

An *In Silico* Analysis of Cytotoxic Effects of Peruvoside on A549 Cell Line

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Abstract:

Background: Lung cancer is a challenging disease. Treatment strategies are effective in enhancing patient survival. Nonetheless, they possess drawbacks. The low response rate, unexpected intrinsic and acquired resistance. Therefore, there is a need for the development of novel cancer therapies that minimize harm to normal cells. Peruvoside, a cardiac glycoside derived from *Thevetia Peruviana*, has been utilized in this experiment for its promising anticancer effect. This study aimed to evaluate the anticancer potential of Peruvoside using integrated *in silico* ADME-Tox prediction and *in vitro* validation in A549 lung cancer cells.

Method: *In vitro* analysis includes the cytotoxicity assay using WST-8 on cancer and normal cells, and the genotoxicity of lung cancer cells was measured by a comet assay. In addition, *in silico* analysis (SwissADME and Pro-Tox 3.0) was applied to estimate the pharmacokinetic and toxicity profile of Peruvoside.

Result: The cytotoxicity assay showed that there is a significant reduction in cell viability of A549 cells with a selective cytotoxicity toward cancer cells determined by the selectivity index (SI), which was $SI \geq 10$, suggesting its safety toward normal cells. The genotoxicity result showed that Peruvoside has damaged the DNA of A549 cells; *in silico* analysis confirms these results by categorizing Peruvoside in class 4 toxicity with an LD₅₀ of 2000 mg/kg and estimating lack of blood-brain barrier penetration and inhibiting the CYP450 enzyme.

Conclusion: It was concluded that Peruvoside exhibits a potent cytotoxic impact on lung cancer cells while remaining harmless to normal cells. which is confirmed by DNA damage of the comet assay and the safety margin of Peruvoside with the pharmacokinetic profile of *in silico* analysis.

Keywords: A549; cytotoxicity; genotoxicity; plant extract; Peruvoside

1. Introduction

Lung cancer is one of the most critical global health concerns (1). Right now, in terms of cancer-related mortality and morbidity, lung
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cancer ranks first among males, whereas it ranks second for females (2). The most ubiquitous type of lung cancer is non-small cell lung cancer (NSCLC) (2). In 2022 the



most prevalent cancers were breast, lung, and colon cancer and leukemia, which constituted over half of all newly diagnosed cancer cases; this highlights the necessity to find more therapeutic modalities for the management of cancer (3, 4).

Lung carcinoma is often characterized by activation of oncogenes along with inactivation of tumor suppressor genes (5). Notably, the epidermal growth factor (EGF) and transforming growth factor alpha (TGF- α) are essential for cancer progression; these molecules activate and transphosphorylate tyrosine kinase receptors and initiate two fundamental primary signaling pathways: rat sarcoma virus (Ras)/mitogen-activated protein kinase (MAPK) and protein kinase B (PI3K)/the mammalian target of rapamycin (mTOR). Tumor suppressor genes that regulate a cell's internal defense system include tumor protein (TP53), retinoblastoma (RB1), and cyclin-dependent kinase inhibitor 2A (CDKN2A) genes (6). The fundamental treatment of lung cancer is encompassed by surgery, radiation, chemotherapy, and targeted therapy (7, 8). Despite their effectiveness in attenuating lung cancer and enhancing patient survival, these strategies have limitations. Low response rate, unexpected innate and acquired resistance, and these medications are toxic to normal tissue (9). Furthermore, there are substantial challenges in terms of side effects of skin damage, fatigue, irritation of mucous membranes, hypertension, and gastrointestinal issues; therefore, there is an urgent need to detect novel therapeutic candidates that are more effective in treating cancer and less toxic to normal cells (10, 11, 12).

Nature is considered a vast reservoir for drug discovery (13). Since plants are a cornerstone of this diversity, one such plant of particular interest is cardiac glycoside (CG). CGs are a diversity of organic compounds generated from diverse plant and amphibian species

(14). CGs significantly block the $\text{Na}^+/\text{Ca}^{2+}$ ATPase pump in cardiac tissue and have promising therapeutic potential against cancer cells (14).

Among cardiac glycosides, Peruvoside derived from *Thevetia Peruviana*, which also has an affinity to cardiac tissue, and current evidence points to the importance of Peruvoside as a plant extract that may have potential anticancer effects (15). Previous studies show that Peruvoside downregulates the proliferation of various types of cancer at low doses (16, 17). Peruvoside suppresses pivotal oncogenic pathways, among them mitogen-activated protein kinase (MAPK), stress-activated protein kinase/c-Jun N-terminal kinase (SAPK/JNK), and wingless-related integration site/cytoplasmic protein (Wnt/ β -catenin) (16, 18). Peruvoside inhibits breast cancer cell survival by disruption of the cell cycle (17, 19). Also, recent studies indicated that Peruvoside has a notable safety profile toward normal cells, while it has a toxic effect toward leukemic and solid cancer cells (19).

In modern drug evaluation, the integration of *in silico* and *in vitro* assays gives indication to the drugs' therapeutic potential (20). *In vitro* assays are essential to quantify actual cellular impact. *In silico* ADME and toxicity profiling offer a predictive computational framework to decode the pharmacokinetic properties and theoretical safety margin. Together, these platforms allow a more precise assessment of Peruvoside safety and efficacy (21).

The aim of this study is to investigate Peruvoside's anti-cancer activity through integration of a dual-platform approach by combining the *in silico* ADME-Tox predictions with *in vitro* experimental validations to measure the cytotoxic effect of Peruvoside and its genotoxic impact on lung cancer A549 cells.



2. Material and Methods

2.1. study design

The study was conducted through a cross-platform approach for examining the effect of naturally occurring Peruvoside as a pharmaceutical substance. The lung cancer (A549) cell line was employed as an *in vitro* model and human blood peripheral mononuclear cells (PBMCs) as a model representing normal cells (10, 22). The *in vitro* experimental design encompassed examining the cytotoxic effect of Peruvoside on A549 cells and human blood peripheral mononuclear cells (PBMCs) using the WST-8 assay for 24, 48, and 72 hrs. after treatment with ten concentrations of Peruvoside (23). Followed by estimation of the DNA damage of A549 cells by single-cell gel electrophoresis (comet assay) (24). In silico ADME & toxicity prediction to confirm the pharmacokinetics and selective toxicity of Peruvoside by using SwissADME and ProTox 3.0 (25, 26).

2.2. Cell Culture

The human lung cancer cell line (A549) was obtained from the American Type Culture Collection (ATCC; USA); PBMCs were isolated in the Al-Mustansiriya Postgraduate Research Laboratory and serve as a model for

normal cells. Periodically, cells were cultured in complete DMEM media (Capricorn Scientific, GmbH) enriched with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin antibiotic solution. Cells were incubated at 37°C in 98% humidity with 5% CO₂ environmental conditions. Phosphate-buffered saline (PBS) was used to wash the cells (Capricorn Scientific, GmbH), and 0.05% trypsin (Capricorn Scientific, GmbH) was used for routine subculturing (27).

2.3. In vitro biological evaluation:

2.3.1 cytotoxicity assay (WST-8)

The cytotoxic effect of Peruvoside on both A549 cells and PBMCs was measured by WST-8 assay (28). Upon completion of trypsinization, both cell lines were seeded in a 96-well plate at a density of 5×10^3 cells/well. On the subsequent day, cells of both cell lines treated with ten concentrations of Peruvoside (9.7, 19.5, 39, 78.125, 156.25, 312.5, 625, 1250, 2500, and 5000 nM, respectively), with each concentration tested in triplicate and incubated for 24, 48, and 72 hrs, WST-8 (Elabscience, USA) was introduced and incubated for 4 hrs. The optical density (OD) was determined for each well via GloMax Microplate Reader (Promega, USA) (28). The formula used to determine cytotoxicity was illustrated below:

$$\text{Cell viability \%} = [\text{OD treated} / \text{OD control}] \times 100$$

$$\text{Cell death \%} = 100 - \text{Cell viability \%}$$

To determine the selective toxicity of Peruvoside against A549 cells and exhibit a non-toxic profile against healthy normal cells, the selectivity index (SI) was used, and

the $SI \geq 10$, indicating that Peruvoside has a favorable safety profile in our study model (PBMCs) (22). The formula used to determine SI is illustrated below:

$$SI = \frac{\text{IC}_{50} \text{ normal cells (PBMCs)}}{\text{IC}_{50} \text{ of cancer cell (A549)}}$$



2.3.2 Assessment of genotoxicity (alkaline version of the comet assay)

Single-cell gel electrophoresis (comet assay) was conducted to determine the effect of Peruvoside on the DNA of A549 cells using comet tail, tail length, and tail moment as parameters for measuring DNA damage between the treated and untreated control groups. Human blood peripheral mononuclear cells (PBMCs) were used in this test as part of the comet assay protocol (29). Concisely, after treating A549 cells with Peruvoside, low-melting comet agarose was introduced to the cell sample in a 1:10 ratio. Cellular protein was eliminated by suspending the slides in lysis solution, and then alkaline electrophoresis was performed. Following that, the slides were neutralized and stained with RedSafe staining, and finally, cells were observed and examined using fluorescence microscopy with a FITC green (24, 30).

2.4. In silico ADME and toxicity prediction of Peruvoside

The pharmacokinetic properties and toxicity profile of Peruvoside were estimated theoretically by using two integrated platforms as a comprehensive computational analysis.

2.4.1. ADME and drug-likeness prediction

Using SwissADME web servers (Swiss Institute of Bioinformatics) has been used to predict the physicochemical properties and pharmacokinetic parameters. The SMILES string of Peruvoside was used as the primary input. The analysis focused on evaluating Lipinski's rule of five for drug-likeness. Gastrointestinal absorption (GI), blood brain barrier (BBB) permeability, and interaction with P450 (CYP) enzymes to assess potential metabolic pathways(25) .

2.4.2. Predictive toxicology (Pro-Tox 3.0)

The Pro-Tox3.0 web server was used to assess the systemic toxicity and specific toxicological endpoints. The Pro-Tox3.0 web server utilized machine learning models and similarity-based fragments to predict toxicity. The analysis included the determination of the predicted median lethal dose (LD₅₀) and assignment of Peruvoside-specific toxicity class (I-IV). Furthermore, the server was used to predict specific toxicity targets, including organ toxicity (hepatotoxicity) and toxicogenomic endpoints (mutagenicity, carcinogenicity, and cytotoxicity). These computational predictions were utilized to build a theoretical framework that complements observed *in vitro* results from the WST-8 and comet assays (26).

2.5. Statistical analysis

All statistical methodology data were performed using GraphPad Prism 10 software to calculate IC₅₀ and SPSS software to draw bar graphs and calculate P-values. Experimental data was represented by the mean of biological and technical replicates. The difference between cells and PBMCs was performed by one-way ANOVA. A difference with a P-value less than 0.05 is considered statistically significant. For *in silico* ADME and toxicity predictions, data were generated as absolute predictive values and scores from the respective web servers.

2. Result

3.1. Cytotoxicity of Peruvoside on A549 and PBMCs

To examine the cytotoxic effect of Peruvoside on lung cancer cells and human peripheral blood mononuclear cells (PBMCs), the WST-8 assay was used to determine the concentration that will cause 50% inhibition of cell viability of both A549 cells and PBMCs at 3 time points: 24, 48, and 72 hrs. As illustrated in Fig. 1 and Fig. 2,



Peruvoside inhibits cell proliferation in a concentration- and time-dependent manner. The IC_{50} was 92.14 nM and 68.77 nM at 24 and 48 hours, respectively, and at 72 hours,

the percentage of cell death started at 70%; therefore, the IC_{50} is less than 9.7 nM, as shown in Table 1. The IC_{50} of 48 hours was used in a subsequent experiment.

Table 1: The average IC_{50} values, cell viability, 95% confidence interval, and R^2 of A549 cells after treatment with Peuvoside.

Time (h.)	IC_{50} of A549	Cell viability	R^2	95% CI (nM)
24hrs.	92.14 nM	50%	0.9591	27.85-308.3
48hrs.	68.77 nM	50%	0.9742	12.74-371.3
72hrs.	0.066 nM	50%	0.9332	0.00732-0.2745

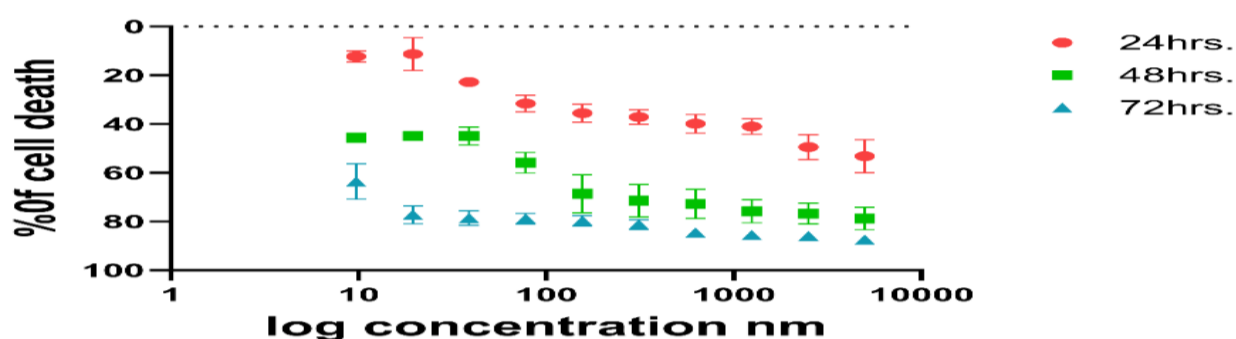


Figure1: Percentage of cytotoxicity of A549 after treatment with Peruvoside at 24, 48, and 72 hrs. The experiment was performed at 3 time points (24, 48, and 72 hrs.) The results represent the mean cell death $\pm$ SEM of 3 independent experiments using GraphPad Prism 10 software.

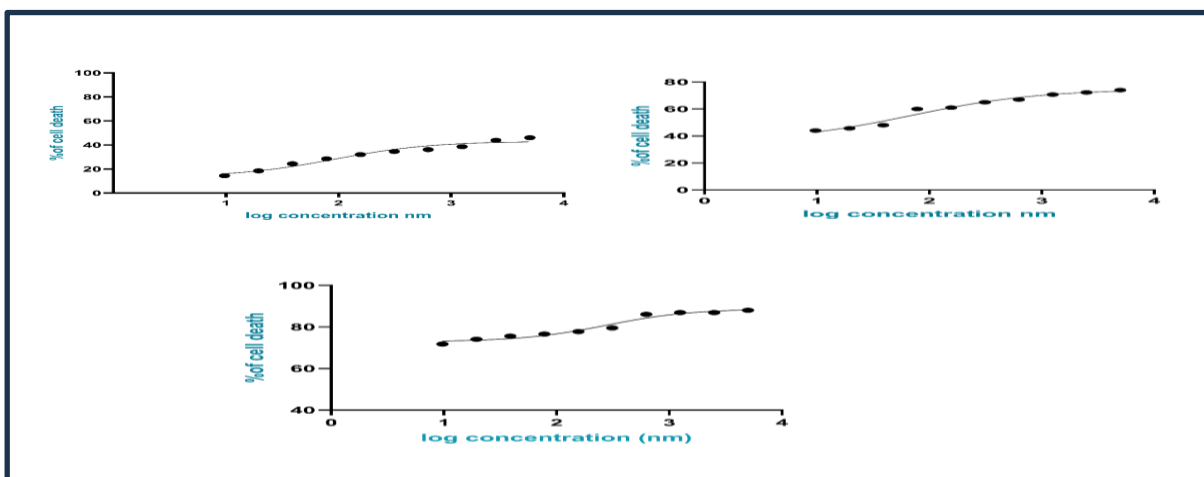


Figure 2: Dose-response curve of A549 cells treated with Peruvoside at 24, 48, and 72 hours. Cells were treated with 10 different concentrations of Peruvoside ranging from 9.7, 19.5, 39, 78.125, 156.25, 312.5, 625, 1250, 2500, to 5000 nM. A. dose-response curve at 24 hrs. B. dose-response curve at 48 hrs. C. The dose-response curve at 72 hrs. IC_{50} was determined using nonlinear regression analysis by GraphPad Prism 10.

The PBMC cell line, a human peripheral blood mononuclear cell that is derived from human blood, was used in this experiment as a model for normal cells and to assess the potential cytotoxic effect of Peruvoside against healthy normal cells. The results showed that cell viability of A549 cells was markedly reduced, while cell viability of PBMCs was minimally inhibited. As shown in Fig. 3, there is a very high significant difference ($P < 0.001$) between A549 cells and PBMCs in cell viability.

In order to determine if Perovoside has selective toxicity toward cancer cells, the

selectivity index (SI) parameter was used. If $SI \geq 10$ (22), this indicates that Peruvoside has a negligible toxic effect on normal cells. As shown in Figure 3A, Peruvoside has minimal effect on the percentage of cell viability of PBMCs; therefore, it is difficult to calculate the IC_{50} of PBMCs. Therefore, this study used the highest tested concentration as IC_{50} for PBMCs.

At 24 hrs the selectivity index was 52.2, and at 48 hrs it was 72.7, because the IC_{50} of Peruvoside at 72 hrs could not be precisely calculated; thus, SI was expected to be >100 .

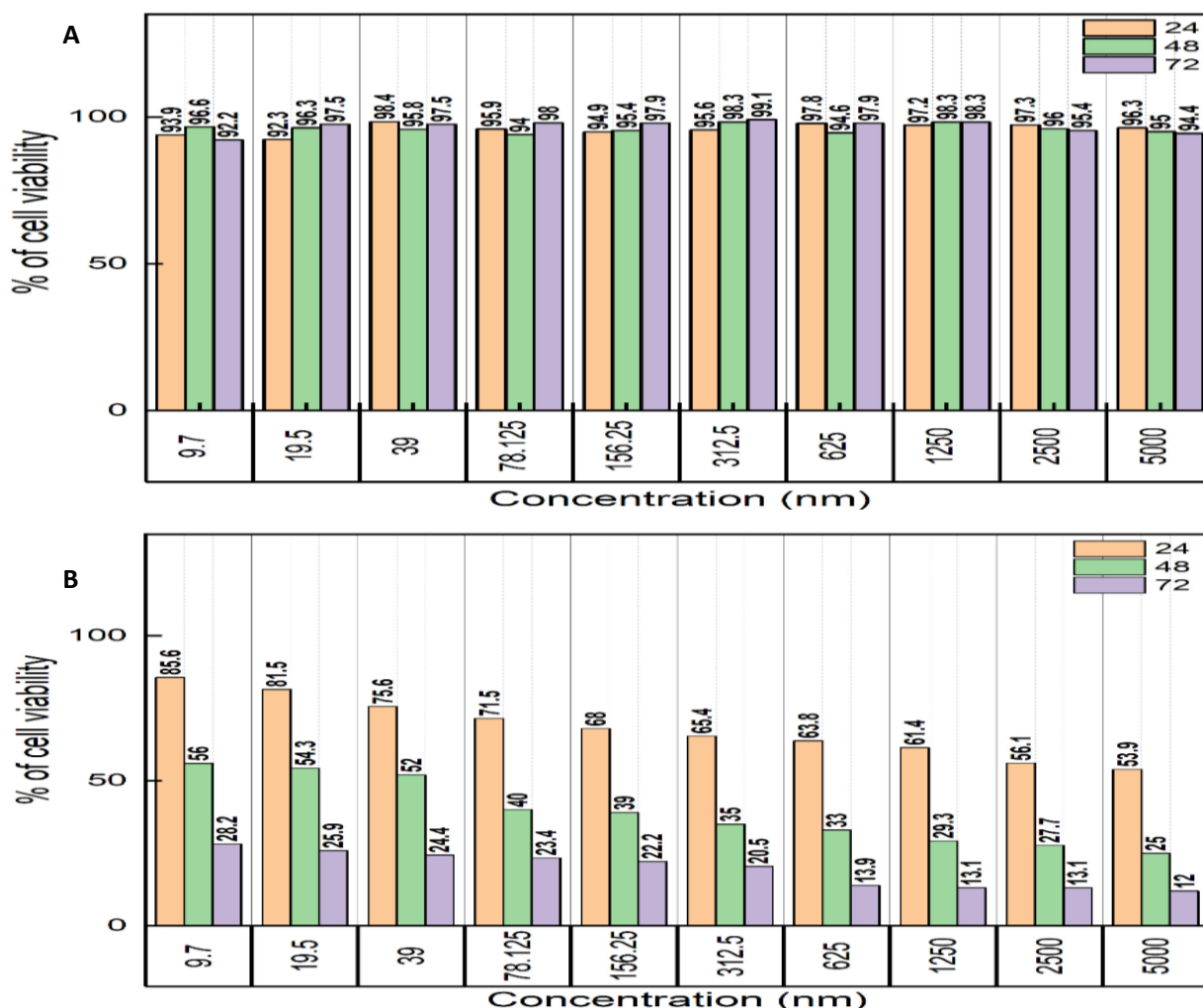


Figure 3: Percentage of cell viability after treatment with Peruvoside. A bar plot represents the percentage of cell viability of PBMCs. B. The bar plot represents the percentage of cell viability of A549 cells. Results represent mean $\pm$ SEM. The experiment was performed in 3 independent experiments; SPSS software was used to draw the bars and calculate the P-value using one-way ANOVA.



3.2. Effect of Peruvoside on Genotoxicity of A549 Cells

DNA damage was one of the strategies that was used to confirm that Peruvoside has a toxic effect on A549 cells. The alkaline version of the comet assay was used to determine the effect of Peruvoside on DNA. In the current study, comet tail, tail length, and tail moment are used as parameters to determine the percentage of DNA damage. Furthermore, in the comet assay, peripheral blood mononuclear cells (PBMCs) were used as a component of the standard protocol.

After treating the cells with 68.77 nm of Peruvoside for 48 hrs, the treated A549 exhibits morphological changes compared

with untreated A549 cells. As illustrated in Fig. 4.

Cells show a significant difference between treated and non-treated groups of A549 cells. As shown in Figure 5, PBMCs values for the comet parameter were comet length ($71.4 \pm 26.7 \mu\text{m}$), tail length ($6 \pm 11.9 \mu\text{m}$), and tail moment (0.0646 ± 0.114). While untreated A549 values were comet length ($76.3 \pm 20.5 \mu\text{m}$), tail length ($9.38 \pm 11 \mu\text{m}$), and tail moment (0.109 ± 0.20). The values for A549 cells after treatment with Peruvoside comet length was ($102.37 \pm 66.8 \mu\text{m}$), tail length was ($21.64 \pm 18.8 \mu\text{m}$), and the value for tail moment was (0.439 ± 0.797)

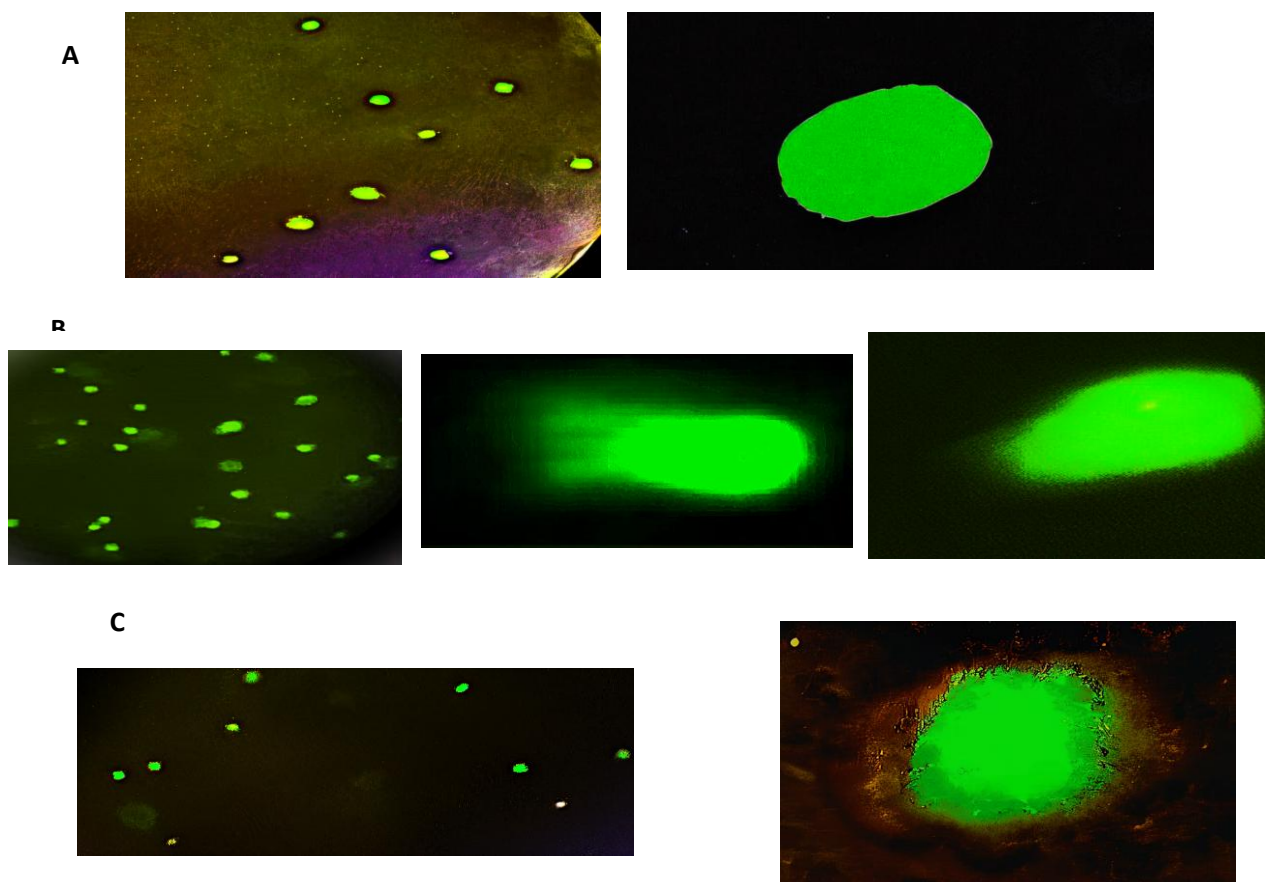


Figure 4: Morphological changes of A549 cells. Peruvoside induced DNA damage on A549 cells as compared with untreated group A. represents A549 cells without Peruvoside. B. Represent A549 cells with Peruvoside. C. PBMCs as a standard control group. Cells were observed by fluorescence microscope (VANGUARD).

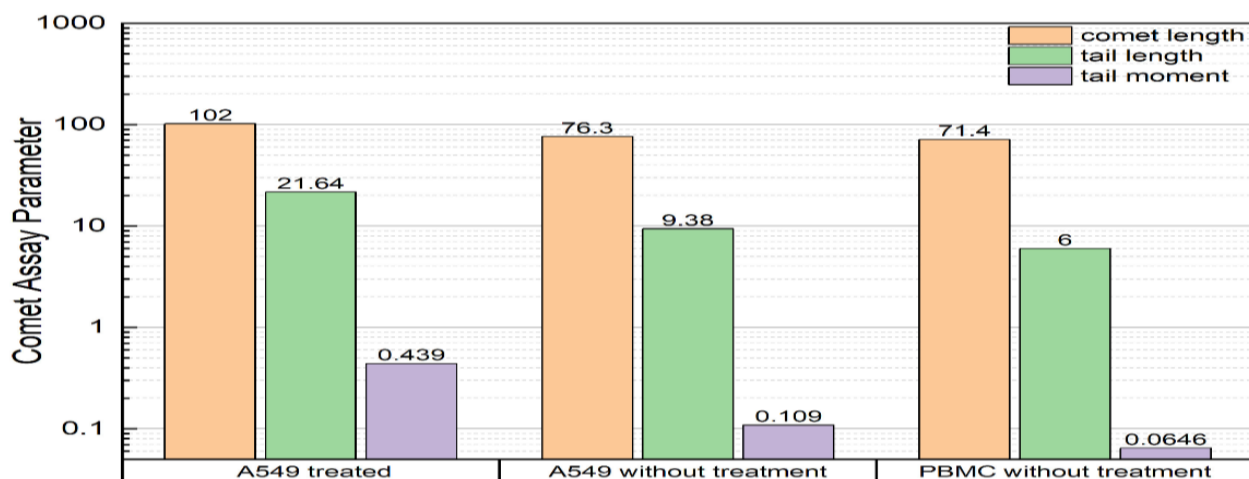


Figure 5: Values of comet assay parameters. After treatment with Peruvoside, the comet assay parameter was given higher values when compared with the untreated group, indicating the occurrence of DNA damage. Results are represented as mean $\pm$ SEM. The cells that were used for calculation were 20 cells for each group. The comet assay parameter was estimated by CometScore software. SPSS software was used to draw the bar plot.

3.3. *In silico* ADME and drug-likeness (SwissADME)

To further investigate the pharmacokinetics and physicochemical properties of

Peruvoside, an *in silico* SwissADME study was conducted. As summarized in Table 2.

Table 2: Computational ADME of Peruvoside using SwissADME

Category	Parameter	Result
Physicochemical	Molecular weight	548.66 g/mol
	TPSA	Å ² 131.75
	Logp (lipophilicity)	2.29
	Lipinski Rule	Yes (1 violation: MW > 500)
Pharmacokintic	GI absorption	High
	Blood-Brain-Barrier	NO(not-pentrate)
	Cyp 450	Not inhibited

The pharmacokinetic analysis shows that Peruvoside exhibits a high gastrointestinal absorption and low probability of penetrating the blood-brain barrier, as visualized in the boiled-egg model (Fig. 6). Furthermore,

Peruvoside acts as a non-inhibitor for the majority of cytochrome P450 isoenzymes (CYP1A2, CYP2C19, CYP2C9, CYP2D6, CYP3A4).

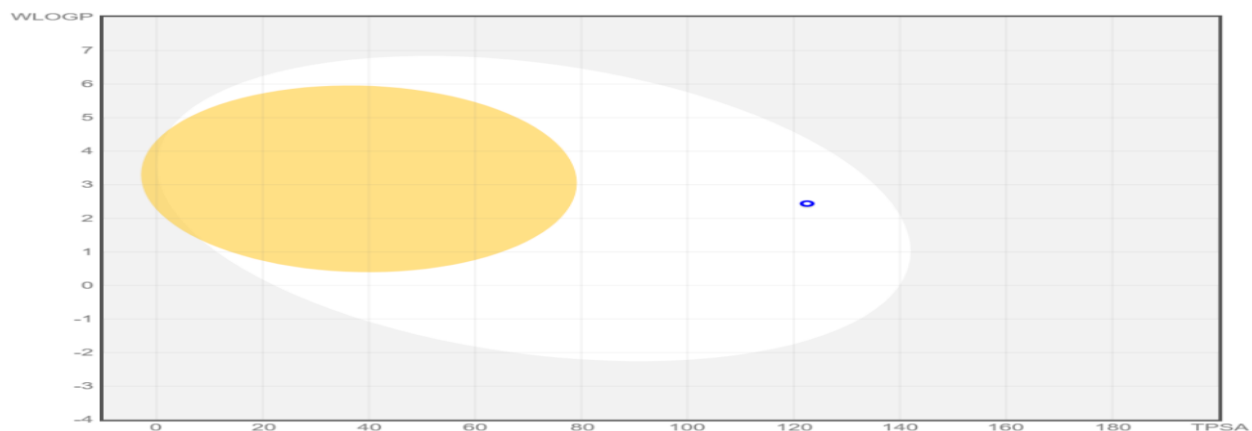


Figure 6: The boiled egg model. Illustrating the pharmacokinetic profile of Peruvoside. The blue dot is situated within the white region (yolk) suggesting a high probability of gastrointestinal (GI) absorption, while its departure from the yellow region of the egg (white) suggests that the compound is not predicted to cross the blood-brain barrier (BBB). WLOGD: Wildman-Crippen LogP, TPSA: Topological Polar Surface Area. The model generated by SwissADME.

The physicochemical properties of Peruvoside evaluation showed that the compound follows Lipinski's rule of five with one violation, which suggests high oral bioavailability. As illustrated in Fig. 7, the bioavailability radar exhibits lipophilicity,

size, and flexibility parameters situated within the optimal range, while slight deviations were observed in polarity and insolubility. The synthetic accessibility score for the compound was 6.8.

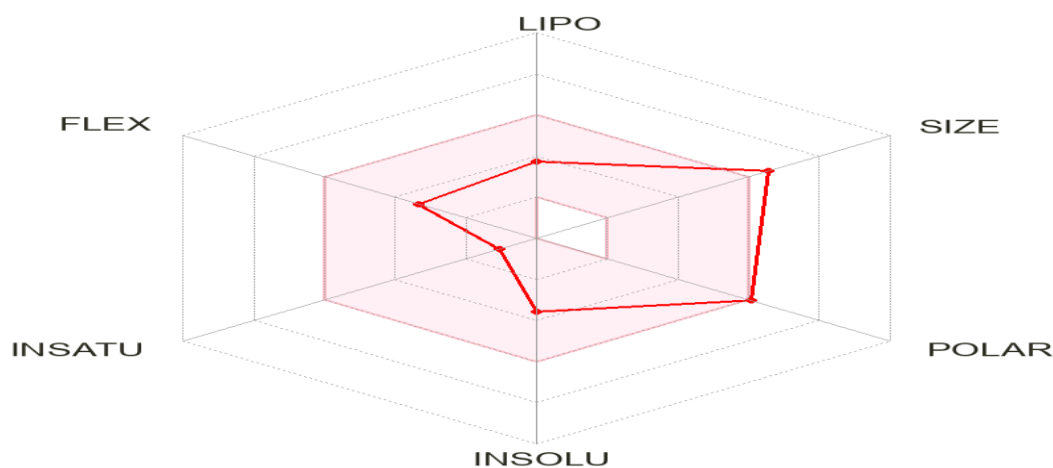


Figure 7: Bioavailability Radar of Peruvoside. showing the physicochemical profile for cellular uptake. The balanced lipophilicity and polarity, as shown within the pink zone, enable Peruvoside's penetration through the A549 lung cancer cell membranes, directly reinforcing its powerful cytotoxic activity and disruption of intracellular signaling pathways. Bioavailability radar generated by SwissADME.

3.4. Predictive toxicology profiling (Pro-Tox 3.0)

Concerning the toxicity profile of Peruvoside, the Pro-Tox 3.0 platform was predicted to be classified under toxicity class 4 with a predicted median lethal dose (LD₅₀) of 2000 mg/m. Fig. 8 illustrates the toxicity

radar chart of Peruvoside, which was predicted to have a low probability of hepatotoxicity (95% inactive). In addition, Peruvoside has a probability of 62% of inactive cytotoxicity, and it is also inactive (67%) carcinogenicity.

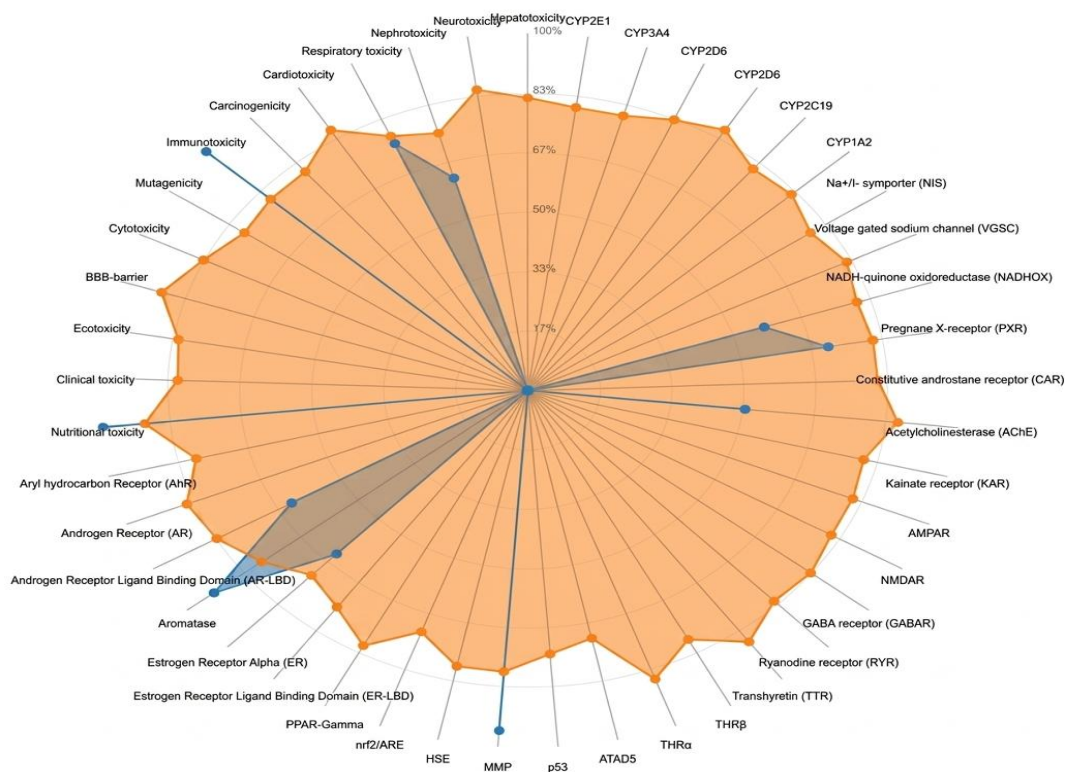


Figure 8: Toxicity radar chart of Peruvoside. The radar illustrates the predicted probability of several toxicological endpoints, involving organ toxicity, stress response pathways, and molecular targets. The orange areas depict the total predicted space, while the blue dots and gray shaded areas suggest the specific probability scores for Peruvoside across different toxicity endpoints. The points situated toward the periphery denote a high probability of Peruvoside being inactive, conversely; points moving toward the center indicate potential toxic activity. Toxicity radar generated by Pro-Tox 3.0.

4. Discussion

Lung cancer is one of the most formidable diseases around the world. Genetic damage from exposure to toxic substances, tobacco consumption, and genetic etiology—these factors lead to the proliferation of genetically modified cells and the development of lung

cancer (2, 31). Despite the presence of a number of treatments for lung cancer, they often cause injuries, and treatment resistance is still a problem (11). Therefore, there is an imperative need to find new drug therapies with a delicate balance between high therapeutic efficacy and a safe toxicological



profile, either by drug repurposing or natural drugs or chemically modified drugs (32). Therefore, Peruvoside, a plant extract, was investigated as a promising candidate for lung cancer therapy.

The current study used an integrative approach, combining *in vitro* assays with *in silico* modeling via SwissADME and Pro-Tox 3.0. This synergistic methodology was implemented to reconcile biological activity and ADME properties. Which established a solid foundation to assess the safety profile and pharmacological viability of Peruvoside.

4.1. Cytotoxicity assay of peruvoside against A549 and PBMCs

To perform its cytotoxicity, the cytotoxic assay (WST-8) was used, and results show that there is a significant inhibition of cancer cell proliferation at 3 time points and at a low nanomolar concentration. This potent effect of Peruvoside is aligned with an established mechanism of CGs, which is a Na^+/K^+ ATPase pump inhibitor that induces downstream signaling pathways resulting in cancer cell death (33). Moreover, according to the result of the WST-8 assay on peripheral blood mononuclear cells (PBMCs), Peruvoside does not affect cell viability, and the difference between these two cell lines was highly significant. It is essential to emphasize that the selectivity index (SI), which is a parameter used to determine the selective toxicity toward cancer cells, revealed that $\text{SI} \geq 10$ for 24, 48, and 72 hrs, which gave a favorable safety profile toward normal cells. A recent study documented that Peruvoside inhibits proliferation of lung cancer and suppresses cancer cell survival by affecting the PI3K/AKT/mTOR pathway (34). Another study also documented that Peruvoside has an inhibitory effect across many cancer types by modulating specific proteins involved in cell cycle progression (18). A previous study on primitive myeloid leukemia cells (KG1a, K562) found that,

Peruvoside suppressed cell viability in KG1a and K562 cells with IC_{50} of 31 and 60 nM, respectively, at 48 hrs and did not appear to have a cytotoxic effect on normal human peripheral blood mononuclear cells (PBMCs) at an effective dose (19). Moreover, evidence suggested that CGs were potent at low concentrations as an anticancer effect, suggesting a dose-dependent toxicity profile (35).

4.2. Genotoxic effect of Peruvoside against A549

To evaluate whether Peruvoside has an anticancer effect, a genotoxicity assay (comet assay) in an alkaline version was performed. The results showed that in A549 cells after treatment with Peruvoside, the comet assay parameter was significantly increased compared with untreated A549 cells. PBMC cells were used in this assay as a standard group within the protocol of the comet assay. As a standard group, PBMC cells were used within the protocol of the comet assay to compare with untreated A549 cells and to confirm that the comet assay reagent does not affect the genomic stability of untreated A549 cells and any change in the genomic stability of treated A549 cells is revealed to Peruvoside, consequently indicating that Peruvoside has a notable effect as a genotoxic agent. This result aligns with a study on various malignancies, which determined that Peruvoside has an anti-tumor mechanism by DNA damage and cell cycle arrest (16). A further study on cardiac glycoside demonstrated that CGs induce downregulation of PI3K/AKT/mTOR; thus, it causes double- and single-strand breaks (36).

4.3. Pharmacokinetic and physicochemical properties of Peruvoside

To elucidate the molecular foundations of these findings, SwissADME was implemented to predict the ADME profile of



Peruvoside (25). It exhibits a favorable pharmacokinetic profile, in compliance with the drug-likeness rule, including Lipinski's Rule of Five. A notable outcome in the safety profile is the inability of Peruvoside to penetrate the blood-brain barrier (BBB), which markedly decreases the likelihood of CNS adverse effects. Consistency with a previous study mentions that clinical application of cardiac glycoside to neurodegenerative disease was limited due to poor penetration to the BBB (37). Moreover, the data suggested that Peruvoside does not inhibit key cytochrome P450 enzymes. Indicating that Peruvoside has low potential to cause drug interaction. These criteria, along with advantageous gastrointestinal absorption, strengthen the position of Peruvoside as a safe and feasible therapeutic candidate. A study on the metabolism of CGs stated that CGs do not influence the activity of P450 isoenzymes; in addition, they exhibit notable intestinal absorption (38, 39).

4.4. *In silico* toxicity assessment

The safety margin of Peruvoside was substantiated through the Pro-Tox 3.0 web server (26). The toxicity radar offers insights into Peruvoside's safety profile, given that the majority of toxicity parameters (hepatotoxicity, carcinogenicity, and cytotoxicity) are situated within inactive zones. Of particular significance, the estimated LD₅₀ value was 2000 mg/kg, which positions it within toxicity class 4 (26). These categorizations and the high degree of probability for being inactive at the periphery of the toxicity radar suggest that Peruvoside exhibits a lower toxicity profile; these findings reinforce the *in vitro* cytotoxicity results of Peruvoside toward PBMCs. Recent research incorporating cardiac glycosides shifted the focus toward inhibiting cancer cell growth without compromising normal cells through modulating key signaling pathways (40).

Integration of experimental observations and *in silico* simulations supports the potential anticancer effect of Peruvoside. The cytotoxicity results (WST-8) and genotoxicity profile (comet assay) are bolstered through the *in silico* safety profile. In particular, the negligible toxicity of Peruvoside on PBMCs with the high selectivity index $SI \geq 10$ aligns with the estimated toxicity class 4 alongside the radar-inactive zone. In addition, a complete lack of penetration to the BBB and inhibition of the CYP 450 isoenzyme elucidate the molecular basis for its selective action. Collectively, these findings validate that Peruvoside serves as a therapeutic candidate as an antineoplastic agent via inhibiting the proliferation and preventing cancer cell survival. In contrast, it exhibits an excellent safety profile toward normal cells.

This experimental study has several limitations. First, the experiments were conducted exclusively *in vitro* and *in silico* by using the A549 cell line and PBMCs, which may not reflect the complex biological environment of cancer *in vivo*. Second, the experiment investigated the cytotoxic and genotoxic effect of Peruvoside restricted to a limited *in vitro* environment. Moreover, studies utilizing cell lines other than lung cancer cell lines and *in vivo* studies are also required to confirm these results and should understand the underlying mechanisms.

5. Conclusion

In summary, the presented study documented that Peruvoside a natural compound, has a promising anticancer effect while sparing normal cells. The integration of *in vitro* (WST-8 and COMET assays) and *in silico* studies (SwissADME and Pro-TOX3.0 software) confirmed that Peruvoside exhibits a remarkable cytotoxic and genotoxic effect against the lung cancer (A549) cell line with a significant safety profile toward normal



cells as presented by the selectivity index along with a radar inactive zone, resulting in an inhibition of cancer cell proliferation and survival.

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References

1. Marghescu A-S, Leonte DG, Radu AD, Măgheran ED, Tudor AV, Teleagă C, et al. Atypical Histopathological Aspects of Common Types of Lung Cancer—Our Experience and Literature Review. *Medicina*. 2024;60(1):112.
2. Lee PC, Lin MW, Liao HC, Lin CY, Liao PH. Applying machine learning to construct an association model for lung cancer and environmental hormone high-risk factors and nursing assessment reconstruction. *Journal of Nursing Scholarship*. 2025.
3. Bray F, Laversanne M, Sung H, Ferlay J, Siegel RL, Soerjomataram I, Jemal A. Global cancer statistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA: a cancer journal for clinicians*. 2024;74(3):229-63.
4. cancer registry report, Iraqi cancer board, 2024_03_31: p: 3,5,15,27-28.
5. Alam M, Hasan GM, Eldin SM, Adnan M, Riaz MB, Islam A, et al. Investigating regulated signaling pathways in therapeutic targeting of non-small cell lung carcinoma. *Biomedicine & Pharmacotherapy*. 2023;161:114452.
6. Lee JY, Bhandare RR, Boddu SH, Shaik AB, Saktivel LP, Gupta G, et al. Molecular mechanisms underlying the regulation of tumour suppressor genes in lung cancer. *Biomedicine & Pharmacotherapy*. 2024;173:116275.
7. El-Hussein A, Manoto SL, Ombinda-Lemboumba S, Alrowaili ZA, Mthunzi-Kufa P. A review of chemotherapy and photodynamic therapy for lung cancer treatment. *Anti-Cancer Agents in Medicinal Chemistry (Formerly Current Medicinal Chemistry-Anti-Cancer Agents)*. 2021;21(2):149-61.
8. Tsimberidou A-M. Targeted therapy in cancer. *Cancer Chemotherapy and Pharmacology*. 2015;76(6):1113-32.
9. Wannas AN, Mahdi MF, Maraie NK. Advances in Tracking Anticancer Drugs to Their Targeted Site: A Review. *Al Mustansiriyah Journal of Pharmaceutical Sciences*. 2025;25(5):931-45.
10. TORRES-MARTINEZ, SUSANA MORENO MANUEL, ANDREA. EXPERIMENTAL PROTOCOL: Isolation of PBMCs by Ficoll-Paque density gradient centrifugation. experimental protocol. Madrid: CENTRO DE INVESTIGACION BIOMEDICA EN Red Cancer(ciberonc); 2021.
11. Liu R. The limitations and prospects of targeted therapy for treating cancer. *Theoretical and Natural Science*. 2024;70:160-6.
12. Du R, Wang X, Ma L, Larcher LM, Tang H, Zhou H, et al. Adverse reactions of targeted therapy in cancer patients: a retrospective study of hospital medical data in China. *BMC Cancer*. 2021;21(1):206.
13. Moatasem M, Dhifaf J, Emaduldeen H, Mohammed M, Ibrahim S, Dhuha J, et al. The Synergistic Anticancer Effect of Some Plant Extracts in Combination against Human Liver Cancer Cell Line.



- Asian Journal of Dairy & Food Research. 2023;42(4).
14. Kumavath R, Paul S, Pavithran H, Paul MK, Ghosh P, Barh D, Azevedo V. Emergence of cardiac glycosides as potential drugs: Current and future scope for cancer therapeutics. *Biomolecules*. 2021;11(9):1275.
15. Gaur R, Rathore A, Sharma AK, Pathak D, Kulshreshtha M. Scientific Update on the Pharmacognostic, Pharmacological and Phytochemical Properties of *Thevetia peruviana*. *Pharmacognosy Communications*. 2023;13(1).
16. Reddy D, Kumavath R, Tan TZ, Ampasala DR, Kumar AP. Peruvoside targets apoptosis and autophagy through MAPK Wnt/ β -catenin and PI3K/AKT/mTOR signaling pathways in human cancers. *Life sciences*. 2020;241:117147.
17. Liu M, Huang Q, Li L, Li X, Zhang Z, Dong J-T. The cardiac glycoside deslanoside exerts anticancer activity in prostate cancer cells by modulating multiple signaling pathways. *Cancers*. 2021;13(22):5809.
18. Lai Y, Chang H, Chen H, Chang G, Chen JJ. Peruvoside is a novel Src inhibitor that suppresses NSCLC cell growth and motility by downregulating multiple Src-EGFR-related pathways. *Am J Cancer Res*. 2022;12(6):2576-93.
19. Feng Q, Leong WS, Liu L, Chan W-I. Peruvoside, a cardiac glycoside, induces primitive myeloid leukemia cell death. *Molecules*. 2016;21(4):534.
20. Zhang Z, Deng Y, Wang J, Dou P, Fan H, Zeng X, et al. Screening and identification of novel anti-inflammatory peptides from sea cucumber gonads: In silico and in vitro analysis. *Food Chemistry: X*. 2026:103510.
21. Pavithran H, Ghosh P, Kumavath R. Network and modeling analysis of MAPK signaling cascade uncovers EGR1 regulation through ERK2 protein in breast cancer. *Computers in biology and medicine*. 2025;196:110830.
22. Acacio BR, Prada AL, Neto SF, Gomes GB, Perdomo RT, Nazario CED, et al. Cytotoxicity, anti-inflammatory effect, and acute oral toxicity of a novel *Attalea phalerata* kernel oil-loaded nanocapsules. *Biomedicine & Pharmacotherapy*. 2024;174:116308.
23. AL-Asady SH, Al-Sudani BT, Mshimesh BA-R. Knockdown of α -enolase (ENO1) to suppress glycolytic pathway in human hepatocellular carcinoma cell line (HepG2). *Iraqi Journal of Pharmaceutical Sciences*. 2024;33(2):156-60.
24. Afanasieva K, Sivolob A. Physical principles and new applications of comet assay. *Biophysical chemistry*. 2018;238:1-7.
25. Daina A, Michielin O, Zoete V. SwissADME: a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. *Scientific reports*. 2017;7(1):42717.
26. Banerjee P, Kemmler E, Dunkel M, Preissner R. ProTox 3.0: a webserver for the prediction of toxicity of chemicals. *Nucleic acids research*. 2024;52(W1):W513-W20.
27. Omietimi HB, Afolalu SA, Kayode JF, Monye SI, Lawal SL, Emeteri ME, editors. An overview of nanotechnology and its application. *E3S web of conferences*; 2023: EDP Sciences.
28. Chamchoy K, Pakotiprapha D, Pumirat P, Leartsakulpanich U, Boonyuen U. Application of WST-8 based colorimetric NAD (P) H detection for quantitative dehydrogenase assays. *BMC biochemistry*. 2019;20:1-14.
29. Saenz-Martinez E, López de Cerain A, Azqueta A. The use of DNA repair



- inhibitors and the comet assay—an overview. *Mutagenesis*. 2025;40(5-6):577-91.
30. De Boeck M, Touil N, De Visscher G, Vande PA, Kirsch-Volders M. Validation and implementation of an internal standard in comet assay analysis. *Mutation Research/Genetic Toxicology and Environmental Mutagenesis*. 2000;469(2):181-97.
 31. Guan C, Chen H, Chen H, Li S, Chen Y, Chen J, et al. Treatment of lung cancer by acupuncture combined with medicine based on pathophysiological mechanism: A review. *Medicine*. 2024;103(6):e37229.
 32. Wu K, House L, Liu W, Cho WC. Personalized targeted therapy for lung cancer. *International Journal of Molecular Sciences*. 2012;13(9):11471-96.
 33. Fujii T, Shimizu T, Sakai H. Pathophysiological Roles of Two Intracellular P-Type ATPases: The Cancer-Associated Na⁺, K⁺-ATPase α 3 Isoform and the Parkinson's Disease-Related ATP13A2. *International Journal of Molecular Sciences*. 2026;27(4):1800.
 34. Khan AN, Jawarkar RD, Zaki ME, Al Mutairi AA, Mali SN. Targeting PI3K/AKT/mTOR signalling pathway in non-small-cell lung carcinoma: Exploring promising bioactive natural compounds as anti-cancer agents. *Chemical Physics Impact*. 2025;10:100793.
 35. Calderón-Montaña JM, Burgos-Morón E, Orta ML, Maldonado-Navas D, García-Domínguez I, López-Lázaro M. Evaluating the cancer therapeutic potential of cardiac glycosides. *Biomed Res Int*. 2014;2014:794930.
 36. Ainembabazi D, Zhang Y, Turchi JJ. The mechanistic role of cardiac glycosides in DNA damage response and repair signaling. *Cellular and Molecular Life Sciences*. 2023;80(9):250.
 37. Eid S, Zerbès T, Williams D, Wang X, Sackmann C, Meier S, et al. Identification of a cardiac glycoside exhibiting favorable brain bioavailability and potency for reducing levels of the cellular prion protein. *International Journal of Molecular Sciences*. 2022;23(23):14823.
 38. Diabetes Nlo, Digestive, Diseases K. LiverTox: clinical and research information on drug-induced liver injury: National Institute of Diabetes and Digestive and Kidney Diseases; 2012.
 39. Haass A, Lüllmann H, Peters T. Absorption rates of some cardiac glycosides and portal blood flow. *European Journal of Pharmacology*. 1972;19(3):366-70.
 40. Newman RA, Yang P, Pawlus AD, Block KI. Cardiac glycosides as novel cancer therapeutic agents. *Mol Interv*. 2008;8(1):36-49.

