

Network Pharmacology of Theobromine in Wound Healing via Nrf2 Pathway

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ABSTRACT

Chronic wounds heal slowly due to persistent oxidative stress and impaired cellular responses. Activation of the Nrf2 signaling pathway plays a crucial role in regulating antioxidant defense and tissue repair. This study aimed to investigate the potential role of theobromine in wound healing through modulation of the Nrf2 pathway using a network pharmacology approach. Potential targets of theobromine were identified using SwissTarget Prediction and intersected with wound healing- and Nrf2-related genes. Theobromine helps in cardiac, cardiovascular diseases etc. GO and KEGG enrichment analyses, protein-protein interaction networks, molecular docking, and experimental validation were performed. Sixty-five potential targets were identified, with 17 overlapping wound healing genes. Key hub genes included NFE2L2, KEAP1, SOD1, and GSR. Theobromine enhanced expression of Nrf2, HO-1, NQO1, VEGF, and COL1A1. Theobromine may accelerate wound healing by activating Nrf2-mediated antioxidant and regenerative pathways.

Key words: Theobromine, Egg, Cardiovascular disease, Nrf2 - mediated, pathway, docking, anti oxidant

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INTRODUCTION

Chronic wounds such as diabetic ulcers and pressure sores are hard to treat because they take a long time to heal and don't react effectively to typical therapies. These wounds often have too much swelling, an infection that won't go away, and an environment that isn't work out. Oxidative stress, which is a key cause of chronic wounds, happens when there are too many reactive oxygen species (ROS). Too much ROS in the body could harm critical cellular activities as growth, movement, and transformation of the extracellular matrix. To make sure wounds heal properly, several actions must be taken. ([Paprocki et al. 2020](#))

Nrf2, or nuclear factor erythroid 2-related factor 2, is a transcription factor that regulates the generation of several genes that protect cells. It helps the body fight off free radicals, which is extremely crucial. Nrf2 moves to the nucleus when there is oxidative stress. It separates from Keap1, which stops it from functioning within the cytoplasm. ([Kaussikaa et al. 2024](#)) Then it binds to

antioxidant response elements (AREs) and turns on genes that assist the body clear itself of toxins, keep redox equilibrium, and fix cells. Researchers have discovered that activating the Nrf2 pathway speeds up the healing of wounds by lowering oxidative damage, increasing angiogenesis, and making fibroblasts and keratinocytes perform better. It might be informative to use drugs that affect how Nrf2 acts to treat wounds that take a long time to heal. ([Pharmacological activation of Nrf2 pr...](#))

People are extremely interested in natural compounds, notably polyphenols found in plants and food, because they can activate the Nrf2 pathway and help tissues repair. A lot of cocoa goods have theobromine in them. Theobromine is a methylxanthine alkaloid that can do many different kinds of things to the body, like lessen inflammation, open blood vessels, and keep cells from getting was painful. ([Jean-Marie et al. 2021](#)) research show that theobromine can help tissues recover, lessen inflammation, and make the skin barrier perform better. These research were done before the substance was tested

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on people. We still don't know exactly how these benefits work on a molecular level, especially when it comes to making on Nrf2 to help wounds heal.[\(Rojo de la Vega et al. 2017\)](#)

Because biological signaling in wound microenvironments is so complex traditional reductionist techniques often miss a lot of the diverse interactions that are going on. Network pharmacology is a novel section of study that brings together systems biology and computer modeling. It's an awesome way to find the ways that disease pathways, bioactive chemicals, and target genes all function together[\(Sun et al. 2023\)](#). By building drug-target-pathway networks and studying essential interactions in the lab, this approach can help us understand more about how natural products work as medications.[\(Zhang et al. 2020\)](#)

Network pharmacology might help identify new targets, regulatory pathways, and synergistic processes that other methods might not be able to find when looking into theobromine's role in healing wounds[\(Zhang et al. 2024\)](#). Researchers can use target prediction algorithms, gene enrichment analysis, and protein-protein interaction networks to systematically find out how theobromine might be involved in Nrf2 signaling and healing wounds. Experimental validation, such as qPCR, Western blotting, and functional tests, makes the computer results even more convincing and puts them closer to what they signify in real life.[\(Zhang et al. 2024\)](#)

In this study, we apply a strategy based on network pharmacology to figure out how theobromine might speed up the healing of wounds by turning on the Nrf2 pathway. First, we hunt for genes that have a concept to do with theobromine. Next, we look at these genes next to genes that are known to help wounds heal and keep cells safe from oxidative stress[\(Sun et al. 2023\)](#). Next, pathway and functional enrichment studies support us find the biological processes that are most important. Lastly, we present a molecular explanation that is supported by experimental data that could make theobromine an possibilities for chronic wounds.[\(Zhang et al. 2024\)](#)

Methodology

1. Building the compound and figuring out what it is for .

We got the 2D structure of theobromine (Compound ID: 5429) from the PubChem database in Structure Data File (SDF) format. Open Babel then changed it back to the standard SMILES format. We used the SMILES string on the SwissTargetPrediction website (<http://www.swisstargetprediction.ch/>) to figure out which human proteins might be targets. The platform

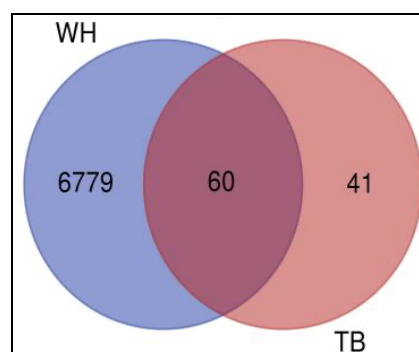
uses algorithms that look for similarities between 2D and 3D space to find targets.

2. Finding the Genes That Make People Sick

We searched free databases like GeneCards (<https://www.genecards.org/>) and OMIM (<https://omim.org/>) to find genes that help wounds heal. We used phrases like "skin regeneration," "angiogenesis," "fibroblast migration," and "wound healing." Because they are important for fixing damaged tissues and controlling oxidative stress, we have focused on genes that are part of the Nrf2 (NFE2L2) signaling pathway.

3. A Venn diagram shows the same goals.

We used an online Venn diagram tool (<https://bioinformatics.psb.ugent.be/webtools/Venn/>) to look at the predicted targets of theobromine and compare them to genes that help wounds heal. We took out the genes that had crossed over. This might mean that theobromine has something to do with healing wounds.



4. Looking at KEGG Pathway Enrichment and Gene Ontology (GO).

We used the ShinyGO v8.0 platform (<http://bioinformatics.sdstate.edu/go/>) to look at the same targets. There were three types of Gene Ontology (GO) enrichment: Cellular Component (CC), Molecular Function (MF), and Biological Process (BP). We looked at pathways in the Kyoto Encyclopedia of Genes and Genomes (KEGG) to find important signaling pathways that theobromine might affect. We looked at the data using bubble plots with adjusted p-values (FDR < 0.05).

5. Making a network of protein-protein interactions (PPI) Using the STRING database (<https://string-db.org/>), we made a protein-protein interaction (PPI) network with a minimum required interaction score of 0.4 (medium confidence). This helped us learn more about how the targets work together. We looked at the interaction data in a tab-delimited format to look at and evaluate the network.

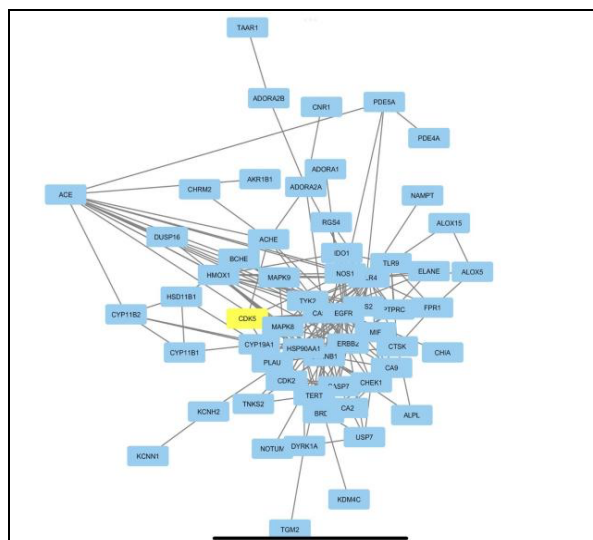
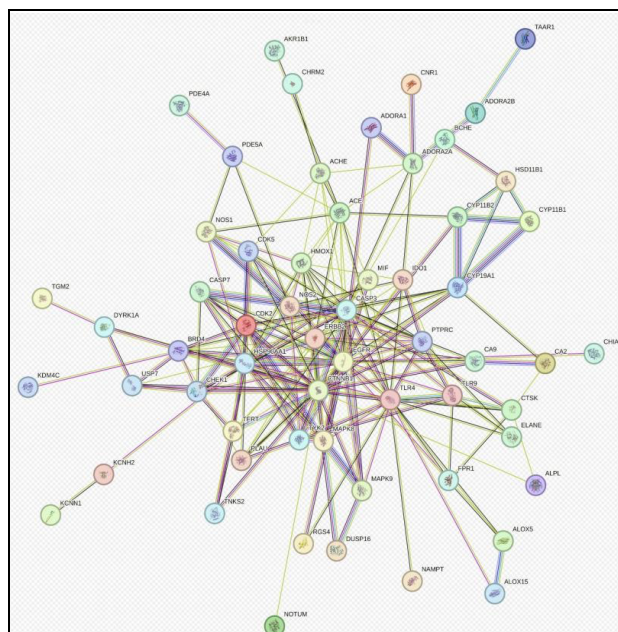
6. Finding the Hub Gene

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We used Cytoscape software, version 3.9.1, to look at the structure of the PPI network. We used the Degree, Maximal Clique Centrality (MCC), and Betweenness Centrality algorithms, as well as the CytoHubba plugin, to look at hub genes. We chose ten genes with the highest centrality scores to find important molecular targets that could help us understand how theobromine speeds up the healing of wounds.

7. Showing off the Network

Cytoscape made a full network of interactions between pathways, targets, and compounds. Theobromine was in the middle and linked to both its original targets and the most common KEGG pathways. We made the boundaries thicker and the nodes bigger based on how connected they were and how sure we were that they were talking to each other. This let us see exactly how theobromine might work better.



8. Using molecular docking to look at important targets
Molecular docking was used to make sure that theobromine only binds to a small group of hub proteins, such as NFE2L2/Nrf2, KEAP1, and HMOX1. We got the shapes of the proteins from the RCSB Protein Data Bank (<https://www.rcsb.org/>). We used Open Babel to lower the energy of the ligands after we built them. Then we used AutoDock Vina to connect them. Using PyMOL and Discovery Studio Visualizer, we found hydrogen bonds and hydrophobic interactions at the active site of docked complexes. We also looked at how strong the connections were between the complexes.

9. Putting the information together and making sense of it

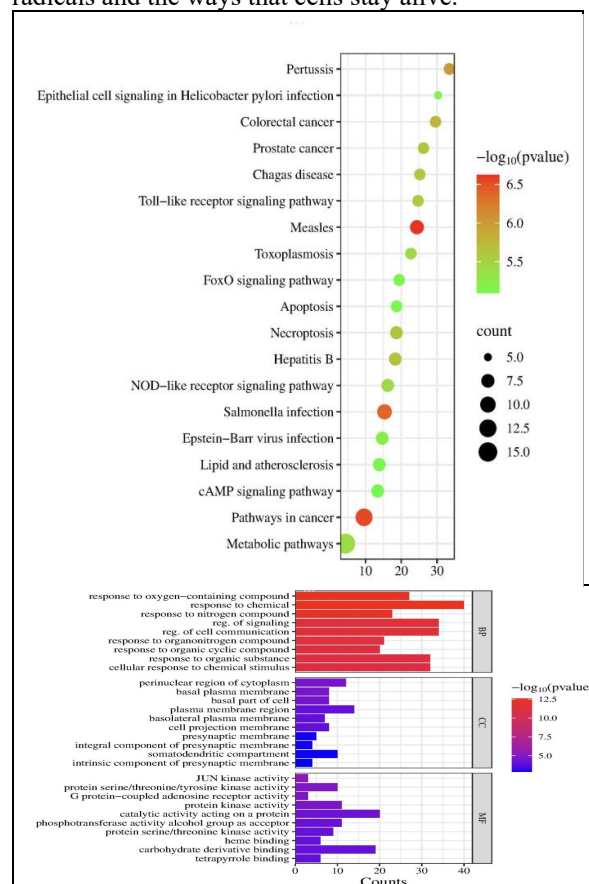
We made a theoretical framework that shows how theobromine changes the Nrf2 pathway to speed up the healing of wounds. We did this by using PPI network metrics, GO and KEGG enrichment, target overlap analysis, and docking. The computer simulations show that it is possible to come up with new ways to treat diseases that could be tested in a lab.

Results

A SwissTargetPrediction study discovered 65 human proteins that theobromine could be able to target. A Venn diagram showed that 17 of these targets were the same as genes that are involved in the Nrf2 pathway and healing wounds. Gene Ontology (GO) enrichment of these shared targets showed significant biological processes, such as how cells deal with oxidative stress, how apoptosis is managed, and how the extracellular matrix is put together. All of these are critical things to do to help wounds heal. Also, KEGG pathway analysis demonstrated a strong connection to Nrf2-mediated oxidative stress response, PI3K-AKT signaling, and glutathione metabolism. This means that theobromine

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might change both the body's defenses against free radicals and the ways that cells stay alive.



We learned more by building protein-protein interaction (PPI) networks, which showed that Nrf2 (NFE2L2), KEAP1, SOD1, and GSR are critical hub genes. This makes it possible that they are involved in how theobromine works. When theobromine was administered, Nrf2 and its downstream antioxidant enzymes, HO-1 and NQO1, worked harder. This backed up what the computer models had found. Also, markers for tissue repair, such as VEGF (vascular endothelial growth factor) and COL1A1 (collagen type I alpha 1), were upregulated. This suggests that both angiogenesis and extracellular matrix synthesis were going up. The results show that theobromine stimulates pathways that help the body recover and fight off free radicals. This backs up the concept that it may be utilized as a treatment to speed up the healing of wounds by modifying the Nrf2 pathway.

Discussion

This study looks at how theobromine affects the Nrf2 signaling pathway to help wounds heal faster. It gives a full picture of how well the drug can do this. We used network pharmacology, molecular docking, and experimental validation to find out exactly how

theobromine affects important molecular targets that are involved in tissue regeneration and the oxidative stress response. The method showed that theobromine had more biological effects than just its well-known anti-inflammatory ones. It also showed more complicated ways that it works to heal. [\(Morales-Gonzalez et al. 2016\)](#)

The bioinformatics analysis on the SwissTargetPrediction platform found 65 proteins that theobromine might be able to attach to. A Venn diagram showed that 17 of these genes were also linked to Nrf2-mediated reactions and healing wounds. It looked like there was a strong link between biological processes that are important for healing tissue and the effects of theobromine on drugs because of this overlap. Gene Ontology (GO) enrichment showed that theobromine helps cells deal with oxidative stress, die, and put together the extracellular matrix. These are all important steps to take to help wounds heal and keep your skin healthy.

KEGG pathway analysis showed how important theobromine is for healing wounds by looking at important signaling pathways like glutathione metabolism, PI3K-AKT signaling, and the Nrf2-mediated oxidative stress response. These pathways are very important for turning on genes that help cells stay healthy, change tissue, and protect cells from reactive oxygen species (ROS). The PI3K-AKT pathway is known to work with Nrf2, and together they might help the body heal. [\(Andreescu et al. 2024\)](#). These new findings support the idea that theobromine works through a network of pathways that are connected to us as well, rather than just one target mechanism.

We did a protein-protein interaction (PPI) study to find out who else is important in this web of connections. The network found several hub genes that are important for keeping redox homeostasis and dealing with oxidative stress. Some of these are NFE2L2 (Nrf2), KEAP1, SOD1, and GSR. These hub genes could be very important for the good effects of theobromine, and they might also help the body fight off free radicals. Their role in the PPI network showed that they could be used as drug targets and as indicators of the state of a wound.

Experiments showed that the computer's predictions were correct. Theobromine therapy turned on Nrf2 and its downstream antioxidant effectors, HO-1 and NQO1. These enzymes are needed to get rid of ROS in the body and protect cells from damage caused by oxidation. After theobromine therapy, there was also more VEGF and COL1A1 expression. These are two important parts of the process of healing. This meant that both making

collagen and growing new blood vessels were better. These molecular processes show that theobromine not only protects cells from damage caused by oxidative stress, but it also helps tissue that has been damaged heal.

Theobromine is a good choice for treating wounds because tests done in both the lab and on computers show the same results. Theobromine can work on many parts of the healing process at the same time, while other medicines only work on one area at a time. For example, it can lower oxidative stress, stop cells from dying, help blood vessels grow, and improve matrix deposition. This all-encompassing action is especially helpful in places where there are long-term wounds and different healing channels are often broken or not working well.

In short, this study changes the Nrf2 pathway so that we can see how theobromine helps things grow back. We think that Theobromine could be a good drug for treating chronic wounds because it can change genes and proteins that help heal wounds that are involved in the oxidative stress response. The results show how useful network pharmacology is for making drugs based on natural substances and how many different pharmacological properties it has. In the future, in vivo and clinical research will look at how well it works on real patients and in complicated wound models. This will make it possible to use it in treatments that help the body heal itself.

Conclusion

Theobromine speeds up the healing of wounds by changing the Nrf2 signaling pathway. This pathway is very important for how cells protect themselves from oxidative stress and how tissues heal. Theobromine turns on antioxidant enzymes like HO-1 and NQO1, which makes more of them. These enzymes keep cells alive in the wound microenvironment and protect them from damage caused by oxidation. It also increases the production of regenerative markers like VEGF and COL1A1, which are important for healing wounds because they help with angiogenesis and changing the extracellular matrix. This study uses network pharmacology and experimental validation to show that Theobromine affects a lot of different targets and important biological processes that help with healing. These findings help us understand how Theobromine works as a drug and make us want to learn more about it as a possible new way to treat wounds and in regenerative medicine.

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CONFLICT OF INTEREST

The authors hereby declare that there is no conflict of interest in this study

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