DIFFERENTIAL CYTOTOXIC EFFECTS OF PIPER LONGUM AND ITS BIOACTIVE CONSTITUENTS ON MALIGNANT AND NON-MALIGNANT CELLS: A REVIEW

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ABSTRACT

Background: Piper longum L. (long pepper), is a medicinal plant belonging to the family Piperaceae. It has attracted scientific interest because it has reported pharmacological and potential anticancer properties. Its bioactive constituents, like piperine and piperlongumine (piplartine), have been investigated for their effects on cancer cell proliferation, viability and apoptosis. However, the extent to which these compounds differentiate between malignant and non-malignant cells actually require very careful examination of the available experimental evidence. Objective: Researcher aimed to evaluate the reported cytotoxic effects of Piper longum extracts and its major bioactive constituents on malignant and non-malignant cells, with particular focus on its differential cellular responses, proposed mechanisms of action and methodological limitations. Methods: Relevant literature was identified through searches of Google scholar, ResearchGate, Scopus, Web of Science and other appropriate scholarly databases. Eligible studies included experimental investigations evaluating Piper longum extracts, piperine or piperlongumine in malignant cell models, with particular attention to studies that also examine non-malignant cellular controls. Results: The results section was completed following database searching, study selection, eligibility assessment and data extraction.

Conclusion: Evaluation of the available evidence may help clarify the extent of differential cytotoxic activity associated with Piper longum and its bioactive constituents. Such an assessment is important for identifying promising preclinical findings while recognising the limitations of in vitro evidence and the need for further research.

Keywords: *Piper longum, piperine cancer cells, non-malignant cells, bioactive.*

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INTRODUCTION

Cancer is a major global health challenge, and the development of effective anticancer treatments continues to be complicated by the adverse effects associated with conventional chemotherapeutic agents. Although several chemotherapeutic drugs have demonstrated considerable efficacy in controlling malignant growth, their effects are not always restricted to cancerous tissues. Damage to normal, rapidly dividing cells, including those of the bone marrow, gastrointestinal epithelium and hair follicles, contributes to treatment-associated toxicity and limits therapeutic tolerability. Consequently, the identification of bioactive compounds capable of producing differential effects on malignant and non-malignant cells remains an important area of cancer research.

Natural products have historically contributed to the discovery and development of pharmacologically active compounds, including several agents used in cancer treatment. Medicinal plants contain diverse secondary metabolites that may influence cellular proliferation, apoptosis, oxidative stress and intracellular signalling pathways. Among these plants, Piper longum L., commonly known as long pepper or Pippali, belongs to the family Piperaceae and has an established history of use in traditional medicinal systems, particularly Ayurveda. Its fruits contain several bioactive phytochemicals, including alkaloids and amides, which have attracted attention for their diverse pharmacological properties (Biswas et al., 2022).

The pharmacological interest in Piper longum is particularly associated with its bioactive constituents, including piperine and piperlongumine (also known as piplartine). These compounds have been investigated for their potential effects on cancer cell viability, proliferation and apoptosis. However, their biological activities should

not be considered identical, as differences in chemical structure, concentration, experimental conditions and cellular models may influence their observed pharmacological effects.

Research on ethanolic extracts of Piper longum has demonstrated potential anticancer activity in experimental models. Ovadje et al. (2014) investigated the effects of an ethanolic fruit extract of Piper longum on cancer cell models and examined the biochemical mechanisms associated with cell death. Their findings gave experimental evidence supporting further investigation of long pepper extracts as potential sources of anticancer compounds. Nevertheless, findings from individual experimental models cannot establish the general effectiveness or safety of the plant or its constituents.

Piperlongumine has received particular attention because of its reported influence on oxidative stress and apoptosis. Experimental research has investigated its effects on reactive oxygen species (ROS), cellular antioxidant responses and signalling pathways associated with cell survival. Lee et al. (2015), for example, examined the differential response of human breast cancer MCF-7 cells and non-malignant MCF-10A breast epithelial cells following piperlongumine exposure. Their findings implicated heme oxygenase-1 (HO-1) in the differing cellular responses, highlighting the importance of cellular antioxidant mechanisms in understanding compound-associated cytotoxicity.

Piperine has also been investigated for its potential anticancer activity through mechanisms involving cell-cycle regulation, apoptosis and intracellular signalling. A study by Li et al. (2018) reported that piperine inhibited the viability of human ovarian cancer A2780 cells in a concentration- and time-dependent manner, while no comparable effect was observed in the normal ovarian cell model used in that investigation. The study also associated piperine-induced apoptosis with mitochondrial signalling and the JNK/p38 MAPK pathways. These findings provide an additional basis for examining the differential cellular effects of piperine, although they should be interpreted within the limitations of the specific experimental model.

An important consideration in evaluating these findings is the distinction between cytotoxicity against malignant cells and genuine selectivity towards cancer cells. Demonstrating reduced cancer cell viability does not confirm that a compound is less harmful to normal cells. So, the studies involving both malignant and non-malignant cellular models provide particularly relevant evidence for evaluating differential cytotoxic responses. The choice of normal-cell comparator, cell origin, proliferation characteristics, treatment concentration and duration of exposure may all influence the observed outcomes.

The proposed biological mechanisms underlying differential cytotoxicity also need careful consideration. Oxidative stress, depletion of intracellular glutathione, mitochondrial dysfunction, activation of apoptotic pathways and modulation of antioxidant defence systems have been investigated in relation to the anticancer activities of several phytochemicals, including piperlongumine. However, these mechanisms may vary according to the compound, cell type and experimental conditions. Consequently, mechanism findings from individual studies cannot automatically be generalised to all Piper longum preparations or cancer models.

A comparative evaluation may also help identify inconsistencies in experimental approaches and limitations in the interpretation of reported anticancer effects.

Therefore, the present study aims to critically examine the available preclinical evidence concerning the cytotoxic effects of Piper longum extracts, piperine and piperlongumine on malignant and non-malignant cells. It further seeks to evaluate the reported differences in cellular sensitivity, explore the biological mechanisms proposed to explain these responses, and identify methodological limitations and research gaps that should be addressed in future investigations. By distinguishing experimental observations from proposed therapeutic implications, this review is an effort to provide a balanced assessment of the current evidence and its relevance to future natural-product-based anticancer research.

MATERIALS AND METHODS

This study is designed as a review of preclinical experimental literature investigating the cytotoxic effects of *Piper longum* L. extracts and its principal bioactive constituents, particularly piperine and piperlongumine (piplartine), against malignant and non-malignant cellular models.

The review focused on identifying, evaluating and synthesising experimental evidence concerning cytotoxicity, differential cellular responses and the biological mechanisms potentially associated with these effects.

Because of anticipated differences in botanical preparations, phytochemical composition, cell lines, experimental conditions and cytotoxicity assessment methods, the findings were planned for narrative synthesis rather than quantitative meta-analysis.

Relevant literature was identified through searches of Google scholar, ResearchGate, Scopus, Web of Science and other appropriate scholarly databases. Additional relevant publications were identified by examining the reference lists of eligible articles and relevant review papers.

The search strategy combined botanical names, phytochemical names and terms relating to cytotoxicity and cancer research. The search strategy was adapted to the indexing requirements and search interfaces of

individual databases. All retrieved records were compiled, and duplicate publications were removed before screening.

Relevant information from the included studies was collected using a standardised data extraction form.

The following information was recorded:

1. Study identification: First author, publication year, journal and country of study.
2. Botanical and phytochemical information: Plant species, plant part used, extract type, extraction solvent and identified bioactive constituents.
3. Intervention characteristics: Compound or extract tested, concentration range, treatment duration and experimental conditions.
4. Cellular models: Malignant and non-malignant cell lines, tissue of origin and species.
5. Experimental methods: Cytotoxicity assay, cell viability assessment, apoptosis detection and other relevant analytical techniques.
6. Study outcomes: Reported IC_{50} values, percentage cell viability, proliferation inhibition, apoptosis, oxidative stress and other relevant biological findings.
7. Mechanistic observations: Reported effects on reactive oxygen species, glutathione levels, mitochondrial function, antioxidant enzymes and apoptosis-associated signalling pathways.
8. Study limitations: Experimental limitations, missing information and potential sources of methodological bias.

RESULTS

The identified experimental studies demonstrated the cytotoxic and pro-apoptotic effects of *Piper longum* fruit extract and its bioactive constituents, particularly piperlongumine and piperine, across different cancer models. Ovadje et al. (2014) reported concentration- and time-dependent reductions in cancer cell viability following treatment with ethanolic *Piper longum* fruit extract in colorectal, pancreatic, ovarian and melanoma cell lines. The effects involved apoptosis and mitochondrial dysfunction, while normal-derived colon cells were comparatively unaffected. The study also reported inhibition of colon tumour growth in animal models. Jin et al. (2014) demonstrated greater sensitivity of MCF-7 and BT-474 breast cancer cells than MCF-10A non-malignant breast epithelial cells to piperlongumine, reporting approximately 35% and 45% cell death, respectively, at 10 µM, with ROS-mediated CHOP activation implicated in apoptosis. Similarly, Lee et al. (2015) reported differential apoptotic responses between MCF-7 and MCF-10A cells, with apoptosis indices of 21.9% and 6.7%, respectively, following exposure to 5 µM piperlongumine for 36 hours, implicating HO-1 signalling in the differential response. Additionally, Si et al. (2018) found that piperine reduced A2780 ovarian cancer cell viability and induced mitochondrial apoptosis involving cytochrome c release, caspase activation and JNK/p38 MAPK signalling. Overall, the findings suggest promising cytotoxic and pro-apoptotic activities, with differential responses between malignant and non-malignant cellular models. However, the evidence varies according to the preparation, compound, cell type, concentration and exposure duration. Moreover, piperine findings represent evidence concerning a shared phytochemical constituent rather than direct evidence from a specifically tested *Piper longum* extract. Further preclinical and clinical investigations are required to establish therapeutic efficacy and safety.

DISCUSSION

The experimental evidence suggests that *Piper longum* fruit extracts and their bioactive constituents, particularly piperlongumine and piperine, possess cytotoxic and pro-apoptotic activities against selected cancer cell models. Ovadje et al. (2014) demonstrated that ethanolic *Piper longum* fruit extract reduced cancer cell viability while comparatively sparing normal colon epithelial cells under the tested conditions. The observed effects involved mitochondrial membrane depolarisation and the release of apoptosis-inducing factors, suggesting the involvement of mitochondrial cell-death pathways. The study also reported inhibition of tumour growth in animal models, providing additional preclinical evidence. However, the activity of the crude extract cannot be attributed to a single constituent because of its complex phytochemical composition. Regarding piperlongumine, Jin et al. (2014) associated its differential cytotoxic effects in breast cancer cells with ROS-mediated CHOP activation and the involvement of pro-apoptotic proteins, including Bim and DR5. In contrast, Lee et al. (2015) highlighted the role of Nrf2/HO-1 signalling in the differential responses of malignant and non-malignant breast epithelial cells, indicating that piperlongumine may influence multiple cellular pathways rather than acting exclusively through ROS generation. Similarly, Si et al. (2018) reported that piperine induced apoptosis in ovarian cancer A2780 cells through mitochondrial cytochrome c release, caspase activation and JNK/p38 MAPK signalling. These findings suggest that oxidative stress, mitochondrial dysfunction, apoptotic regulation and cellular stress-response pathways may contribute to the observed anticancer activities. Nevertheless, the findings require cautious interpretation because the studies differ in their experimental models, compounds, concentrations and exposure periods. Furthermore, piperine is present in both *Piper longum* and *Piper nigrum*,

and evidence from isolated piperine cannot be considered direct evidence of the activity of a complete *Piper longum* extract. Overall, the observed differential cytotoxicity is promising but remains context-dependent and does not establish clinical efficacy, safety or selective anticancer activity in humans. Further standardised phytochemical, mechanistic, animal and clinical investigations are necessary to clarify its therapeutic potential.

Findings

The experimental evidence suggests that *Piper longum* extracts and their bioactive constituents exhibit cytotoxic and pro-apoptotic activities against selected cancer cell models. The observed effects involve oxidative stress, mitochondrial dysfunction, apoptosis and Nrf2/HO-1 signalling pathways.

However, the responses vary according to the phytochemical preparation, cancer model, concentration and exposure duration.

Crude extracts and isolated compounds must therefore be evaluated separately, and further studies are required to establish their therapeutic potential and safety.

Implications for Future Research

Future research should prioritise botanical authentication, phytochemical characterisation and standardisation of *Piper longum* extracts to improve reproducibility. Comparative studies should include appropriate malignant and non-malignant cellular models, with systematic evaluation of dose-response relationships, exposure duration and selectivity indices. Further mechanistic investigations are needed to clarify the roles of oxidative stress, mitochondrial dysfunction and apoptotic signalling. Finally, pharmacological, toxicological and animal studies should establish safety, bioavailability and achievable therapeutic concentrations before clinical applications are considered.

CONCLUSION

The available preclinical evidence suggests that *Piper longum* extracts and selected bioactive constituents, particularly piperlongumine and piperine, possess cytotoxic activity against certain malignant cell models. Some experimental investigations have also reported comparatively lower effects on non-malignant cells under specific laboratory conditions, indicating the possibility of differential cellular responses.

The reported biological effects involve several mechanisms, including oxidative stress regulation, mitochondrial dysfunction and apoptosis-associated signalling pathways. However, these mechanisms appear to vary according to the compound investigated, the cellular model and the experimental conditions.

Despite these promising observations, the evidence remains predominantly experimental and is limited by variations in plant preparations, phytochemical composition, cell models and outcome measurements. The available findings are insufficient to establish consistent cancer-cell selectivity or to support claims of clinical safety and therapeutic effectiveness.

Further research involving standardised extracts, well-characterised bioactive compounds, appropriate normal-cell comparators and reproducible experimental designs is required. Additional pharmacological, toxicological and clinical investigations will also be necessary to determine the potential translational value of these compounds.

Overall, *Piper longum* and its bioactive constituents represent an area of scientific interest in natural-product-based cancer research. Their potential should be investigated through rigorous experimental studies rather than interpreted as established anticancer therapy.

LIMITATIONS

The conclusions of this review are dependent on the availability, methodological quality and completeness of the published experimental literature. The possibility of publication bias, selective reporting and differences in experimental methodology cannot be excluded.

These limitations highlight the need for more standardised and reproducible investigations before definitive conclusions can be drawn regarding the selective cytotoxic potential of *Piper longum* and its bioactive constituents.

Ethical Approval

Ethical approval was not required for this review because it is based on previously published research and does not involve new experiments with human participants or animals.

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