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Comparative Hepatoprotective Effects of Dapagliflozin to Silymarin Against Cyclophosphamide-Induced Liver Injury in Rats: Biochemical, Antioxidants and Histopathological Studies

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Abstract

Introduction. Hepatotoxicity is primarily-caused by oxidative stress and mitochondrial dysfunction; and, it is the principal factor that restricts the clinical efficacy of cyclophosphamide (Cpd), which is a chemotherapeutic drug that is frequently-used. The antioxidant capabilities have been demonstrated by dapagliflozin (Dapa), which is an inhibitor of sodium-glucose co-transporter-2 (SGLT2). Silymarin (Sil) is a chemical that is extracted from milk thistle. Researches have demonstrated that silymarin has hepatoprotective and antioxidant properties.

Aim. This study aimed to compare the hepatoprotective effects of dapagliflozin to silymarin in a rat model of Cpd-induced liver injury.

Material and methods. Negative control, vehicle (2 % aqueous sodium carboxy methylcellulose CMC), Cpd (30 mg/kg/day, Intraperitoneal (ip), Dapa + Cpd (3 mg/kg/day, oral), and Sil + Cpd (200 mg/kg/day, oral) were the five groups that were randomly assigned to fifty rats. Each group consisted of ten rats. Following a period of ten days, evaluation the levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in the serum; while, malondialdehyde (MDA), reduced glutathione (GSH), and superoxide dismutase (SOD) enzyme were all measured in liver tissues; and histological inspection was also performed on the samples.

Results and discussion. Levels of ALT, AST, and MDA were each considerably-increased by Cpd, while the levels of GSH and SOD were decreased ($P < 0.05$). Treatment with either Dapa or Sil each with Cpd resulted in a significant amelioration of these alterations ($P < 0.05$ compared to controls). The results of the study demonstrate that Dapa was more effective than Sil in lowering MDA levels and increasing of GSH and SOD levels ($P < 0.05$). In the Dapa (Group IV), the histological examination revealed that the hepatic architecture had been intact, and there was only slight increase in vascular congestion.

Conclusion. Both dapagliflozin and silymarin each confer comparable hepatoprotective effects against Cpd-induced liver injury, possibly through attenuation of oxidative stress and preservation of hepatocyte integrity. The study adds to current evidence supporting the use of each of these agents in hepatic injury models. However, unlike the study of Satyam et al (2024) which investigated their combined effect, this study highlights their individual efficacy in a cyclophosphamide-specific toxicity model.

Keywords: cyclophosphamide, dapagliflozin, silymarin , oxidative stress

Conflict of interest. The authors declare that they have no obvious and potential conflicts of interest related to the publication of this article.

Contribution of the authors. The research was conceptualized, designed, conducted and written in part by both writers.

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Сравнительное исследование гепатотропного действия дапаглифлозина и силимарина на модели циклофосфамид-индуцированного поражения печени у крыс

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Резюме

Введение. Гепатотоксичность циклофосфамида в первую очередь обусловлена влиянием его метаболитов, в том числе акролеина. Антиоксидантные свойства были продемонстрированы дапаглифлозином (Dapa), ингибитором натрий-глюкозного котранспортера-2 (SGLT2). Силимарин (Sil) – это химическое вещество, извлекаемое из расторопши пятнистой. Исследования показали, что силимарин обладает гепатопротекторными и антиоксидантными свойствами.

Цель. Целью данного исследования было сравнение гепатопротекторных эффектов дапаглифлозина и силимарина на модели поражения печени, вызванного ЦФД, у крыс.

Материалы и методы. В качестве отрицательного контроля использовались: растворитель (2%-й водный раствор карбоксиметилцеллюлозы натрия, КМЦ), циклофосфамид (30 мг/кг/день, внутрибрюшинно), дапаглифлозин + циклофосфамид (3 мг/кг/день, перорально) и силимарин + циклофосфамид (200 мг/кг/день, перорально). 50 крыс были случайно распределены на 5 групп по 10 животных в каждой. Через десять дней оценивали уровни аланин-аминотрансферазы (ALT) и аспартат-аминотрансферазы (AST) в сыворотке крови; в тканях печени измеряли уровни малонового диальдегида (MDA), восстановленного глутатиона (GSH) и супероксиддисмутазы (СОД); также проводили гистологическое исследование образцов.

Результаты и обсуждение. Уровни ALT, AST и MDA значительно повышались под действием циклофосфамида, в то время как уровни GSH и СОД снижались ($P < 0,05$). Лечение дапаглифлозином или силимарином на фоне применения ЦФД привело к значительному улучшению этих изменений ($P < 0,05$ по сравнению с контрольной группой). Результаты исследования показывают, что Dapa был более эффективен, чем Sil, в снижении уровня MDA и повышении уровня GSH и SOD ($P < 0,05$). В группе Dapa (группа IV) гистологическое исследование показало, что строение печени осталось неизменным, и наблюдалось лишь незначительное увеличение сосудистого застоя.

Заключение. И дапаглифлозин, и силимарин оказывают сопоставимое гепатопротекторное действие против вызванного циклофосфамидом поражения печени, возможно, за счет ослабления окислительного стресса и сохранения целостности гепатоцитов. Данное исследование дополняет имеющиеся данные, подтверждающие использование каждого из этих препаратов в моделях поражения печени. Однако, в отличие от исследования Сатьяма и др. (2024), в котором изучалось их комбинированное действие, данное исследование подчеркивает их индивидуальную эффективность в модели токсичности, специфичной для циклофосфамида.

Ключевые слова: циклофосфамид, дапаглифлозин, силимарин, окислительный стресс

Конфликт интересов. Авторы декларируют отсутствие явных и потенциальных конфликтов интересов, связанных с публикацией настоящей статьи.

Вклад авторов. Оба автора разработали, спланировали исследование и написали текст статьи.

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INTRODUCTION

Cyclophosphamide (Cpd), an oxazaphosphorine of nitrogen mustard derivative, is utilized in the treatment of various human cancers, including solid tumors, lymphomas, and leukemia [1]. It is also extensively-employed to manage non-neoplastic conditions such as rheumatoid arthritis (RA) and systemic lupus erythematosus [2].

A limitation of Cpd in clinical settings is the risk of therapeutic doses causing liver damage; researchers indicate that alterations in cellular nucleic acids accompany oxidative stress (OS), which is commonly associated with Cpd-induced hepatotoxicity and may significantly contribute to its etiology [3]. This consequently disturbs the intracellular oxidant/antioxidant equilibrium in the liver by diminishing the functions of endogenous antioxidant proteins and enzymes (such as ALT and AST), resulting from Cpd's detrimental effects on the mitochondria and compromised cellular respiration [4, 5].

Dapagliflozin is the inaugural sodium-glucose cotransporter-2 (SGLT2) inhibitor sanctioned by the EMA for the treatment of type 2 diabetes. It functions by inhibiting the kidneys' capacity to reabsorb filtered glucose, hence augmenting urine glucose excretion and reducing blood glucose levels. This medicine has obtained FDA approval for treating type 2 diabetes in adults, focusing on reducing blood glucose levels and minimizing the risk of heart failure hospitalization in individuals with type 2 diabetes with a history of cardiovascular disease (CVD) or other CVD risk factors, including smoking, hypertension, or dyslipidemia; and is intended to be used alongside a healthy lifestyle [6]. Dapagliflozin, has just been licensed as a potential preventive treatment against organ toxicity induced by radiation therapy, particularly in conjunction with doxorubicin and cisplatin. Dapagliflozin mitigated oxidative stress and apoptosis in diabetic rat models by inhibiting doxorubicin-induced apoptosis and the generation of reactive oxygen species (ROS), resulting in decreased heart fibrosis and enhanced cardiac function [7]. This effect is accompanied with the alleviation of oxidative stress pathways in the endoplasmic reticulum (ER). Dapagliflozin mitigated Electrocardiographic abnormalities and doxorubicin-induced reductions in ejection fraction in non-diabetic rats. Histopathological analyses indicated that those administered dapagliflozin exhibited reduced myocardial injury [8]. The expression of antioxidant enzymes was enhanced, while markers of heart hypertrophy and fibrosis were diminished following the stimulation of the PI3K/AKT/Nrf2 signaling pathway by dapagliflozin. The modulation contributes to its capacity to safeguard the heart. It exhibits hepatoprotective properties alongside its cardioprotective benefits [8, 9].

In addition it enhanced histological liver architecture and reduced elevated liver enzymes (ALT, AST) in Wistar rats following cisplatin treatment. The advantages of dapagliflozin and silymarin combination in rehabilitating liver function and architecture were noted. Dapagliflozin mitigated renal fibrosis, renal dysfunction, and glomerular shrinkage induced by doxorubicin, as indicated by research findings. It may be beneficial in preventing nephrotoxicity in patients treated with doxorubicin, as it diminished ROS production and apoptosis in renal tissues [10, 11].

Silymarin, a flavonolignan complex obtained from the seeds of Silybum marianum (milk thistle), has been thoroughly examined for its beneficial effects on liver health; Mutlu Deger et al. (2022) shown that Dapagliflozin safeguarded the kidneys of mice against cyclosporine A-induced nephrotoxicity [12]. The therapeutic potential and mechanisms of action against several hepatotoxins have been further investigated from 2022 to 2025 [13].

Silymarin demonstrates significant antioxidant properties by neutralizing reactive oxygen species (ROS) and preventing lipid peroxidation. Yang et al. (2022) and H. M. Jaffar et al.(2024) discovered that silymarin mitigated acetaminophen-induced hepatic injury by reducing glutathione levels and diminishing the synthesis of deleterious metabolites via the downregulation of CYP2E1 enzyme expression and activity [14,15].

The anti-inflammatory attributes of silymarin are realized through the suppression of NF-κB and the reduction of pro-inflammatory cytokines such as TNF-α and IL-6. El-Kot et al. (2023) shown that rats administered silymarin alongside CCl_4 exhibited markedly reduced inflammatory markers and improved liver histology [16].

Silymarin safeguards the hepatic tissue from oxidative stress-induced damage by regulating enzyme activity. It specifically augments the concentration of intrinsic antioxidant enzymes, including catalase and superoxide dismutase (SOD) [17]. Okiljević et al. (2024) report that administering silymarin to mice subjected to paracetamol-induced oxidative stress resulted in enhanced antioxidant enzyme activity and reduced liver damage [18].

Several hepato-toxins have been investigated concerning silymarin's putative protective properties; where, Silymarin can mitigate acetaminophen-induced hepatotoxicity by augmenting antioxidant defenses and inhibiting CYP2E1-mediated bioactivation. In carbon tetrachloride (CCl_4) liver injury models, it mitigates inflammation and oxidative stress, thereby enhancing liver function and histological outcomes [19].

Research conducted by B. Okiljević, et al (2024) indicated that silymarin exhibited hepatoprotective properties against acetaminophen-induced toxicity by reducing serum liver enzyme levels and enhancing liver histology in rats model [20].

Objectives of the Study

The purpose of this study was to compare dapagliflozin to silymarin in terms of its effect on oxidative stress parameters, serum enzyme levels (alanine and aspartate aminotransferases), and histological investigations in male rats against cyclophosphamide-induced hepatotoxicity.

MATERIALS AND METHODS

Ethical approval

Approval Number: REC062463A was granted on 2-3-2024 by the Local Research Ethics Committee of the College of Pharmacy, University of Baghdad, Iraq, for the processes pertaining to animal care and research.

Materials

Carboxymethylcellulose sodium salt was acquired from HiMedia Laboratories Pvt. India, cyclophosphamide was obtained from Baxter, Germany, and dapagliflozin was sourced from Sigma company, USA. Silymarin was procured from MACKLIN Company, China. AST and ALT kits were acquired from LINEAR CHIMICALS, S.L.U. in Spain, while ELIS/A kits for GSH, SOD, and MDA were sourced from Elabscience in the USA.

Animals

Fifty (50) male Wistar albino rats, aged five to six-weeks-old, weighing 150–180 grams, were obtained from the University of Baghdad's local animal facility, which is part of the Department of Pharmacology and Toxicology. The standard laboratory lighting conditions of a 12:12 hour light: dark cycle and a temperature of 25 ± 2 °C were applied to all of the rats. One week before the trial started, all of the animals were acclimated. Each of the five groups consisted of ten rats, which were randomly assigned to the groups. Negative control: (I). Rats were administered distilled water (DW) orally and provided with a standard diet. (II). Rats were supplied a 2 % aqueous solution of sodium-carboxymethylcellulose (Na+-CMC) orally via gavage daily for 10 days, based on the weight of each animal [21]. (III). (Induction): Rats were administered 30 mg/kg/day of Cpd *via* intraperitoneal injection for duration of 10 consecutive days [22]. (IV) Rats were supplied Dapa (3 mg/kg/day B.W) orally, dissolved in Na+-CMC, for 10 days, alongside an intraperitoneal injection of the cpd (30 mg/kg/day) for 10 consecutive days, dosed according to the weight of each rat [23]. (V) Rats were supplied with Sil (200 mg/kg) orally, dissolved in Na+-CMC, for 10 days, alongside a 30 mg/kg/day Cpd injected intraperitoneally for 10 consecutive days, adjusted according to the weight of each rat [24].

Twenty-four hours post the final administered dose (i.e., on day 11), following euthanasia (Rats were anesthetized *via* intraperitoneal administration of 80 mg/kg of ketamine and 10 mg/kg of xylazine; upon achieving complete anesthesia, the rats were euthanized via cervical dislocation), the liver of each rat was promptly-isolated post-mortem and washed with ice-cold phosphate-buffered saline (PBS) at pH 7.4 to minimize degradation [25].

Preparation of Serum Samples

Following the euthanasia of rats, blood was collected from the neck and transferred to a standard tube; the clot was disrupted with a glass rod and subsequently centrifuged at 3000 rpm for 15 minutes. Serum was stored at –20 °C until it was used for the evaluation of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) [26].

Liver Tissue Homogenate Preparation

Subsequent to euthanization of each rat/Group, the liver was removed and rinsed with an ice-cold phosphate-buffered saline (PBS) solution (pH = 7.4) to eliminate residual blood. The sample was prepared at a ratio of 1 g of liver tissue to 9ml of PBS, which was subsequently-homogenized using a homogenizer. The mixture was then centrifuged for 10minutes in a chilled centrifuge, and the supernatant was collected (26). Liver tissue homogenate was utilized to evaluate the levels of specific oxidative stress parameters (MDA, GSH, and SOD) *via* Enzyme-Linked Immunosorbent Assay (ELISA) [27].

Biochemical tests

Serum samples obtained post-sacrifice of the animals were utilized to assess ALT and AST levels, as per the kit methodology. All reagents, samples, and standards were prepared. Hundred (100 µL) of either the standards or serum samples was added to each well of the ELISA plate, which had been pre-coated with the specific antibody. The plate was subsequently-incubated at 37 °C for one hour, without performing a wash step, with 100 µL of the resultant solution. Detection Reagen A, a biotin-conjugated antibody, was added to each well. The plate was thereafter incubated at 37 °C for one hour. Subsequent to incubation, the wells were aspirated and subjected to three rinses with a 1× Wash Solution utilizing a spray bottle. The excess liquid was removed by flipping the dish onto absorbent paper. Subsequently, 100 µL of the prepared Detection Reagent B (avidin conjugated to horseradish peroxidase, HRP) was added to each well, and the plate was incubated for 30 minutes at 37 °C.The wells were then aspirated and rinsed five times as previously described. Subsequently, 90 µL of the Substrate Solution containing tetramethylbenzidine (TMB) was added to each well, and the plate was incubated for 10 to 20 minutes at 37 °C. Subsequently, 50 µL of the Stop Solution (sulfuric acid) was added to each well to terminate the reaction. The absorbance of each well was thereafter measured immediately [28].

Histological examination

A portion of hepatic tissue from each rat group was fixed in 10 % formalin prepared in PBS and stored at 4 °C for one week for histological analysis. Hepatocel-

lular necrosis was assessed by sectioning formalin-fixed liver tissues into 5 μm slices and staining them with hematoxylin and eosin (H&E) and viewed under a light microscope [29].

Statistical Analysis

All data are expressed as means ± standard deviation (SD). Statistical significance was evaluated using one-way analysis of variance (ANOVA) with GraphPad Prism version 9.5.1, supplemented by the Post Hoc Tukey test for inter-group comparisons. A P-value below 0.05 was considered significant [30].

RESULTS

Effects of Dapagliflozin and Silymarin against Cyclophosphamide-Induced Hepatotoxicity:

Effects on the serum ALT level

Data analysis revealed no statistically-significant change ($P > 0.05$) in serum ALT levels between the CMC-treated rats (Group II) and the negative control (Group I), as illustrated in Figure 1. A notable elevation ($P > 0.05$) in serum ALT level was detected in rats administered Cpd intraperitoneally (30 mg/kg/day, Group III) relative to both the negative control (Group I) and the CMC (Group II). Group III exhibited serum ALT level of 47.14 ± 1.351, compared to 25.49 ± 0.6817 in Group I and 25.56 ± 1.868 in Group II, respectively. Furthermore, results depicted in Figure 1 indicated a substantial reduction ($P < 0.05$) in serum ALT level in Group IV rats, which received oral administration of Dapa with Cpd for 10 days, in comparison to the serum level in rats of the induction Group III/Cpd. Values were (31.54 ± 0.8265) compared to (47.14 ± 1.351). Furthermore, data presented in Figure 1 indicated a substantial drop ($P < 0.05$) in serum ALT levels in rats of Group V, which were orally-supplied silymarin with Cpd for 10 days, compared to the serum levels in rats of Group III (the induction Group). The relative levels are (32.13 ± 1.436) and (47.14 ± 1.351). Moreover, data depicted in Figure 1 indicated that there were no significant variations (P > 0.05) in serum ALT levels between Group IV rats and Group V rats. Levels were (31.54 ± 0.8265) and (32.13 ± 1.436), respectively.

Effects on the serum AST level

Data analysis indicated non-significant changes ($P > 0.05$) in serum AST levels between the CMC/Group II rats and the negative control/Group I rats, with respective levels of (14.33 ± 1.227) and (13.86 ± 0.7663), (Figure 2). Furthermore, a substantial rise ($P < 0.05$) in serum AST levels in Cpd/Group III rats compared to the serum levels of the negative control/Group I; where, values were respectively, (46.7 ± 2.519) compared to (13.86 ± 0.7663). In addition, the data depicted in Figure 2 indicated a substantial reduction ($P < 0.05$) in serum AST levels in Group IV, which received oral administration of Dapa with Cpd for 10 days, compared to the serum levels in rats of the induction, IP Cpd (Group III). Levels were respectively, (24.62 ± 2.4) compared to (46.7 ± 2.519). Furthermore, a substantial rise ($P < 0.05$) in serum AST levels in Group IV rats, which were orally provided Dapa with Cpd for 10 days, compared to the serum levels in Group V rats, which received Sil with Cpd for

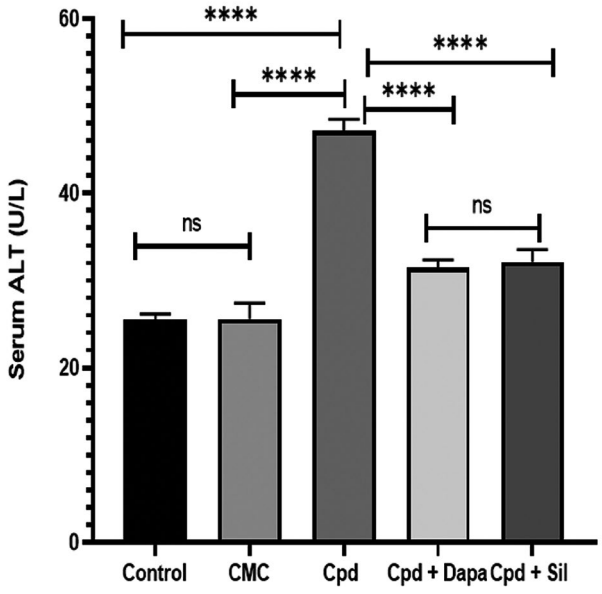

Figure 1. The Serum Levels of Alanine aminotransferase (ALT) according to Rats' Group (Data are expressed as Mean ± SD, *n* = 10; **: The highest Significant (*P* < 0.0001); ns: Non Significant)**

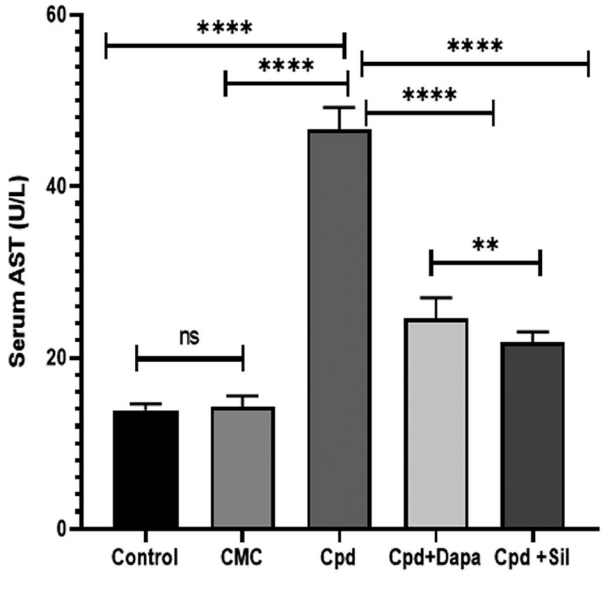

Figure 2. The Serum Levels of Aspartate aminotransferase (AST) according to Rats' Group (Data are expressed as Mean ± SD, *n* = 10; **: The highest Significant (*P* < 0.0001); **: Highly-Significant (*P* < 0.01); ns: Non Significant (*P* > 0.05))**

the same duration. The levels are (24.62 ± 2.4) compared to (21.79 ± 1.221). Besides, the outcomes depicted a significant reduction ($P < 0.05$) in serum AST level in rats of Group V, which were orally-administered sil with Cpd for 10 days, compared to the serum levels in rats of Group III (the induction); the respective levels were (21.79 ± 1.221) versus (46.7 ± 2.519).

Impact on Tissue Malondialdehyde (MDA) Concentration

Data analysis indicated that there were no significant differences ($P > 0.05$) in tissue MDA levels between the CMC/Group II rats and the negative control/Group I rats (Figure 3).

Furthermore, Figure 3 demonstrated a substantial rise ($P > 0.05$) in tissue MDA levels in Cpd/Group III animals compared to the tissue levels in the negative control/Group I. Values were (804.1 ± 111) compared to (241.8 ± 39.63).

Moreover, results depicted in Figure 3 indicated a substantial reduction ($P < 0.05$) in tissue MDA levels in Group IV rats fed Dapa orally with Cpd for 10 days, in comparison to the equivalent tissue levels in the induction Group III animals treated with Cpd. Levels are (161.7 ± 30.36) compared to (804.1 ± 111), respectively. Moreover, a significant reduction ($P < 0.05$) in tissue MDA levels in Group V rats, which were supplied silymarin orally with IP injection of Cpd for 10 days, compared to the equivalent tissue levels in Group III rats, the induction group. Values were (260.3 ± 14.52) compared to (804.1 ± 111). In addition, there was a significant reduction ($P < 0.05$) in tissue MDA levels in Group IV rats administered Dapa orally with Cpd for 10 days, compared to the tissue levels in Group V rats administered silymarin with Cpd for the same duration, measuring (161.7 ± 30.36) versus (171.3 ± 16.29).

Effects on the Reduced Glutathione (GSH) in Rats' Liver Tissue

Data analysis of Figure 4 revealed that, there were non-significant differences ($P > 0.05$) in tissue GSH level in rats of CMC/Group II compared to the corresponding tissue level in negative control/Group I rats. Moreover, a substantial drop ($P < 0.05$) in tissue GSH levels in Cpd/Group III animals in comparison to the negative control/Group I, with values of 16.31 ± 2.763 versus 34.87 ± 0.6828, respectively. Furthermore, a substantial elevation ($P < 0.05$) in tissue GSH levels in the Dapa orally supplied with Cpd for 10 days (Group IV) compared to the comparable tissue levels in the induction group (Group III) receiving Cpd. The relative levels are (47.2 ± 1.318) and (16.31 ± 2.763). Besides, the data represented in Figure 4 indicated a substantial rise ($P < 0.05$) in tissue GSH levels in rats from Group V which received oral silymarin with Cpd for 10 days, compared to the comparable tissue levels in rats from Group III, the induction group receiving only Cpd. The values were (44.69 ± 3.68) compared to (16.31 ± 2.763), respectively. Besides, a substantial elevation ($P < 0.05$) in tissue GSH levels in the group of rats that received oral Dapa with Cpd for 10 days (Group IV) compared to the levels in Group II rats that were supplied CMC orally. Levels are accordingly 47.2 ± 1.318 versus 33.7 ± 3.65. Moreover, there were no significant changes ($P > 0.05$) in tissue GSH levels between Group IV rats and Group V rats. In addition, there was a substantial rise ($P < 0.05$) in GSH tissue levels in the group of rats, which were orally supplied sil with Cpd for 10 days (Group V) compared to the comparable tissue levels in Group II rats that received CMC orally. Levels are (44.69 ± 3.68) compared to (33.7 ± 3.65).

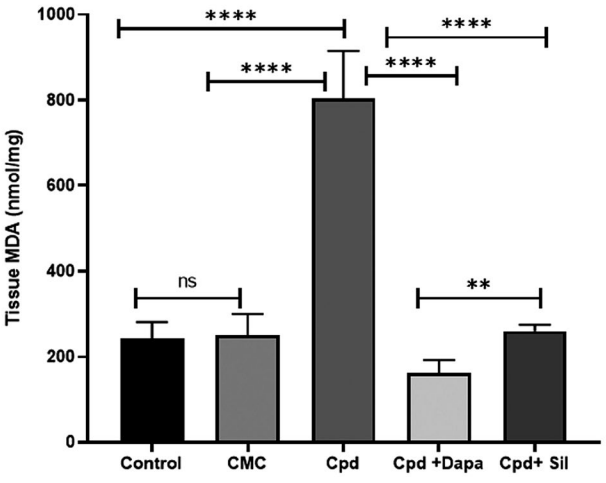

Figure 3. The Hepatic tissue Levels of malondialdehyde (MDA) according to Rats' Group (Data are expressed as Mean ± SD, $n = 10$; **: The highest Significant ($P < 0.0001$); **: Highly-Significant ($P < 0.01$); ns: Non Significant ($P > 0.05$))**

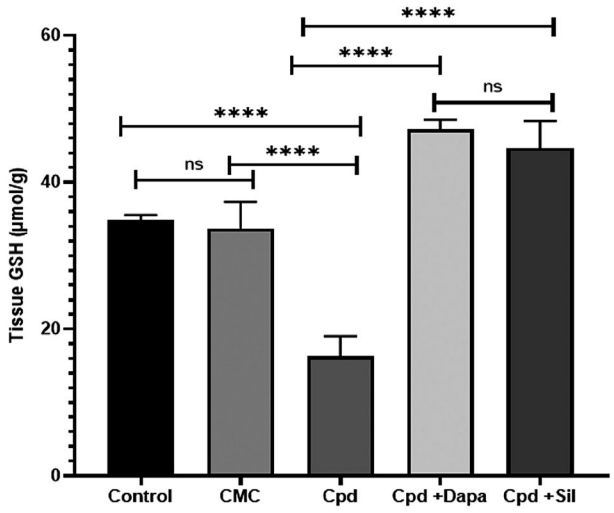

Figure 4. The Tissue Reduced Glutathione (GSH) Levels according to Rats' Group (Data are expressed as Mean ± SD, $n = 10$; **: The highest Significant ($P < 0.0001$); ns: Non Significant ($P > 0.05$))**

Effects on Hepatic Tissue Superoxide Dismutase level (SOD)

Figure 5 indicated that there were no significant differences ($P > 0.05$) in tissue SOD levels between the CMC/Group II rats and the similar tissue levels in the negative control/Group I rats.

Moreover, Figure 5 indicated a substantial decrease ($P < 0.05$) in tissue SOD levels in the Cpd/Group III rats compared to the negative control/Group I tissue levels. The levels were (7.936 ± 0.4482) compared to (64.88 ± 4.466). Furthermore, data presented in Figure 5 indicated a substantial elevation ($P < 0.05$) in tissue SOD levels in Group IV rats fed Dapa orally with Cpd for 10 days, compared to the equivalent tissue levels in the induction Group III animals receiving Cpd. The levels were (75.16 ± 4.096) and (7.936 ± 0.4482), respectively. Furthermore, results depicted in Figure 5 indicated a significant increase ($P < 0.05$) in tissue SOD levels in rats of Group V, which were orally administered Sil with Cpd for 10 days, compared to the corresponding tissue levels in rats of Group III, the induction group; where the levels were (65.35 ± 4.05) versus (7.936 ± 0.4482), respectively. Additionally, there were significant increase (P<0.05) in tissue SOD level in Group IV rats/Dapagliflozin orally-administered with Cpd compared to such tissue level in rats orally-administered silymarin with Cpd/ Group V. (75.16 ± 4.096) versus (65.35 ± 4.05).

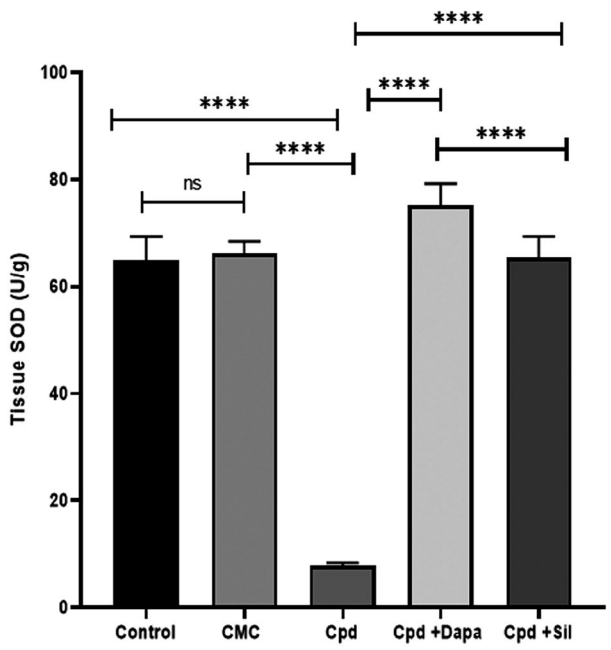

Figure 5. The Superoxide Dismutase (SOD) Tissue Levels according to Rats' Group

Histological Examination of Rats' Hepatic Tissues

The hepatic tissue segment of the experimentally healthy male rats (Group I/Negative Control) which were supplied distilled water orally, exhibited a normal histological architecture of the liver; additionally, there was an absence of congestion, inflammation, and a normal duct was observed (Figure 6).

The liver section of Group II/Positive Control male rats, which orally-administered 2 % aqueous solution of a CMC via rats oral gavage daily for 10 days showed that, there were – no congestion in the central vein or in sinusoids; but, there were mild inflammatory cells infiltrate were seen (Figure 7).

Besides, in liver tissue sections of male rats IP injected with Cpd 30 mg/kg/day for 10 consecutive days (Group III/Induction group) showed that, there were mild-congestion with mild-inflammatory cell infiltrate; and, with feathery degenerative changes in hepatocytes (a form of hepatocyte death that associated with cholestasis); moreover, there were proliferation of the bile duct, (Figure 8).

Furthermore, the section of liver tissue of male rats of Group IV/ [orally-administered Dapa (3 mg/kg/day B.W) dissolved in CMC for 10 days with Cpd (30 mg/kg/day) IP for 10 consecutive days], showed mild congestion (black arrow) with no inflammatory cell infiltrate. In

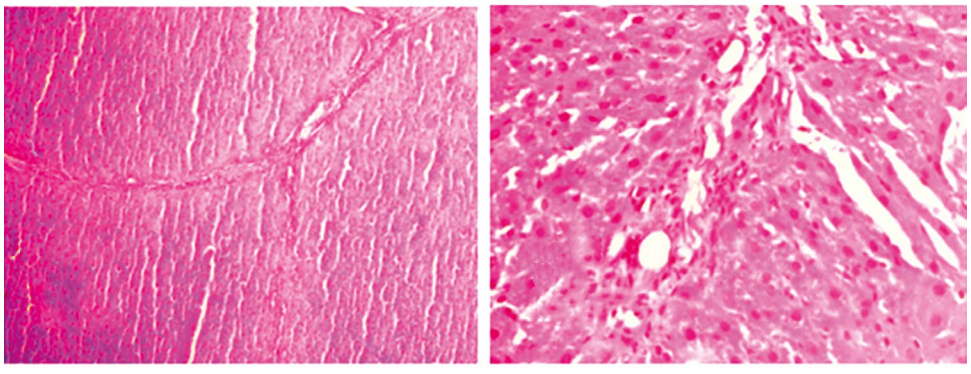

Figure 6. Hepatic section of Group I rats (Negative Control/Distilled water). Hematoxylin and Eosin stain; showed normal histological architecture of the liver mice. No congestion, no inflammation and normal duct; A: 1X, B: 40X

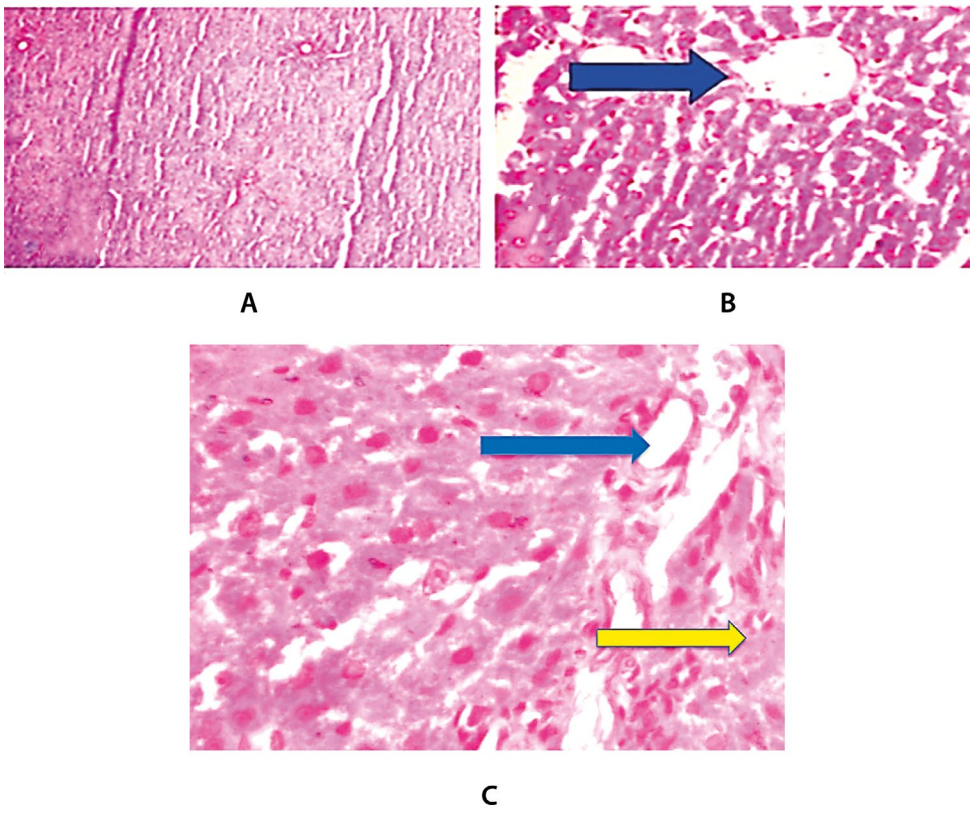

Figure 7. The effect of carboxymethyl cellulose (CMC) pretreatment on the histological architecture. No congestion in all sections in central vein or in sinusoids (blue arrows). Mild inflammatory cell infiltrate (yellow arrow), A: 10X; B: 20X; C: 40X

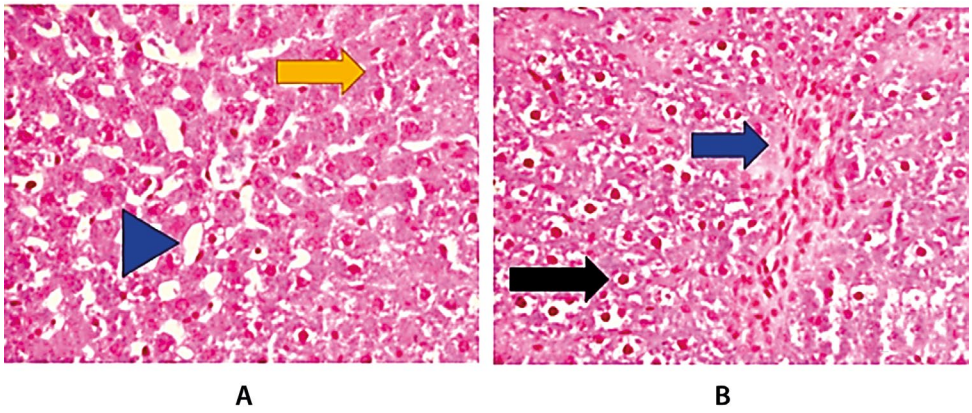

Figure 8. Photomicrographs showed the effect of intraperitoneal injection of cyclophosphamide (Cpd) 30 mg/kg/day for 10 days on the histological architecture. A: Mild congestion is seen (blue arrows). Mild inflammatory cell infiltrate (yellow arrow). B: Feathery degenerative changes in hepatocytes (black arrow) and bile duct proliferation (head-blue arrow), 40X

addition, the hepatocytes are normal with normal bile duct, (Figure 9).

In addition, sections of liver tissue of male rats of Group V Rats, which orally-administered [sil (200 mg/kg) dissolved in CMC with Cpd (30 mg/kg/day) IP injected for 10 consecutive days], there was mild congestion in the central vein (blue arrows); mild inflammatory cell infiltrate (yellow arrow) with normal hepatocytes (black arrow) were also seen (Figure 10).

DISCUSSION

Administration of Cpd (Group III) resulted in a marked increase in serum ALT and AST levels, signifying hepatocellular damage. Cpd metabolites (phosphoramide mustard, acrolein) generate reactive oxygen species (ROS) that compromise hepatocyte membrane integrity, resulting in cytosolic leakage of these enzymes [31, 32]. Treatment with dapagliflozin (Group IV)

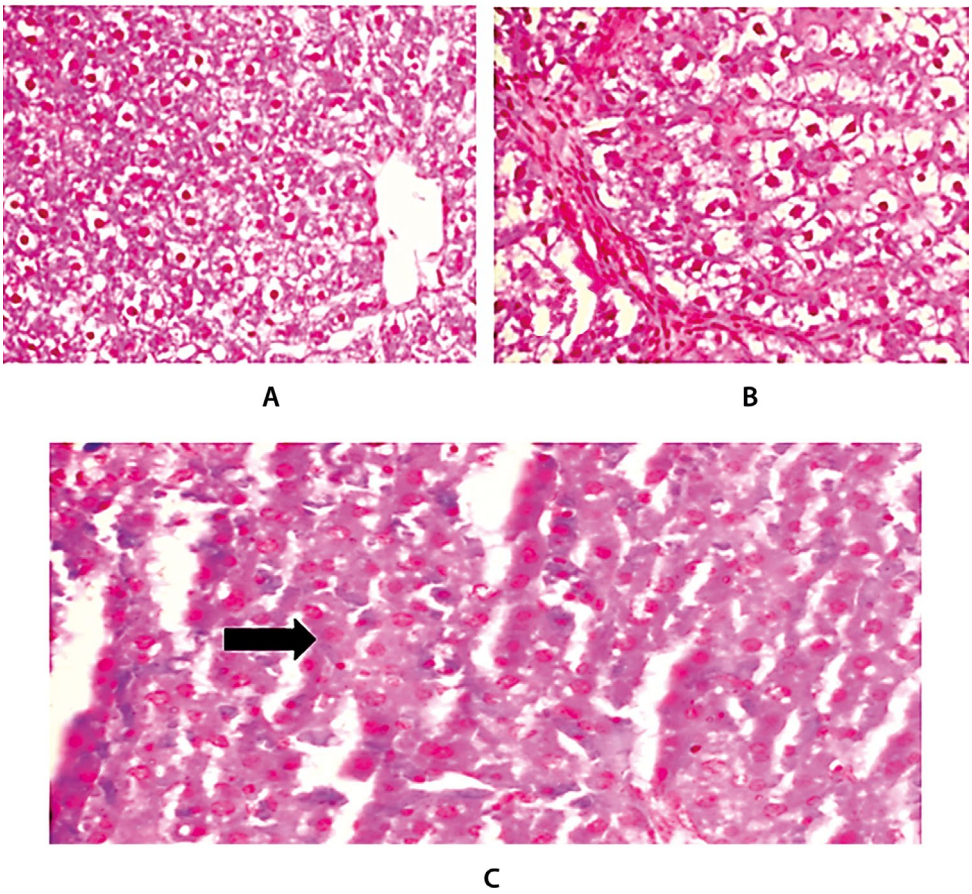

Figure 9. The effect of dapagliflozin pretreatment on the histological architecture. Mild congestion is seen (black arrow)/ (photomicrograph, no inflammatory cell infiltrate, the hepatocytes are normal with normal bile duct, 40X)

and silymarin (Group V) each with Cpd significantly reduced these enzyme levels compared to the Cpd/ Group III rats, indicating strong hepatoprotective effects. Notably, no statistically-significant difference ($P > 0.05$) was observed between dapagliflozin and silymarin in improving these parameters. These results are consistent with those of prior studies documenting the hepatoprotective effects of dapagliflozin and silymarin. For example, dapagliflozin has been shown to markedly decrease ALT and AST levels in a cisplatin-induced hepatotoxicity model [33], while silymarin has demonstrated consistent efficacy in reducing liver enzymes across various types of hepatic injury [34].

With regard to oxidative stress markers (MDA, GSH, and SOD), Cpd exposure led to elevated MDA and decreased GSH and SOD levels, reflecting enhanced oxidative stress [35]. Treatment with either dapagliflozin or silymarin each significantly-mitigated these changes, restoring antioxidant enzyme levels and reducing lipid peroxidation. These outcomes confirm the antioxidant properties of both agents, aligning with previous research [36]. Dapagliflozin has been reported to elevate antioxidant enzyme activity and decrease oxidative stress in diabetic rat models. For instance, El-Sawy et al. (2024) demonstrated that dapagliflozin reduced oxidative stress and boosted antioxidant defenses in a doxorubicin-induced nephrotoxicity model [37]. Similarly, the antioxidant effects of silymarin have been widely demonstrated. Recent studies reported that silymarin increased GSH and SOD while lowering MDA levels, thus alleviating oxidative injury [38]. A 2024 and 2025 meta-analysis confirmed that silymarin significantly reduced MDA (SMD: −1.69; $p < 0.001$) and increased SOD levels (SMD: 3.39; $p = 0.001$) [39, 40]. Other investigations further validated its protective efficacy in nephrotoxicity and salinomycin-induced toxicity models, with consistent antioxidant modulation [41].

Histopathological analysis revealed that Cpd-induced hepatic injury was characterized by congestion, inflammatory cell infiltration, and hepatocyte degeneration, as confirmed by recent studies [42]. In our experiment, treatment with dapagliflozin or silymarin alleviated these histological abnormalities and helped preserve normal hepatic architecture. This protective effect is supported by previous research confirming both drugs' ability to reverse histological damage in liver injury models [43]. For dapagliflozin, Zhao et al. (2023) showed that it improved liver histology in diabetic mice with NAFLD through downregulation of dipeptidyl peptidase-4 (DPP4), reducing hepatic steatosis and insu-

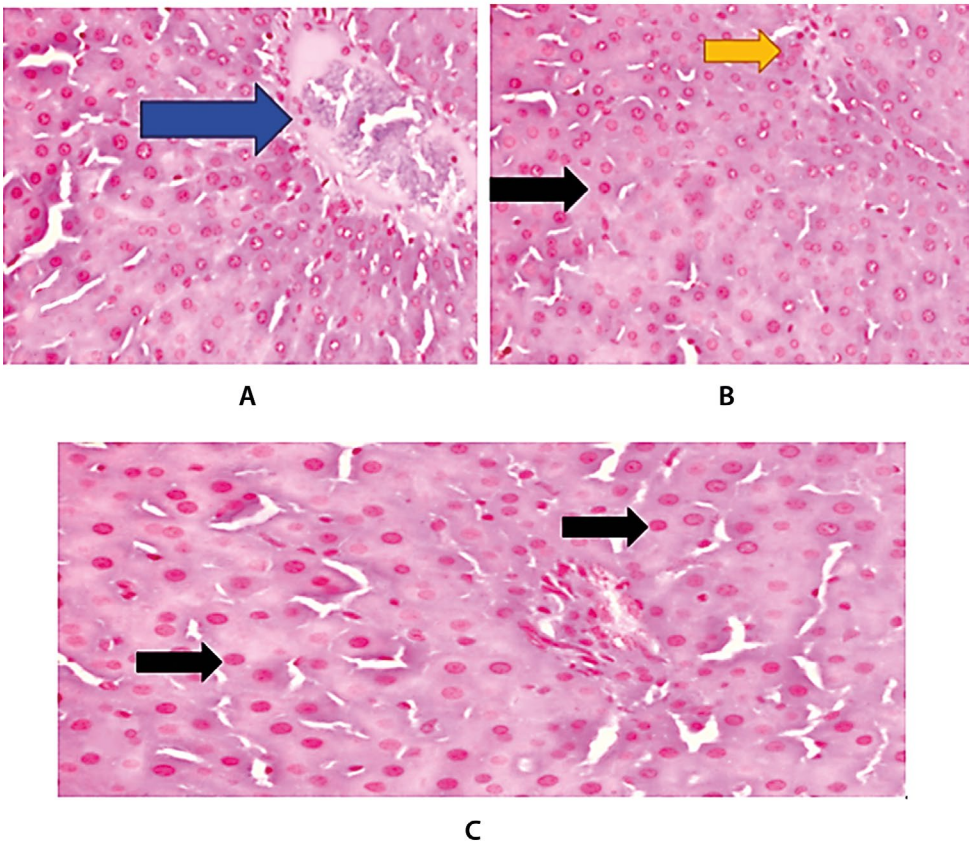

Figure 10. The effect of silymarin [(200 mg/kg) dissolved in CMC] and administered with IP injection of Cpd (30 mg/kg/day) for 10 consecutive days on the histological architecture. A: Mild congestion is seen in central vein (blue arrows). B: Mild inflammatory cell infiltrate (yellow arrow) with normal hepatocytes (black arrow). C: 40X. All the results summarized in the below graphical abstract

lin resistance [44]. Sayour et al. (2025) demonstrated that dapagliflozin mitigates steatosis, inflammation, and fibrosis, highlighting its multifaceted hepatoprotective action [45].

Silymarin, a flavonoid complex extracted from milk thistle, has been extensively evaluated for its hepatoprotective potential [46]. A 2024 systematic review by Amjad et al. involving 26 randomized controlled trials reported that silymarin significantly reduced ALT and AST levels, with improved hepatic steatosis in histology [47]. A global consensus in 2022 further endorsed silymarin's use for a range of liver disorders, including alcoholic liver disease, NAFLD, and drug-induced hepatotoxicity, attributing its efficacy to its antioxidant, anti-inflammatory, and antifibrotic effects [48].

Unlike the research conducted by Satyam et al. (2024), which investigated the synergistic hepatoprotective effects of dapagliflozin and silymarin in a carbon tetrachloride-induced hepatotoxicity model with an emphasis on the modulation of the Nrf2/HO-1 signaling pathway, the current study is the inaugural effort to comparatively assess the distinct hepatoprotective effects of dapagliflozin and silymarin against cyclophosphamide-induced hepatic injury in rats. Our model features a unique mechanism of liver injury–primarily through acrolein-induced oxidative stress and inflammation–offering a new framework for evaluating hepatoprotection. Furthermore, our study incorporates a thorough biochemical, oxidative stress, and histological analysis to evaluate the protective efficacy of each medicine independently, rather than collectively. This methodology provides novel insights into the potential of dapagliflozin and silymarin as therapeutic alternatives instead of synergistic drugs, thereby addressing a therapeutically pertinent concern overlooked by prior studies [38]. Satyam et al. investigated the synergistic benefits of a combination therapy (dapagliflozin + silymarin) in a CCl_4-induced liver injury model; however, their study presented several drawbacks.

1. Absence of Individual Assessment: They did not assess the individual hepatoprotective efficacy of dapagliflozin and silymarin. The efficacy of each medication individually vs the superiority of their combination remains ambiguous.
2. Model-Specific Limitations: The research was performed using a carbon tetrachloride (CCl_4) model, which primarily produces hepatic injury through free radical production and lipid peroxidation. This does not comprehensively depict the mechanism of hepatotoxicity caused by chemotherapeutic me-

dicines such as cyclophosphamide (CPd), which produces acrolein and phosphoramide mustard, resulting in direct oxidative stress, mitochondrial malfunction, and inflammation.

3. Insufficient Clinical Relevance for Chemotherapy-Induced Injury: Their model failed to replicate the hepatotoxicity commonly seen by cancer patients undergoing chemotherapy (e.g., CPd-induced hepatic damage). Consequently, the relevance to oncology or chemotherapy contexts is restricted.

How Our Research Addresses This Gap:

1. A head-to-head comparison of dapagliflozin and silymarin as monotherapies is conducted to elucidate their individual efficacy.
2. We employ a CPd-induced liver injury model that more accurately represents chemotherapy-associated hepatotoxicity, yielding clinically relevant insights.
3. Our study provides a thorough assessment of hepatoprotection through biochemical, oxidative stress, and histological analyses.
4. Dapagliflozin alone demonstrates comparable efficacy to silymarin, indicating its potential repurposing as a standalone hepatoprotective agent in chemotherapy protocols.

CONCLUSION

This study demonstrates that both dapagliflozin and silymarin possess substantial hepatoprotective properties against cyclophosphamide-induced hepatic damage in rats. Each of these medicines significantly diminished hepatic enzyme increases, reduced oxidative stress, and improved histopathological changes. Our data indicate no statistically significant difference in hepatoprotection between the two agents, suggesting that dapagliflozin – a medicine typically utilized for diabetes – may be equally effective as the established hepatoprotective agent silymarin. In contrast to the study by Satyam et al. (2024), which examined the synergistic effects of combination therapy in a CCl_4-induced model, our research provides the inaugural direct comparison of the individual efficacy of dapagliflozin and silymarin in a cyclophosphamide-induced hepatotoxicity model. This additional scenario underscores the therapeutic potential of dapagliflozin as an independent drug for alleviating chemotherapy-induced liver damage and broadens its applicability beyond glycemic regulation.

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