps with the literature. Although there is no explanation for the comparatively lower concentrations chosen in that study, it is plausible that very low doses were tested, as the study was a continuation of an *in vivo* study. In this study, the IC_{50} dose was preferred to inassesshe effect on HDAC activity because of the high doses that could be administered to cells and the predictability of the cell numbers required fotoreparineellular lysates. In both studies, TSA, a well-known HDAC inhibitor, was used as a control, and TSA-mediated HDAC inhibition was demonstrated. On the other hand, the findings on the effect of PL on HDAC activity are contradictory. The reason for this contradiction is thought to be the very different PL concentrations used.

To the best of this knowledge, the effect of PL a bioactive nutrient component with positive effects on various diseases on total cellular HDAC enzyme activity in normal and cancer cell lines was experimentally investigated for the first time in this study, using IC_{50} values as a reference and a colorimetric HDAC activity assay kit.

CONCLUSION

Recent studies have demonstrated that piperlongumine exhibits a broad spectrum of pharmacological activities; however, the molecular pathways mediating PL-associated cellular events have not yet been fully elucidated. Current evidence suggests that PL exerts its biological effects through multiple and distinct molecular pathways rather than a single mechanism. The findings of the present study indicate that PL may influence HDAC activity through direct and/or indirect mechanisms. PL has been reported to be associated with various diseases and conditions, and numerous studies have recently investigated its clinical effects. Nevertheless, given that PL appears to act through distinct mechanisms across different disease contexts, it is necessary—as with other dietary components—to investigate PL in greater detail at the molecular and cellular levels. On the other hand**,** examining the effects of dietary components on HDAC activity as potential epigenetic regulators may contribute to developing diet-based strategies for the prevention, delay, and treatment of diseases.

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Contribution Rate Statement Summary of Researchers

The authors declare that they have contributed equally to the article.

Conflict of Interest

The authors of this manuscript declare that there are no conflicts of interest associated with this manuscript.

REFERENCES

Afolabi, L. O., Bi, J., Chen, L., & Wan, X. (2021). A natural product, Piperlongumine (PL), increases tumor cells sensitivity to NK cell killing. *International Immunopharmacology*, *96*, 107658. https://doi.org/10.1016/j.intimp.2021.107658

Allaman-Pillet, N., & Schorderet, D. F. (2021). Piperlongumine promotes death of retinoblastoma cancer cells. *Oncotarget*, *12*(9), 907–916. https://doi.org/10.18632/oncotarget.27947

Alnasser, S. M. (2025). The role of glutathione S-transferases in human disease pathogenesis and their current inhibitors. *Genes & Diseases*, *12*(4), 101482. https://doi.org/10.1016/j.gendis.2024.101482

Bassett, S. A., & Barnett, M. P. G. (2014). The role of dietary histone deacetylases (HDACs) inhibitors in health and disease. *Nutrients*, *6*(10), 4273–4301. https://doi.org/10.3390/nu6104273

Bezerra, D. P., Pessoa, C., De Moraes, M. O., Saker-Neto, N., Silveira, E. R., & Costa-Lotufo, L. V. (2013). Overview of the therapeutic potential of piplartine (piperlongumine). *European Journal of Pharmaceutical Sciences, 48*(3), 453–463. https://doi.org/10.1016/j.ejps.2012.12.003

Chen, D., Wei, X., Yang, K., Liu, X., Song, Y., Bai, F., Jiang, Y., Guo, Y., & Jha, R. K. (2022). Piperlongumine combined with vitamin C as a new adjuvant therapy against gastric cancer regulates the ROS-STAT3 pathway. *Journal of International Medical Research*, *50*(4), 3000605221093308. https://doi.org/10.1177/03000605221093308

Dong, L., He, J., Luo, L., & Wang, K. (2023). Targeting the interplay of autophagy and ROS for cancer therapy: An updated overview on phytochemicals. *Pharmaceuticals, 16*(1), 92. https://doi.org/10.3390/ph16010092

Falkenberg, K. J., & Johnstone, R. W. (2014). Histone deacetylases and their inhibitors in cancer, neurological diseases and immune disorders. *Nature Reviews Drug Discovery*, *13*(9), 673–691. https://doi.org/10.1038/nrd4360

Hardy, T. M., & Tollefsbol, T. O. (2011). Epigenetic diet: impact on the epigenome and cancer. *Epigenomics*, *3*(4), 503–518. https://doi.org/10.2217/epi.11.71

Henrique, T., Zanon, C. de F., Girol, A. P., Stefanini, A. C. B., Contessoto, N. S. de A., da Silveira, N. J. F., Bezerra, D. P., Silveira, E. R., Barbosa-Filho, J. M., Cornélio, M. L., Oliani, S. M., & Tajara, E. H. (2020). Biological and physical approaches on the role of piplartine (piperlongumine) in cancer. *Scientific Reports*, *10*(1), 22283. https://doi.org/10.1038/s41598-020-78220-6

Huang, J. R., Wang, S. T., Wei, M. N., Liu, K., Fu, J. W., Xing, Z. H., & Shi, Z. (2020). Piperlongumine alleviates mouse colitis and colitis-associated colorectal cancer. *Frontiers in Pharmacology*, *11*, 586885. https://doi.org/10.3389/fphar.2020.586885

Khatun, S., Prasad Bhagat, R., Dutta, R., Datta, A., Jaiswal, A., Halder, S., Jha, T., Amin, S. A., & Gayen, S. (2024). Unraveling HDAC11: Epigenetic orchestra in different diseases and structural insights for inhibitor design. *Biochemical Pharmacology*, *225*, 116312. https://doi.org/10.1016/j.bcp.2024.116312

Kim, E., Bisson, W. H., Löhr, C. V., Williams, D. E., Ho, E., Dashwood, R. H., & Rajendran, P. (2016). Histone and non-histone targets of dietary deacetylase inhibitors. *Current Topics in Medicinal Chemistry, 16*(7), 714–731. https://doi.org/10.2174/1568026615666150825125857

Levidou, G., Arsenakis, D., Bolovis, D. I., Meyer, R., Brucker, C. V. M., Papadopoulos, T., & Theocharis, S. (2024). Clinical significance of the immunohistochemical expression of histone deacetylases (HDACs)-2, -4, and -5 in ovarian adenocarcinomas. *Biomedicines*, *12*(5), 947. https://doi.org/10.3390/biomedicines12050947

Nasir, A., Bullo, M. M. H., Ahmed, Z., Imtiaz, A., Yaqoob, E., Jadoon, M., Ahmed, H., Afreen, A., & Yaqoob, S. (2020). Nutrigenomics: Epigenetics and cancer prevention: A comprehensive review. *Critical Reviews in Food Science and Nutrition*, *60*(8), 1375–1387. https://doi.org/10.1080/10408398.2019.1571480

Ntagwabira, F., Trujillo, M., McElroy, T., Brown, T., Simmons, P., Sykes, D., & Allen, A. R. (2022). Piperlongumine as a neuro-protectant in chemotherapy induced cognitive impairment. *International Journal of Molecular Sciences*, *23*(4), 2008. https://doi.org/10.3390/ijms23042008

Orlikova, B., Schnekenburger, M., Zloh, M., Golais, F., Diederich, M., & Tasdemir, D. (2012). Natural chalcones as dual inhibitors of HDACs and NF-κB. *Oncology Reports*, *28*(3), 797–805. https://doi.org/10.3892/or.2012.1870

Piska, K., Gunia-Krzyżak, A., Koczurkiewicz, P., Wójcik-Pszczoła, K., & Pękala, E. (2018). Piperlongumine (piplartine) as a lead compound for anticancer agents - Synthesis and properties of analogues: A mini-review. *European Journal of Medicinal Chemistry*, *156*, 13–20. https://doi.org/10.1016/j.ejmech.2018.06.057

Prasad, C., Imrhan, V., & Rew, M. (2011). Introducing nutritional genomics teaching in undergraduate dietetic curricula. *Journal of Nutrigenetics and Nutrigenomics*, *4*(3), 165–172. https://doi.org/10.1159/000330237

Rajendran, P., Ho, E., Williams, D. E., & Dashwood, R. H. (2011). Dietary phytochemicals, HDAC inhibition, and DNA damage/repair defects in cancer cells. *Clinical Epigenetics, 3*(1), 4. https://doi.org/10.1186/1868-7083-3-4

Seber, S., Sirin, D. Y., Yetisyigit, T., & Bilgen, T. (2020). Piperlongumine increases the apoptotic effect of doxorubicin and paclitaxel in a cervical cancer cell line. *Nigerian Journal of Clinical Practice*, *23*(3), 386–391. https://doi.org/10.4103/njcp.njcp_80_19

Singh, A. K., Bishayee, A., & Pandey, A. K. (2018). Targeting histone deacetylases with natural and synthetic agents: an emerging anticancer strategy. *Nutrients*, *10*(6), 731. https://doi.org/10.3390/nu10060731

Singhal, S., Pathak, M., Agrawala, P. K., & Ojha, H. (2020). Design and in silico screening of aryl allyl mercaptan analogs as potential histone deacetylases (HDAC) inhibitors. *Heliyon*, *6*(5), e03517. https://doi.org/10.1016/j.heliyon.2020.e03517

Thatikonda, S., Pooladanda, V., Sigalapalli, D. K., & Godugu, C. (2020). Piperlongumine regulates epigenetic modulation and alleviates psoriasis-like skin inflammation via inhibition of hyperproliferation and inflammation. *Cell Death & Disease*, *11*(1), 21. https://doi.org/10.1038/s41419-019-2212-y

Vahid, F., Zand, H., Nosrat-Mirshekarlou, E., Najafi, R., & Hekmatdoost, A. (2015). The role dietary of bioactive compounds on the regulation of histone acetylases and deacetylases: A review. *Gene*, *562*(1), 8–15. https://doi.org/10.1016/j.gene.2015.02.045

West, A. C., & Johnstone, R. W. (2014). New and emerging HDAC inhibitors for cancer treatment. *The Journal of Clinical Investigation*, *124*(1), 30–39. https://doi.org/10.1172/jci69738

Xu, P., Xiao, J., & Chi, S. (2021). Piperlongumine attenuates oxidative stress, inflammatory, and apoptosis through modulating the GLUT-2/4 and AKT signaling pathway in streptozotocin-induced diabetic rats. *Journal of Biochemical and Molecular Toxicology*, *35*(6), 1–12. https://doi.org/10.1002/jbt.22763

Yang, T., Sun, S., Wang, T., Tong, X., Bi, J., Wang, Y., & Sun, Z. (2014). Piperlonguminine is neuroprotective in experimental rat stroke. *International Immunopharmacology*, *23*(2), 447–451. https://doi.org/10.1016/j.intimp.2014.09.016

Ye, W., Tang, T., Li, Z., Li, X., & Huang, Q. (2022). Piperlongumine attenuates vascular remodeling in hypoxic pulmonary hypertension by regulating autophagy. *Journal of Cardiology*, *79*(1), 134–143. https://doi.org/10.1016/j.jjcc.2021.08.023

Yoon, S., & Eom, G. H. (2016). HDAC and HDAC inhibitor: From cancer to cardiovascular diseases. *Chonnam Medical Journal*, *52*(1), 1–11. https://doi.org/10.4068/cmj.2016.52.1.1

Zam, W., & Khadour, A. (2017). Impact of phytochemicals and dietary patterns on epigenome and cancer. *Nutrition and Cancer*, *69*(2), 184–200. https://doi.org/10.1080/01635581.2017.1263746

Zhu, P., Qian, J., Xu, Z., Meng, C., Zhu, W., Ran, F., Zhang, W., Zhang, Y., & Ling, Y. (2021). Overview of piperlongumine analogues and their therapeutic potential. *European Journal of Medicinal Chemistry*, *220*, 113471. https://doi.org/10.1016/j.ejmech.2021.113471