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Original Article

Synergistic Antioxidant and Anti-Apoptotic Effects of Captopril and Spironolactone in Streptozotocin-Induced Damage to Human Granulosa-Like KGN Cells

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ABSTRACT

Background: Diabetes mellitus causes ovarian granulosa cells to undergo oxidative stress, inflammation, and apoptosis, all of which have a negative impact on female reproductive health. Although there are currently few treatment options, granulosa dysfunction under hyperglycemia contributes to infertility. This study evaluates the cytoprotective potential of captopril and spironolactone, individually and in combination, against Streptozotocin (STZ)-induced damage in human granulosa-like KGN cells. **Materials and Methods:** Following a 24-hr exposure to STZ (5 mM), KGN cells were treated with either 50 µM captopril, 25 µM spironolactone, or both. Hoechst 33258 staining and the TUNEL assay were used to measure apoptosis. Western blot analysis was used to examine the expression of Nrf2, cytochrome c, and cleaved caspase-3. One-way ANOVA and Tukey's *post hoc* test ($p<0.05$) were used for statistical analysis. **Results:** Exposure to STZ markedly decreased Nrf2 levels while increasing nuclear condensation, DNA fragmentation, and pro-apoptotic protein expression. Separately, captopril and spironolactone decreased apoptotic indices and partially restored antioxidant defences. Interestingly, the combination treatment had a synergistic effect, increasing Nrf2 expression and significantly decreasing apoptotic markers ($p<0.001$). **Conclusion:** Combined treatment with captopril and spironolactone confers enhanced protection against STZ-induced apoptosis in granulosa cells, primarily by modulating oxidative stress and apoptosis pathways. This supports the potential for drug repurposing in preserving ovarian function under diabetic conditions.

Keywords: Diabetes Mellitus, Granulosa Cells, Inflammation, Apoptosis, Captopril, Spironolactone, TUNEL, Hoechst Staining, NF-κB.

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INTRODUCTION

The female reproductive axis is one of the organ systems impacted by Diabetes Mellitus (DM), a chronic metabolic disease (Andlib *et al*., 2024). One of its less well-known side effects is ovarian dysfunction, which can show up as decreased ovarian reserve, irregular menstruation, and infertility. Damage to granulosa cells, which are specialised somatic cells necessary for follicular development, steroidogenesis, and oocyte maturation, is closely linked to these results (Wang, 2025).

Granulosa cell dysfunction is largely caused by oxidative stress brought on by hyperglycemia. Reactive Oxygen Species (ROS) overproduction, mitochondrial damage, and apoptotic pathway activation are all brought on by elevated glucose levels (Tao *et al*., 2024). At the same time, the NF-κB signalling cascade amplifies inflammation, which raises the expression of iNOS and COX-2. Together, these effects impair cell viability by upregulating apoptotic markers like cytochrome c and cleaved caspase-3 and suppressing antioxidant defence mechanisms like Nuclear Factor erythroid 2-Related Factor 2 (Nrf2) (Kim *et al*., 2025).

Even though glycaemic control agents are available, current treatments do not directly target these cellular mechanisms. This shows how important it is to find new ways to protect cells that will keep granulosa cells working properly when someone has diabetes (Cao *et al*., 2025). Drug repurposing is a promising path because it uses drugs that are already known to be safe (Recino *et al*., 2025). Captopril is an ACE inhibitor, and spironolactone is a mineralocorticoid receptor antagonist (Jahan *et al*., 2025). Both drugs have anti-inflammatory and antioxidant properties, but their effects on ovarian granulosa cells when used together have not been fully studied (Elmorsy *et al*., 2025). This study looks at how well captopril and spironolactone, both alone and together, protect human granulosa-like KGN cells from oxidative

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and apoptotic damage caused by Streptozotocin (STZ). We think that the combination treatment will provide extra protection by restoring antioxidant defence and lowering apoptosis. This supports their possible use in stopping reproductive problems linked to diabetes.

MATERIALS AND METHODS

Cell Culture and Study Design

Human granulosa-like tumor cell line (KGN) was obtained from the National Centre for Cell Science (NCCS), Pune, India. Cells were maintained in DMEM/F12 medium supplemented with 10% Fetal Bovine Serum (FBS) and 1% penicillin-streptomycin at 37°C in a humidified atmosphere containing 5% CO_2. To simulate diabetic conditions, KGN cells were exposed to streptozotocin (STZ, 5 mM) for 24 hr. Following STZ treatment, cells were divided into five groups: (i) Control (untreated), (ii) STZ only, (iii) STZ+captopril (50 µM), (iv) STZ+spironolactone (25 µM), and (v) STZ+combination of captopril (50 µM) and spironolactone (25 µM). All treatments were performed in triplicate, and experiments were independently repeated three times (n=9 per group) to ensure reproducibility and statistical reliability (Kumar *et al.*, 2014).

Tunnel assay

The *in situ* Cell Death Detection Kit (Roche, Germany) was used to check for apoptotic DNA fragmentation. We fixed KGN cells with 4% paraformaldehyde, made them permeable with 0.1% Triton X-100, and then put them in the TUNEL reaction mix for an hour at 37°C. Cells that had been treated with DNase I were used as positive controls. We used Hoechst 33258 (5 µg/mL) to stain the nuclei and then looked at them with a Nikon Eclipse Ti fluorescence microscope at 20× magnification. ImageJ was used to count the TUNEL-positive (green) and total (blue) nuclei in five random fields. There were at least 100 cells in each field that were looked at. As described, the data were analysed statistically (Wang *et al.*, 2024).

Hoechst 33258 staining for nuclear morphology assessment

We employed Hoechst 33258 staining to look at the shape of the nuclei and the condensation of chromatin in KGN cells. After treatment, cells were fixed with 4% paraformaldehyde, made permeable with 0.1% Triton X-100, and stained with Hoechst 33258 (5 µg/mL) in the dark for 10 min. We looked at the cells with a Nikon Eclipse Ti fluorescent microscope at 20× magnification. The excitation was 350 nm and the emission was 460 nm. Apoptotic nuclei looked smaller, broken up, or brilliantly coloured, while normal nuclei stayed spherical and blue all over. We counted apoptotic nuclei by hand in five randomly chosen fields using ImageJ. Each field had at least 100 cells. The data were analysed statistically as specified (Zhang *et al.*, 2020).

Infllammatory Assay

Western blotting was used to measure the expression of cytochrome c, Nrf2, and cleaved caspase-3. Protease and phosphatase inhibitors added to RIPA buffer were used to extract the total protein. Thermo Fisher Scientific's BCA assay was used to measure the protein content. SDS-PAGE was used to separate equal amounts of protein (30-50 µg), which were then transferred to PVDF membranes. Membranes were blocked with 5% BSA, then incubated with primary antibodies and HRP-conjugated secondary antibodies for a whole night at 4°C. Bands were scanned and visualised with Enhanced Chemiluminescence (ECL). The loading control was β-actin. ImageJ software was used to quantify band intensities. As stated, statistical analysis was performed on the data (You *et al.*, 2025).

Statistical Analysis

All quantitative data were expressed as Mean±Standard Deviation (SD). Statistical significance between groups was assessed using One-Way Analysis of Variance (ANOVA), followed by Tukey's *post hoc* test for multiple comparisons. A p-value less than 0.05 was considered statistically significant. Analyses were performed using GraphPad Prism version 8.0.

Ethical Statement

This study did not involve human or animal subjects; therefore, ethical approval and informed consent were not required.

RESULTS

Tunnel assay

STZ exposure significantly increased DNA fragmentation in KGN cells, as shown by a higher proportion of TUNEL-positive nuclei compared to control (p<0.001; 95% CI: 10.2-15.6). Treatment with captopril or spironolactone alone reduced apoptotic indices (p<0.05), while combination therapy produced a greater reduction (p<0.001; 95% CI: 2.1-5.8), nearing control values. These results confirm the enhanced anti-apoptotic effect of combined therapy (Figure 1B).

Hoechst 33258 staining for nuclear morphology assessment

Hoechst staining showed that STZ induced chromatin condensation and nuclear fragmentation. Both monotherapies preserved nuclear morphology to some extent, with spironolactone showing greater nuclear protection than captopril (p<0.01). The combination group displayed significantly reduced apoptotic nuclei (p<0.001; 95% CI: 6.5-9.0), with mostly uniform, round nuclei consistent with healthy morphology (Figure 2B).

Infllammatory Assay

Western blot results indicated that STZ significantly upregulated cleaved caspase-3 and cytochrome c and downregulated Nrf2

(p<0.001 vs. control). Captopril and spironolactone monotherapy partially corrected these changes (p<0.05-0.01), but the combination treatment produced the strongest effects, markedly reducing apoptotic markers and significantly increasing Nrf2 expression (p<0.001; 95% CI for Nrf2 increase: 8.7-13.4) (Figures 3B-3D).

DISCUSSION

The present study demonstrates that captopril and spironolactone, both individually and in combination, significantly attenuate STZ-induced oxidative stress and apoptosis in human granulosa-like KGN cells. These findings underscore the therapeutic potential of repurposing cardiovascular drugs to combat diabetes-associated ovarian dysfunction. Among the tested groups, combination therapy exhibited the most potent protective effects, indicating a synergistic interaction between captopril and spironolactone (Schubert *et al.*, 2020).

STZ-induced hyperglycemia is known to trigger mitochondrial dysfunction, increase ROS production, and activate caspase-dependent apoptotic pathways in various cell types, including granulosa cells (Fu *et al.*, 20254). Our findings align with prior reports demonstrating STZ-induced upregulation of cleaved caspase-3 and cytochrome c (Yang *et al.*, 2024), and we further show that this is coupled with downregulation of Nrf2, a master regulator of antioxidant defense. The restoration of Nrf2 expression by captopril and spironolactone supports earlier findings in renal and neuronal models (Huang *et al.*, 202), suggesting that this pathway may be a key mediator of their cytoprotective effects. The TUNEL and Hoechst assays revealed that combination therapy markedly reduced nuclear fragmentation and chromatin condensation, two hallmark features of apoptosis. This morphological evidence reinforces the biochemical findings from Western blotting. Importantly, the combination group restored cellular morphology and Nrf2 levels to near-control values, suggesting effective reversal of oxidative injury. A major strength of this study is the use of a well-characterized human granulosa cell line, providing a relevant model for evaluating diabetic ovarian damage. Additionally, the use of multiple complementary assays strengthens the validity of our findings. However, some limitations should be noted. The *in vitro* design, while controlled, does not capture the complexity of the ovarian microenvironment or endocrine signaling. Further *in vivo* studies are needed to evaluate pharmacokinetics, hormonal

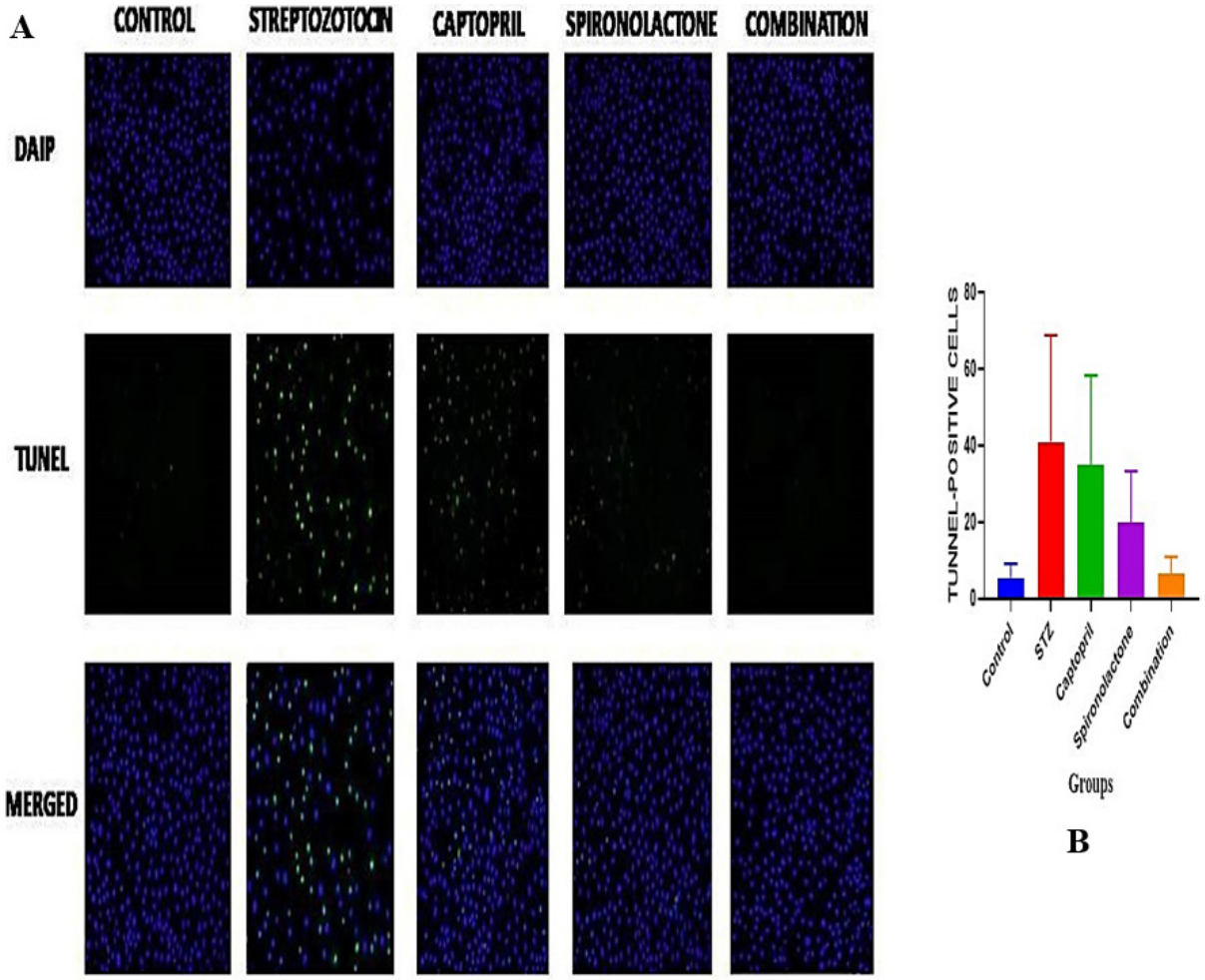

Figure 1: A. TUNEL assay showing apoptotic nuclei in KGN cells. B. Graphs representing percentage of TUNEL-positive cells per group.

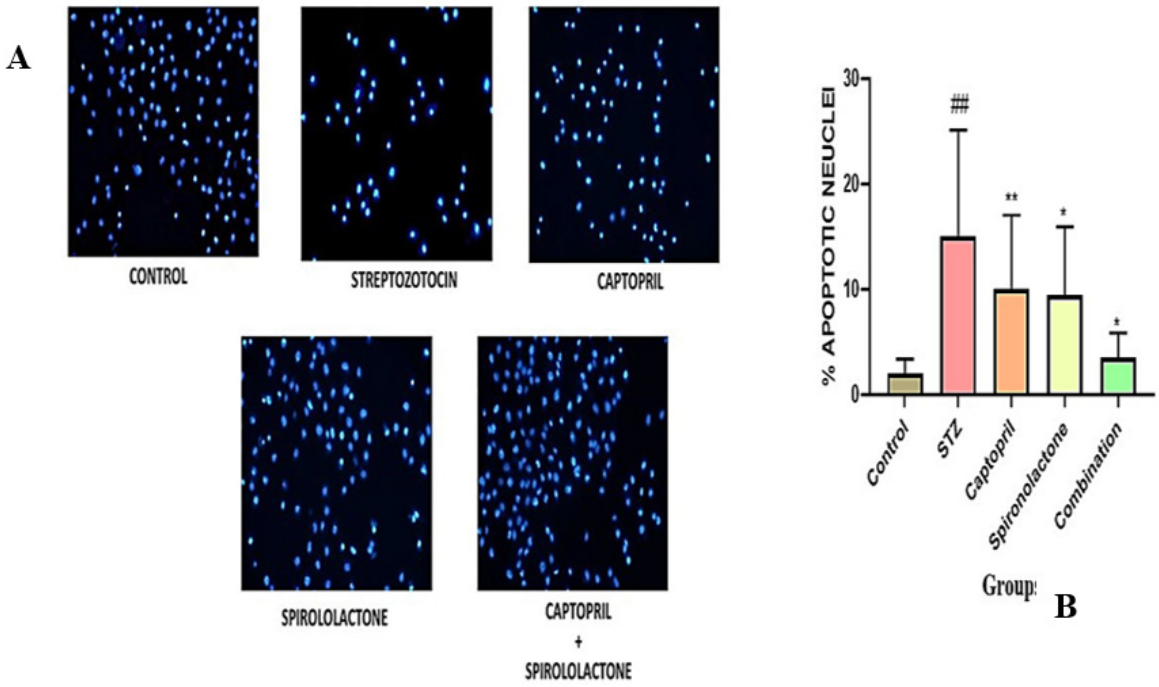

Figure 2: A Hoechst 33258 Staining for KGN cells Using JC-1 Staining Nuclear Morphology Assessment. B Graphs representing percentage of apoptotic nuclei across treatment groups.

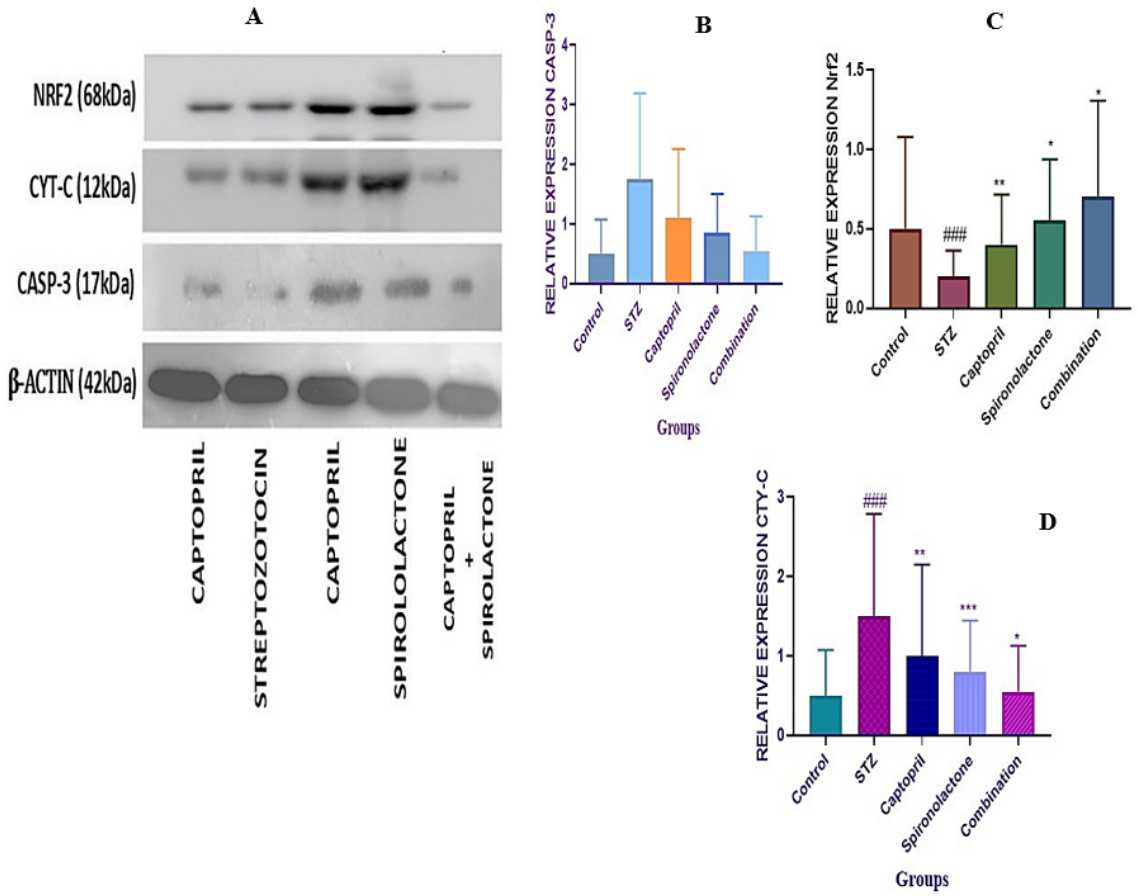

Figure 3: A. Western blot showing cleaved caspase-3, cytochrome c, and Nrf2 expression. B. Graph showing expression CASP-3 across different treatment groups. C. Graph showing Nrf2 expression indicating antioxidant response in KGN cells. D. Graph showing Nrf2 expression indicating antioxidant response in KGN cytochrome c.

interactions, and fertility outcomes. Clinically, these findings point toward the potential for using captopril and spironolactone as adjunctive therapies to preserve ovarian function in diabetic women. Their established safety profiles make them attractive candidates for drug repurposing. Future studies should explore their effects in animal models of Polycystic Ovarian Syndrome (PCOS) or diabetes-induced infertility.

CONCLUSION

In conclusion, captopril and spironolactone, particularly in combination, mitigate STZ-induced oxidative stress and apoptosis in KGN granulosa cells by upregulating Nrf2 and downregulating caspase-mediated apoptotic markers. These findings suggest their potential for repurposing as protective agents against diabetes-associated ovarian damage. However, further *in vivo* studies are necessary to validate their efficacy and safety in a physiological context.

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ABBREVIATIONS

DM: Diabetes Mellitus; **STZ:** Streptozotocin; **ROS:** Reactive Oxygen Species; **KGN:** Human Granulosa-like Tumor Cell Line; **NF-κB:** Nuclear Factor Kappa-Light-Chain-Enhancer of Activated B Cells; **Nrf2:** Nuclear Factor Erythroid 2-Related Factor 2; **COX-2:** Cyclooxygenase-2; **iNOS:** Inducible Nitric Oxide Synthase.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHOR'S CONTRIBUTION STATEMENT

Chandana G and Bharathi D.R. formulated the research question(s), designing the study, carrying it out, analysing the data and writing the manuscript. Mohammad Ali and Bharathi D.R. reviewed the manuscript. All the authors provided approval for publishing the manuscript.

REFRENCES

Andlib, N., Sajad, M., & Thakur, S. C. (2024). Association of diabetes mellitus with risk of reproductive impairment in females: A comprehensive review. Acta Histochemica, 126(5–7), Article 152173. https://doi.org/10.1016/j.acthis.2024.152173

Cao, Z., Zhou, Q., An, J., Guo, X., Jia, X., & Qiu, Y. (2025). Glycolytic dysfunction in granulosa cells and its contribution to metabolic dysfunction in polycystic ovary syndrome. Drug Design, Development and Therapy, 19, 5255–5270. https://doi.org/10.2147/DDDT.S525651

Fu, Y., Hou, L., Han, K., Zhao, C., Hu, H., & Yin, S. (2025). The physiological role of copper: Dietary sources, metabolic regulation, and safety concerns. Clinical Nutrition, 48, 161–179. https://doi.org/10.1016/j.clnu.2025.03.023

Huang, W., Zheng, J., Wang, M., Du, L.-Y., Bai, L., & Tang, H. (2024). The potential therapeutic role of melatonin in organ fibrosis: A comprehensive review. Frontiers in Medicine, 11, Article 1502368. https://doi.org/10.3389/fmed.2024.1502368

Jahan, S., Lu, Z. X., Akram, M., Hasanat, M. A., Fariduddin, M., Fuller, P. J., & Yang, J. (2025). The diagnostic utility of the captopril challenge test for primary aldosteronism in a Bangladeshi population: A prospective study. Pathology, 8. https://doi.org/10.1016/j.pathol.2025.04.003. Elmorsy, E. M., Al Doghaither, H. A., Al-Ghafari, A. B., Ebrahim, N. A., and Amer, S. (2025). The flavonoid hyperoside attenuates the toxic effect of cisplatin on the human ovarian granulosa cells: *In vitro* model study. Toxicology Mechanisms and Methods, 1-11. https://doi.org/10.1080/15376516.2025.2491774

Kim, C. Y., Lee, G. H., Lee, S. Y., Bui, A. T. N., & Jeong, H. G. (2025). Rutaecarpine protects human endothelial cells from oxidative-stress-induced apoptosis via TRPV1- and AhR-mediated Nrf2 activation. Antioxidants, 14(5), 616. https://doi.org/10.3390/antiox14050616

Kumar, N., Sharan, S., Srivastava, S., & Roy, P. (2014). Assessment of estrogenic potential of diethyl phthalate in female reproductive system involving both genomic and non-genomic actions. Reproductive Toxicology, 49, 12–26. https://doi.org/10.1016/j.reprotox.2014.06.008

Recino, A., Rayner, M. L. D., Rohn, J. L., Della Pasqua, O., & University College London Repurposing TIN Committee. (2025). Therapeutic innovation in drug repurposing: Challenges and opportunities. Drug Discovery Today, 30(7), Article 104390. https://doi.org/10.1016/j.drudis.2025.104390

Schubert, M., Hansen, S., Leefmann, J., & Guan, K. (2020). Repurposing antidiabetic drugs for cardiovascular disease. Frontiers in Physiology, 11, Article 568632. https://doi.org/10.3389/fphys.2020.568632

Tao, S., Yang, T., Yin, Y., & Zhang, Q. (2024). Mitochondrial reactive oxygen species promote mitochondrial damage in high glucose-induced dysfunction and apoptosis of human dental pulp cells. Journal of Dental Sciences, 19(1), 292–302. https://doi.org/10.1016/j.jds.2023.04.008

Wang, H. (2025, July). The role of granulosa cells in oocyte development and aging: Mechanisms and therapeutic opportunities. In Seminars in Cell and Developmental Biology. Academic Press, 171. https://doi.org/10.1016/j.semcdb.2025.103614

Wang, Y., Liang, G., Mu, W., Sun, S., Chen, X., & Xu, X. (2024). Bushen Tianjing recipe inhibits human ovarian granulosa cell line KGN apoptosis induced by miR-23a through the regulation of the sirtuin family. Journal of Ethnopharmacology, 319(3), Article 117201. https://doi.org/10.1016/j.jep.2023.117201

Yang, L., Xu, L., Hao, X., Song, Z., Zhang, X., Liu, P., Wang, S., He, Z., & Zou, L. (2024). An aldose reductase inhibitor, WJ-39, ameliorates renal tubular injury in diabetic nephropathy by activating PINK1/Parkin signaling. European Journal of Pharmacology, 967, Article 176376. https://doi.org/10.1016/j.ejphar.2024.176376

You, L., Zhao, W., Li, X., Yang, C., & Guo, P. (2025). Tyrosol protects RPE cells from H2O2-induced oxidative damage *in vitro* and *in vivo* through activation of the Nrf2/HO-1 pathway. European Journal of Pharmacology, 991, Article 177316. https://doi.org/10.1016/j.ejphar.2025.177316

Zhang, Z., Yi, P., Yi, M., Tong, X., Cheng, X., Yang, J., Hu, Y., & Peng, W. (2020). Protective effect of quercetin against H2O2-induced oxidative damage in PC-12 cells: Comprehensive analysis of a lncRNA-associated ceRNA network. Oxidative Medicine and Cellular Longevity, 2020, Article 6038919. https://doi.org/10.1155/2020/6038919

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