Pyrethrins and Breast Cancer Risk Assessment: An Analysis Combining Molecular Simulation and Total Risk Model

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Abstract: Pyrethrins are widely used in agriculture and public health, and while their acute toxicity is relatively low, increasing concerns have been raised about the potential carcinogenic risks of chronic exposure in recent years. This study integrates methods such as network toxicology, deep learning, Mendelian randomization (MR), molecular docking, and molecular dynamics simulations to systematically evaluate the potential association between pyrethrins and breast cancer. The results show that pyrethrins I and II bind stably with high affinity to several key breast cancer targets and can promote multiple cancer-related signaling pathways. By combining this with the total risk assessment model (TR), the study indicates that even under low-dose exposure scenarios, pyrethrins still pose significant risk potential. The study suggests that the environmental concentration and exposure thresholds should be reassessed in policy-making and calls for long-term epidemiological tracking.

Keywords: Pyrethrins, Breast Cancer, Multi-target Toxicology, Molecular Simulation, Risk Assessment, Environmental Exposure

1. Introduction

Pyrethrins, natural-origin broad-spectrum insecticides, have long been considered relatively safe due to their rapid environmental degradation and low acute toxicity in mammals [1]. However, emerging evidence has begun to challenge this perception by revealing their endocrine-disrupting properties [2,3], particularly their interference with estrogen receptor signaling and mammary gland morphogenesis [4], which may promote breast cancer progression through non-genotoxic mechanisms. Notably, previous toxicological assessments of carcinogenicity have primarily focused on synthetic pyrethroids [5,6], while the mechanistic toxicity and carcinogenic potential of natural pyrethrins remain largely unresolved—especially concerning their multi-target interactions with oncogenic signaling pathways such as PI3K-AKT and Wnt [7]. To address this critical knowledge gap, our study employs an integrative computational approach combining molecular docking (validated by [8]), pathway enrichment analysis (based on KEGG [7]), and U.S. EPA-compliant risk quantification frameworks [9], to systematically elucidate compound-specific risk pathways linking pyrethrins to breast carcinogenesis. This approach uniquely addresses pyrethrins' multiplexed risk mechanisms by simultaneously evaluating genomic (EZH2-mediated), proliferative (RPS6KB1-driven), and microenvironmental (TEK-regulated) impacts. In addition, using in silico toxicokinetics, we establish dose–response relationships for pathway-specific effects, enhancing the mechanistic resolution of toxicity predictions.

By incorporating a Total Risk (TR) assessment model, our findings further demonstrate that even under

low-dose exposure scenarios, pyrethrins exhibit significant carcinogenic risk potential. Based on these results, we recommend a policy-driven reassessment of environmental concentration thresholds and human exposure limits, alongside the initiation of long-term epidemiological surveillance to ensure effective public health risk management.

2. Materials and Methods

This study integrates bioinformatics tools with a quantitative toxicological risk model to establish a multi-scale risk assessment framework. The primary objective is to evaluate the potential carcinogenic risk of pyrethrins on breast cancer–related molecular targets. The methodology is composed of two core components:

2.1 Multi-dimensional Target Mining and Mechanistic Elucidation

This part of the research is based on the author's previous work, focusing on the systematic identification of breast cancer–related targets of pyrethrins and the exploration of their underlying biological mechanisms. The analytical process is summarized as follows:

First, target prediction and intersection analysis were conducted. Potential molecular targets of pyrethrin I and II were predicted using platforms such as ChEMBL and SwissTargetPrediction. Breast cancer–associated genes were retrieved from public databases, including GeneCards, OMIM, DisGeNET, and GEO. By intersecting the predicted drug targets with breast cancer–related genes, a set of candidate targets with potential carcinogenic relevance was identified for subsequent analysis. Next, binding affinity evaluation via deep learning was performed. The DeepPurpose model, built on the PyTorch framework, was used to predict drug–target binding scores by inputting the SMILES structures of pyrethrins and the amino acid sequences of target proteins. Based on these predictions, drug–target pairs with high binding potential were selected. To investigate the causal relationship between candidate targets and breast cancer, Mendelian Randomization (MR) analysis was applied. Publicly available GWAS summary data were used to assess the statistical causality between the expression levels of candidate genes and the risk of specific breast cancer subtypes, particularly ER-negative (ER−) and triple-negative breast cancer (TNBC). This served as mechanistic evidence to support target selection. Finally, molecular docking and molecular dynamics (MD) simulations were conducted. Using AutoDock Vina, the docking affinity between pyrethrins and target proteins was calculated based on binding free energy (ΔG). Subsequently, GROMACS was employed to perform 100-nanosecond MD simulations in aqueous environments, allowing the evaluation of complex stability and structural variation, which verified the dynamic structural correlation between ligand and protein.

Overall, this workflow provides a comprehensive analytical framework, spanning from bioinformatic prediction to structural simulation, thereby establishing a scientific foundation for target selection and parameter estimation in the subsequent risk modeling phase.

2.2 Total Risk Model Development and Application

To evaluate the cumulative risk posed by multiple molecular targets, this study independently developed and applied a Total Risk (TR) model. The model is designed to integrate molecular toxicity, exposure dose, and population characteristics in order to estimate a quantitative risk value. The mathematical

expression of the model is as follows:

$$TR = (\sum_{i=1}^{n} EDI \times HW_i \times PF_i) \times (\frac{[Exposed]}{[Total]}) \quad (1)$$

The definitions of model parameters are listed in Table 1, as follows:

Table 1: The definitions of model parameters of Total Risk (TR) model

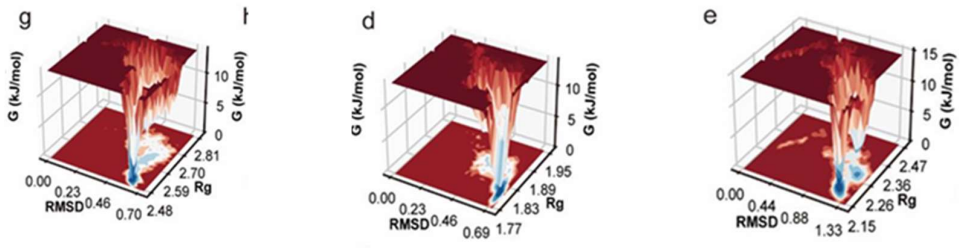
Parameter	Description
EDI（Estimated Daily Intake）	Calculated based on compound concentration (C), inhalation rate (IR), bioavailability (F), and body weight (BW): $EDI = \frac{C \times IR \times F}{BW}$
HW_i	Hazard weight of target iii, calculated by normalizing the molecular docking binding free energy (ΔTOTAL) and multiplying by a pathway-specific kinetic coefficient（k_i）
PF_i	Potency factor of target iii, estimated from the cell doubling time and EC50 value: $PF_i = \frac{1}{EC50_i} \times \frac{\ln(2)}{t_{doubling}}$
[Exposed]/[Total]	Population exposure ratio, set to 5% in the simulated scenario

In the application of this model, three breast cancer–related targets—RPS6KB1, EZH2, and TNKS2—were selected for validation. Molecular simulation data were incorporated into the model, and a low-dose exposure condition (EDI = 0.0017 mg/kg/day) was used as the exposure scenario for TR calculation. To further assess the influence of different parameters on risk outcomes, a sensitivity analysis was performed. This analysis investigated how variations in environmental concentration (C), hazard weight (HW), and potency factor (PF) contributed to changes in the overall risk level. The results confirmed that the TR model is both feasible and sensitive for multi-target integration under low-dose exposure conditions. This demonstrates the model's potential utility in risk prediction for other environmental contaminants.

3. Results

3.1 Target Prediction and Intersection Analysis Results

Through integrated target prediction and gene intersection analysis, a total of 16 candidate targets were identified as having high binding potential with pyrethrin I and II, while also being implicated in breast cancer–related biological processes. These targets are involved in several key oncogenic signaling pathways and physiological regulatory mechanisms. Among them, RPS6KB1, EZH2, and TNKS2 emerged as the most representative and functionally relevant targets, the Gibbs free energy landscape shown in Fig.1. RPS6KB1 is a downstream kinase in the PI3K/AKT signaling pathway, playing a critical role in cell proliferation and anti-apoptotic processes. EZH2 is an epigenetic regulator involved in chromatin modification and is widely associated with tumor progression and malignancy. TNKS2 is engaged in the Wnt/β-catenin signaling cascade and regulates tumor energy metabolism and cell fate determination.



RPS6KB1 EZH2 TNKS2

Fig. 1: The Gibbs free energy landscape RPS6KB1, EZH2, and TNKS2

These findings suggest that pyrethrins may influence breast cancer development via multi-target synergy, underscoring their potential toxicological significance and public health relevance.

3.2 Binding Affinity Prediction and Molecular Simulation Results

Binding affinity between pyrethrins and the selected breast cancer–related targets was evaluated using a combination of DeepPurpose deep learning prediction, AutoDock Vina molecular docking, and 100-nanosecond molecular dynamics (MD) simulations. The results confirmed that pyrethrins possess stable and energetically favorable interactions with key targets including RPS6KB1, EZH2, and TNKS2. A summary of the docking scores and MD simulation stability is presented in Table 2 as below:

Table 2: the docking scores and MD simulation stability results of RPS6KB1, EZH2, and TNKS2.

Target	Binding Free Energy ΔG (kcal/mol)	MD Simulation Stability (100 ns RMSD)
RPS6KB1	−27.37	Stable (< 2 Å)
EZH2	−16.37	Stable
TNKS2	−21.94	Stable

These results suggest that pyrethrins form stable binding conformations with these critical oncogenic targets, supporting their potential mechanistic involvement in breast cancer–related pathways.

3.3 Causal Inference via MR Analysis

Mendelian randomization (MR) analysis was conducted to explore the causal relationship between gene expression levels of selected targets and breast cancer risk. The results revealed a significant positive association between the expression of RPS6KB1, TNKS2, and EZH2 and the risk of developing breast cancer. Notably, these associations were most pronounced in the triple-negative breast cancer (TNBC) subtype. These findings support the role of these targets as potential driver genes in breast cancer pathogenesis.

3.4 Total Risk Model Application and Results

Based on simulated parameter inputs—estimated daily intake (EDI) of 0.0017 mg/kg/day and an assumed exposed population ratio of 5%—the total risk (TR) was quantitatively estimated for each selected target. The hazard weight (HW), potency factor (PF), and calculated individual risk contributions are summarized in Table 3 aas below:

Table 3: The calculated individual risk contributions of RPS6KB1, TNKS2, and EZH2

Target	HW	PF ((mg/kg/day)$^{-1}$)	Individual Risk
RPS6KB1	15.0	0.0012	3.06×10^{-5}
EZH2	7.2	0.0021	2.57×10^{-5}

Target	HW	PF ((mg/kg/day)$^{-1}$)	Individual Risk
TNKS2	12.0	0.0015	3.06×10^{-5}

The total integrated risk is calculated as follows: TR=(3.06+2.57+3.06)×10^{-5}×0.05=4.3×10^{-6}. This result indicates a quantifiable risk contribution under low-dose exposure conditions, supporting the applicability of the TR model for early-stage environmental health risk assessments.

3.5 Sensitivity Analysis Results

A partial derivative–based sensitivity analysis was performed to evaluate the influence of model parameters on the total risk (TR) outcome. The results indicated that TR is most sensitive to changes in environmental concentration (C). The partial derivative with respect to concentration is calculated as:

$$\frac{\partial TR}{\partial C} = 2.15 \times 10^{-4} (mg/m^3)^{-1} \quad (2)$$

This implies that for every 0.01 mg/m³ increase in environmental concentration, the total risk increases by approximately 21.5%. The relative elasticity of each key parameter is ranked, listed Table 4, as follows:

Table 4: The relative elasticity of each key parameter

Parameter	Elasticity Coefficient (η)	Sensitivity Ranking
Concentration (C)	1.0	Highest
Hazard Weight (HW)	0.8	Moderate
Potency Factor (PF)	0.6	Lower

These findings suggest that environmental concentration is the dominant factor influencing TR, while biological parameters such as HW and PF also contribute but to a lesser extent. This supports prioritizing source control strategies in environmental health risk management.

4. Discussion

This study integrated bioinformatics approaches with a quantitative risk assessment model to systematically evaluate the potential contribution of pyrethrins to breast cancer risk. The multi-target prediction and molecular simulation results demonstrated that pyrethrins can form stable interactions with multiple key targets involved in breast cancer–related pathways, including PI3K/AKT, Wnt/β-catenin, and immune regulation. These interactions suggest that pyrethrins may promote breast cancer development or progression through multi-pathway modulation. According to the total risk (TR) model, even under low-dose exposure conditions, the estimated cumulative risk reached 4.3 × 10^{-6}, indicating a non-negligible potential health impact. This risk could be further amplified in agricultural or domestic exposure scenarios where the proportion of the exposed population increases, posing a greater concern particularly for high-risk subgroups such as women with triple-negative breast cancer (TNBC). Sensitivity analysis revealed that environmental concentration (C) is the most critical factor influencing TR, highlighting the importance of source-level control as a core preventive strategy. In addition, the toxicological nature (hazard weight, HW) and biological potency (potency factor, PF) of each target provide valuable information for toxicological research and potential pharmacological interventions.

Although the TR model developed in this study is based on a simplified linear framework, it serves as a practical tool for risk prioritization and early-stage policy assessment. Future improvements may include incorporating toxicokinetic models, interaction terms, and real-world epidemiological data to enhance

its predictive power and applicability in environmental health decision-making.

5. Conclusion

This study establishes a total risk model (Total Risk, TR) that integrates multi-target toxicological information and exposure parameters, and applies it to assess the potential health risks of pyrethroids on breast cancer-related targets. Using methods such as network toxicology, deep learning predictions, causal verification, and molecular simulations, several key targets closely related to the occurrence of breast cancer (e.g., RPS6KB1, EZH2, TNKS2) were identified, and the stable and high-affinity binding ability of pyrethroids to these targets was confirmed.

The results of the total risk model indicate that even under low-dose exposure conditions, pyrethroids may still pose an overall risk with statistical and biological significance ($TR = 4.3\times10^{-6}$). This risk may be further amplified, especially in individuals with higher exposure rates or impaired metabolic functions. Sensitivity analysis highlighted environmental concentration as the primary influencing factor, suggesting that source reduction and exposure control should be priority policy directions.

The total risk model proposed in this study is scalable and versatile, and it can be applied to the risk assessment of other environmental exposures and chronic diseases in the future. Additionally, this model can serve as an important basis for toxic substance management, public health strategy planning, and risk communication.

Declaration of competing interest: The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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