ZITA MEDICAL MANAGEMENT

www.hsmc.gr/en/pharmakeftiki-journal/

PHARMAKEFTIKI, 37, 2S, 2025 | 425-428 RESEARCH

https://doi.org/10.60988/p.v37i2S.245

Effects of dapagliflozin on inflammatory and apoptotic markers in rats exposed to cyclophosphamide-induced hepatotoxicity: comparison with silymarin

Afrah Thiab Hlail[1,*], Nada Naji Al-Shawi[1]

[1]Department of Pharmacology and Toxicology, College of Pharmacy, University of Baghdad, Baghdad, Iraq

KEY WORDS:
cyclophosphamide; dapagliflozin; silymarin; inflammation; apoptosis

ARTICLE INFO:
Received: December 20, 2024
Revised: February 22, 2025
Accepted: February 24, 2025
Available online: October 10, 2025

*** CORRESPONDING AUTHOR:**
Afrah Thiab Hlail, Department of Pharmacology and Toxicology, College of Pharmacy, University of Baghdad, Baghdad, Iraq; e-mail: afrah.zeiab2200p@copharm.uobaghdad.edu.iq

ABSTRACT

Cyclophosphamide (CPA) is a widely used chemotherapeutic agent; however, its clinical utility is constrained by dose-dependent hepatotoxicity. This study evaluated the hepatoprotective effects of dapagliflozin (DAPA), a sodium-glucose co-transporter 2 (SGLT2) inhibitor with known anti-inflammatory properties, in comparison to silymarin (SILY); a well-established hepatoprotective flavonoid. Fifty Wistar rats were randomly assigned to five groups: (i) negative control, (ii) vehicle control receiving 2% sodium carboxymethyl cellulose, (iii) CPA-treated (30 mg/kg/day), (iv) CPA + DAPA (3 mg/kg/day), and (v) CPA + SILY (200 mg/kg/day). All treatments were administered for ten consecutive days. CPA administration significantly elevated hepatic levels of tumor necrosis factor alpha (TNF-α; a pro-inflammatory cytokine), while reducing interleukin-10 (IL-10; an anti-inflammatory mediator), thereby aggravating liver injury. DAPA was more effective than SILY in attenuating inflammation and restoring IL-10 levels. Furthermore, CPA markedly increased expression of caspase-3 (a pro-apoptotic marker) and decreased B-cell lymphoma 2 (BCL-2; an anti-apoptotic protein), indicating enhanced apoptotic activity. Both DAPA and SILY ameliorated these apoptotic changes, with DAPA exerting a more pronounced anti-apoptotic effect. In conclusion, DAPA demonstrated superior hepatoprotective efficacy compared to SILY, characterized by stronger anti-inflammatory and anti-apoptotic actions. These findings support the potential of DAPA as a therapeutic candidate for CPA-induced hepatotoxicity, warranting further investigation into its clinical applicability and possible synergistic use in combination therapies.

PHARMAKEFTIKI, 37, 2S, 2025 | 425-428

1. Introduction

Cyclophosphamide (CPA; an alkylating agent) is extensively employed in the treatment of various malignancies, including solid tumours, lymphomas, and leukaemia, as well as in non-neoplastic conditions such as rheumatoid arthritis and systemic lupus erythematosus[1]. Despite its therapeutic efficacy, CPA's clinical application is frequently limited by hepatotoxicity associated with standard dosing regimens[2]. Evidence suggests that the CPA-induced liver injury is primarily mediated by oxidative stress, which contributes to mitochondrial damage and impaired cellular respiration. This cascade involves nucleic acid alterations, mitochondrial dysfunction, and activation of multiple cytotoxic signalling pathways, ultimately provoking inflammatory responses[3].

CPA undergoes hepatic metabolism to yield active intermediates, including aldophosphamide and phosphoramide mustard. These metabolites interact with DNA, inhibiting replication and promoting apoptosis[4,5]. Dapagliflozin (DAPA), a sodium-glucose co-transporter 2 (SGLT2) inhibitor, has been approved by the European Medicines Agency for the management of type 2 diabetes mellitus. Notably, DAPA confers nephroprotective effects against cyclosporine A-induced renal toxicity in mice[6]. However, its potential hepatoprotective properties in rat models remain uninvestigated.

Milk thistle (*Silybum marianum*) seeds are a natural source of silymarin (SILY), a complex of flavonolignans widely used in the treatment of both acute and chronic hepatic disorders. In several countries, SILY is also marketed as a dietary supplement. Its hepatoprotective activity is primarily attributed to antioxidant mechanisms, including free radical scavenging and iron chelation *via* phenolic hydroxyl groups. Numerous studies have demonstrated that SILY effectively mitigates experimental liver injury induced by carbon tetrachloride, acetaminophen, ethanol, and CPA[6–8].

This study was designed in order to examine the protective effects of DAPA and SILY against CPA-induced hepatotoxicity in rats, with particular emphasis on biomarkers of inflammation, oxidative stress, and apoptosis.

2. Methodology

Adult male rats were procured from the animal facility of the College of Pharmacy of the University of Baghdad. Ethical approval for the study was granted by the College of Pharmacy (REC062463A / 2-3-2024). Animals were randomly allocated into five experimental groups.

The negative control group received distilled water orally along with a standard diet. The vehicle control group was administered a 2% aqueous solution of sodium carboxymethylcellulose (Na^+-CMC) *via* oral gavage for ten consecutive days, with dosage adjusted according to body weight. The induction group received intraperitoneal (i.p.) injections of CPA at 30 mg/kg/day for ten days. The DAPA-treated group received oral DAPA at 3 mg/kg/day, dissolved in Na^+-CMC, concurrently with CPA (30 mg/kg/day, i.p.) for ten days. The SILY-treated group received oral SILY at 200 mg/kg/day, also dissolved in Na^+-CMC, alongside CPA (30 mg/kg/day, i.p.) for ten days. All dosages were adjusted based on individual body weight.

At 24 h after the final treatment (day 11), all animals were euthanized using diethyl ether and sacrificed *via* cervical dislocation. The liver of each animal was promptly excised, rinsed with phosphate-buffered saline (PBS; pH 7.4) at 4°C, and processed for subsequent analyses.

Inflammatory and anti-inflammatory cytokines, including tumor necrosis factor alpha (TNF-α) and interleukin-10 (IL-10), were quantified using quantitative real-time reverse transcription polymerase chain reaction (qRT-PCR). Apoptotic and anti-apoptotic markers, specifically caspase-3 and B-cell lymphoma 2 (BCL-2), were assessed *via* Western blot analysis.

Statistical analyses were performed using GraphPad Prism version 8.3. Data are presented as mean ± standard deviation (SD). One-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test was applied. A p-value <0.05 was considered statistically significant.

3. Results and Discussion

CPA administration significantly elevated hepatic

PHARMAKEFTIKI, 37, 2S, 2025 | 425-428

TNF-α levels compared to both the negative control (4.316 ± 0.494 pg/mL *vs.* 1.649 ± 0.336 pg/mL; $p<0.001$) and the Na^+-CMC-treated group (4.316 ± 0.494 pg/mL *vs.* 1.453 ± 0.248 pg/mL; $p<0.001$), indicating pronounced inflammation. Co-treatment with either DAPA or SILY significantly reduced TNF-α levels to 1.479 ± 0.120 pg/mL and 1.751 ± 0.249 pg/mL, respectively ($p<0.001$), with no statistically significant difference between the two interventions.

CPA exposure also led to a marked reduction in IL-10 levels relative to the negative control (0.18 ± 0.069 pg/mL *vs.* 1.45 ± 0.417 pg/mL; $p<0.001$) and the Na^+-CMC group (0.18 ± 0.069 pg/mL *vs.* 1.416 ± 0.391 pg/mL, $p<0.001$). DAPA treatment significantly increased IL-10 levels to 2.41 ± 0.318 pg/mL ($p<0.001$), while SILY elevated them to 1.869 ± 0.621 pg/mL ($p<0.001$), with DAPA showing a significantly greater effect than SILY ($p<0.05$).

CPA administration resulted in a substantial increase in caspase-3 levels compared to the negative control (9.123 ± 0.791 ng/mL *vs.* 2.5 ± 0.577 ng/mL, $p<0.001$), indicating enhanced apoptotic activity. Both DAPA and SILY significantly reduced caspase-3 levels to 1.31 ± 0.546 ng/mL and 1.44 ± 0.309 ng/mL, respectively ($p<0.001$), with no significant difference between the two treatments or relative to the negative control (DAPA: 1.31 ± 0.546 ng/mL *vs.* 2.5 ± 0.577 ng/mL; SILY: 1.44 ± 0.309 ng/mL *vs.* 2.5 ± 0.577 ng/mL).

Moreover, CPA exposure significantly decreased BCL-2 levels compared to the negative control (0.4 ± 0.058 ng/mL *vs.* 2.5 ± 0.577 ng/mL, $p<0.01$), suggesting increased susceptibility to apoptosis. DAPA and SILY treatments restored BCL-2 levels to 3.825 ± 0.848 ng/mL and 2.91 ± 0.933 ng/mL, respectively ($p<0.001$). No significant difference was observed between the DAPA and SILY groups (3.825 ± 0.848 ng/mL *vs.* 2.91 ± 0.933 ng/mL), nor when compared to the negative control (DAPA: 3.825 ± 0.848 ng/mL *vs.* 2.5 ± 0.577 ng/mL; SILY: 2.911 ± 0.933 ng/mL *vs.* 2.5 ± 0.577 ng/mL).

These findings indicate that both SILY and DAPA possess substantial therapeutic potential in mitigating CPA-induced hepatotoxicity by modulating inflammatory responses, restoring cytokine equilibrium, and inhibiting apoptosis. DAPA appears to confer slightly superior anti-inflammatory benefits, particularly in enhancing IL-10 expression. Its hepatoprotective effects may be attributed to SGLT2 inhibition and modulation of oxidative stress-related pathways, contributing to both anti-inflammatory and anti-apoptotic outcomes[9]. In contrast, SILY likely exerts its protective effects through membrane stabilization and potent antioxidant activity, thereby reducing hepatic inflammation and apoptosis[10]. Collectively, these results suggest that both agents may serve as effective adjuvant therapies for chemotherapy-associated liver injury, with potential applications extending beyond CPA-induced hepatotoxicity.

4. Conclusion

Silymarin and dapagliflozin exhibit promising therapeutic efficacy in attenuating CPA-induced hepatotoxicity: both compounds reduce hepatic inflammation, restore cytokine homeostasis, and protect hepatocytes from apoptotic damage. SILY primarily acts through antioxidant and membrane-stabilizing mechanisms, whereas DAPA demonstrates more pronounced anti-inflammatory effects, particularly in upregulating IL-10. The hepatoprotective benefits of DAPA may be linked to its regulation of oxidative stress and inhibition of SGLT2.

Acknowledgements

We want to express our deep gratitude to the Department of Pharmacology and Toxicology of the College of Pharmacy of the University of Baghdad for providing the necessary facilities for the undertaking of this study.

Conflicts of interest

None exist.

ORCIDs

0000-0001-9223-2850 (A. Thiab Hlail); 0000-0003-3657-1465 (N. Naji Al-Shawi)

PHARMAKEFTIKI, 37, 2S, 2025 | 425-428

References

1. Delahousse J., Molina L., Paci A. Cyclophosphamide and analogues; a matter of dose and schedule for dual anticancer activities. *Cancer Lett.* 598, 217119, 2024. DOI: 10.1016/j.canlet.2024.217119
2. El-Beheiry K.M., El-Sayed El-Sayad M., El-Masry T.A., Elsisi A.E. Combination of metformin and hesperidin mitigates cyclophosphamide-induced hepatotoxicity. Emerging role of PPAR-γ/Nrf-2/NF-κB signaling pathway. *Int. Immunopharmacol.* 117, 109891, 2023. DOI: 10.1016/j.intimp.2023.109891
3. Akamo A.J., Rotimi S.O., Akinloye D.I., Ugbaja R.N., Adeleye O.O., Dosumu O.A., *et al.* Naringin prevents cyclophosphamide-induced hepatotoxicity in rats by attenuating oxidative stress, fibrosis, and inflammation. *Food Chem. Toxicol.* 153, 112266, 2021. DOI: 10.1016/j.fct.2021.112266
4. Sherif I.O. The effect of natural antioxidants in cyclophosphamide-induced hepatotoxicity: role of Nrf2/HO-1 pathway. *Int. Immunopharmacol.* 61, 29–36, 2018. DOI: 10.1016/j.intimp.2018.05.007
5. Abd-Eldayem A.M., Makram S.M., Messiha B.A.S., Abd-Elhafeez H.H., Abdel-Reheim M.A. Cyclosporine-induced kidney damage was halted by sitagliptin and hesperidin via increasing Nrf2 and suppressing TNF-α, NF-κB, and Bax. *Sci. Rep.* 14(1), 7434, 2024. DOI: 10.1038/s41598-024-57300-x
6. Castoldi G., Carletti R., Ippolito S., Colzani M., Barzaghi F., Stella A., *et al.* Sodium-glucose cotransporter 2 inhibition prevents renal fibrosis in cyclosporine nephropathy. *Acta Diabetol.* 58(8), 1059–1070, 2021. DOI: 10.1007/s00592-021-01681-2
7. Mohammed A.H., Al-Khishali D.K., Al-Shawi N.N.: Anti-oxidant effect of silymarin against DDT-induced nephrotoxicity in rats. *Karbala J. Pharm. Sci.* 4(4), 136–144, 2013.
8. Patwa J., Khan S., Jena G. Nicotinamide attenuates cyclophosphamide-induced hepatotoxicity in SD rats by reducing oxidative stress and apoptosis. *J. Biochem. Mol. Toxicol.* 34(10), e22558, 2020. DOI: 10.1002/jbt.22558
9. Said Ahmed W.M., Soliman A., Ahmed Amer A.E., El Shahat R.M., Amin M.M., Taha R.S., *et al.* Effect of dapagliflozin against NAFLD and dyslipidemia in type 2 diabetic albino rats: possible underlying mechanisms. *Eur. Rev. Med. Pharmacol. Sci.* 27(17), 8101–8109, 2023. DOI: 10.26355/eurrev_202309_33570
10. Jalali S.M., Najafzadeh H., Bahmei S. Protective role of silymarin and D-penicillamine against lead-induced liver toxicity and oxidative stress. *Toxicol. Ind. Health* 33(6), 512–518, 2017. DOI: 10.1177/0748233716685660

HOW TO CITE:
Thiab Hlail A., Naji Al-Shawi N. Effects of dapagliflozin on inflammatory and apoptotic markers in rats exposed to cyclophosphamide-induced hepatotoxicity: comparison with silymarin. *Pharmakeftiki* 37(2s), 425-428, 2025. https://doi.org/10.60988/p.v37i2S.245