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Effect of Effervescent Formulation of Silymarin against Experimental Hepatotoxicity in Rats: Involvement of NRF2/HO-1, PI3K/AKT and TLR4/NFκB Pathways

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ABSTRACT

Despite the exploration of pharmaceutical and natural liver-protecting drugs, the number of hepatotoxicity deaths continues to rise. Milk thistle flavonoids, silymarin, have been widely investigated and demonstrated liver disease protection. Poor solubility and absorption restrict silymarin bioavailability. Effervescent formulations may boost silymarin absorption by improving gastrointestinal solubility. Our study compared the effectiveness of silymarin effervescent formulation in protecting the liver from thioacetamide (TAA)-induced oxidative damage at both low and high doses. Study findings may have an implication on chemotherapy-induced off-target harmful oxidative insult. 24 adult male rats were separated into normal control, hepatotoxic (TAA) (100 mg/kg body weight), and TAA plus silymarin (50 and 100 mg/kg b.wt) groups. Serum liver enzyme, hepatic antioxidant, lipid peroxidation, and inflammatory indicators were measured. The modest dose's great bioavailability was shown by its potency being identical to the doubled dose. TAA caused hepatic injury, as evidenced by elevated liver enzymes (ALT and AST) and tissue levels of MDA, TLR4, TGFβ1, TNF-α, IL-6, NF-κB, and p-NFκB. TAA also decreased tissue SOD, GSH, and HO-1 levels as well as Nrf2 level and expression. Increased gene expressions of Akt, PI3K, and TLR4 were observed. Silymarin's antioxidant and anti-inflammatory actions reduced hepatotoxicity via upregulating Nrf2/HO-1 and downregulating PI3K/Akt and TLR4/NFκB pathways. This study shows that effervescent silymarin formulation is a powerful bioavailable treatment for liver oxidative toxicity in chemotherapy-related toxicities.

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INTRODUCTION

The liver plays a significant role in the body's process of detoxifying dangerous medications, xenobiotics, and organic pollutants **(Shirani *et al.*, 2020; Taleb *et al.*, 2023)**. The prevalence and mortality of liver disease remain major global issues. The main factors known to induce liver damage are viral hepatitis, antibiotics, chemotherapeutic drugs, hepatotoxins including carbon tetrachloride (CCI4), and TAA **(Teksoy *et al.*, 2020; Bashandy *et al.*, 2022; Elbaset *et al.*, 2023a; Elbaset *et al.*, 2023b).**

Thioacetamide (TAA) is a thio-sulfur-containing chemical **(Abd El-Rahman and Fayed 2019)**. One dosage of TAA administration induces acute liver damage (Chen *et al.*, 2008). Reactive oxygen species (ROS) and TAA intermediates are formed when cytochrome P450 metabolically activates TAA. Following their binding to physiologically significant molecules, these ROS can amplify cellular oxidative stress, lipid peroxidation, and glutathione depletion **(Pallottini *et al.*, 2006)**. TAA has been a widely used model for research on liver damage and fibrosis **(Sarma**

and Hanzlik 2011). TAA offers a liver-damaging model with a high ability for regeneration that is similar to that of a human with protracted injury.

Further, the TAA liver toxicity is similar to the pathological oxidative burden induced by chemotherapeutic agents **(Delire *et al.*, 2015)**. A significant event in the pathogenesis of many hepatic diseases is oxidative stress. Hepatocytes' mitochondria and endoplasmic reticulum are where the cytochrome P-450 enzymes mostly create ROS **(Arauz *et al.*, 2016)**. Liver cells can regulate the level of oxidative stress and the balance of oxidant and antioxidant molecules under physiologically normal circumstances. While under stressful circumstances, oxidative stress is increased, and the liver's capacity to maintain this balance is compromised **(Cichoz-Lach and Michalak 2014)**.

Natural remedies are increasingly popular for preventing and treating liver disease **(Abdelbaset *et al.*, 2014; Ayoub *et al.*, 2022; Ogaly *et al.*, 2022; Taleb *et al.*, 2023)**. There is evidence that oxidative stress-related liver damage can be mitigated by antioxidants and free radical-scavenging substances **(Bruck *et al.*, 2004; Elbaset *et al.*, 2022; Moussa *et al.*, 2022)**. It is generally known that silymarin, derived from the milk thistle (Silybum marianum), has hepatoprotective and antioxidant activities **(Vargas-Mendoza *et al.*, 2014).** Silymarin is composed of the flavonoids silybin, silydianin, and silychristin. **(Wu *et al.*, 2009)**. **Saller *et al.*, (2001)** showed that silymarin protects the liver from oxidative stress caused by alcohol. Additionally, silymarin has been demonstrated to protect the liver against carbon tetrachloride (CCl_4) damage and the kidney from acetaminophen damage **(Lamia *et al.*, 2021).** These effects are primarily attributable to decreased lipid peroxidation **(Chen *et al.*, 2012)**.

Manna *et al.* (1999) showed that silymarin has limited bioavailability due to poor solubility and absorption. This means a large portion of the ingested silymarin isn't absorbed by the body. Since silymarin solubility is essential for achieving an ideal and effective bioavailability, formulation techniques aimed at enhancing its solubility are vital. According to the reviewed research, silymarin-based formulations have become more advanced and efficient over time, which makes sense given the development of nanotechnologies and nano systems intended to enhance the delivery of active key bioactive molecules that are poorly soluble in water **(Di Costanzo and Angelico 2019).** Drug delivery is an active area of research **(Zahin *et al.*, 2020; Ahmad *et al.*, 2021).**

Further, effervescent formulations offer a potential solution by enhancing silymarin's dissolution in the gastrointestinal tract, leading to increased absorption **(Ahmad *et al.*, 2023).** These findings motivate us to investigate silymarin's effervescent formulation as a potent bioavailable hepatoprotective role in TAA toxicity as well as molecular approaches upon toxicity.

MATERIALS AND METHODS

1. Animals

At the National Research Centre (NRC, Egypt), 24 Wistar rats, males (180-220 g; 7 weeks) were acquired from the "Animal House." The rats were housed in an environment with a constant temperature of 25 ˚C and 12-hour light/dark cycles. Adherence to both national and international ethical standards was ensured in the treatment of the animals. Approval for all experimental procedures was obtained from the Institutional Animal Care and Use Committee at Cairo University. (Approval certificate: Vetcu23052022441).

2. Chemicals

The supplier of thioacetamide was "Sigma-Aldrich, USA". Silymarin, Na HCO3, sodium citrate, citric acid compounds were provided with the best analytical-grade ingredients. Silymarin effervescent formula: Silymarin 78%, Na HCO3 19%, sodium citrate 1%, citric acid 2%.

3. Experimental design

Four sets of six rats each were randomly distributed; The rats in the first set were designated as the control group, received intraperitoneal injections of normal saline three times weekly for a duration of two weeks (0.5 ml/ 100 g rat). The rats in the second set (referred to as the TAA-treated group) were administered intraperitoneal injections of TAA (100 mg/kg body weight) three times weekly over a period of two consecutive weeks. The third sets of rats were given silymarin at a dosage of 50 mg/kg/day orally for two weeks following the TAA injections in a similar manner to the second group. The fourth set of rats were administered silymarin at a dosage of 100 mg/kg/day orally for a period of two weeks after receiving TAA injections following the same protocol as the second group.

4. Preparation of blood samples and tissue specimens

Twenty-four hours after the end of the experiment, during the administration of ketamine for sedation, blood samples were collected from the tail vein of each rat. Serum samples intended for biochemical analysis were maintained at a temperature of -20°C. Following the extraction of blood samples, rats were euthanized through cervical dislocation, and expeditiously, their livers were extracted, cooled using ice-cold saline, and desiccated. The left lobe of the liver from each rat was excised and preserved in buffered neutral formalin at a concentration of 10% for

subsequent histological investigations. A portion of this tissue was also weighed and stored at -80°C for advanced molecular and biological scrutiny.

5. Evaluation of biomarkers for liver function

Colorimetric analysis was performed on the aspartate aminotransferase (AST) (Catalog #AS1061, Biodiagnostic®, Egypt) and alanine aminotransferase (ALT) (Catalog #AL1031, Biodiagnostic®, Egypt) activities in serum, as well as the serum albumin levels (Catalogue #AB1010, Biodiagnostic®, Egypt).

6. Evaluation of the markers of oxidative stress

The levels of "glutathione (GSH) content, malondialdehyde (MDA), and superoxide dismutase (SOD)" were assessed in liver tissue samples using particular colorimetric assays (BioVision, Milpitas, CA, USA; Cat: No: K464-100, K739-100, and K335-100, respectively), in order to assess oxidation stress and antioxidant defense.

7. Biomarkers of liver inflammation and pro-fibrosis by enzyme-linked immunosorbent assay (ELISA)

Using particular Rat ELISA kits (SunLong Biotech Co., LTD, China; Catalogs Number: SL0722Ra, SL0411Ra, SL0537Ra, and SL0705Ra, respectively), the important signals in liver injury and inflammation, tumor necrosis factor (TNF-α), interleukin-6 (IL-6), nuclear factor-kappa B (NF-κB), and transforming growth factor-beta (TGF-β1) were assayed in liver homogenate as per the manufacturer's guidelines. Toll like receptor 4 “TLR4” in hepatic homogenate was tested using the production protocols of Sunlong Biotec Com. LTD in Zhejiang, China (Catalog No. SL0699Ra).

8. Nrf2 and HO-1 in the liver using enzyme-linked immunosorbent assays (ELISA)

The ELISA kits (Sunlong Biotech Co., Zhejiang, China) were used to quantify the liver level of nuclear factor erythroid 2-related factor-2 (Nrf2) (SL0985Ra) and heme oxygenase 1 (HO-1) (SL0341Ra).

9. QRT-PCR for the expression Nrf2, AKT, PI3K and TLR4

RNA extraction

For the reverse transcription and PCR of the isolated RNA, the SuperScript IV One-Step RT-PCR kit (Cat# 12594100) was provided by Thermo Fisher Scientific, located in Waltham, Massachusetts, USA. A thermal profile was executed utilizing a 96-well plate StepOne equipment from Applied Biosystems, USA, the initial denaturation phase is comprised of 40 cycles, with each cycle lasting 10 s at 98 °C, 10 s at 55 °C, and 30 s at 72 °C for the amplification step. Following this, the reverse transcription process extends over a duration of 10 minutes at 45 °C, succeeded by RT inactivation lasting 2 minutes at 98 °C. Upon completion of the RT-PCR procedure, the data concerning the target genes and housekeeping genes were presented using the cycle threshold (Ct) method. Oligonucleotide sequences for the forward and reverse primers are detailed in **Table (1).** The mRNA levels of tested genes Nrf2, AKT, PI3K, and TLR4 were adjusted for variability using the mean critical threshold (CT) expression.

Table 1: List of “oligonucleotide primers” used in qPCR.

Gene		Sequence (5′-3′)	
Nrf2	FD	AAAATCATTAACCTCCCTGTTGAT	NM_010902.5
	RE	CGGCGACTTTATTCTTACCTCTC	
AKT	FD	CGGATACCATGAACGACGTAG	NM_033230.2
	RE	GCAGGCAGCGGATGATAAAG	
PI3K	FD	TTAAACGCGAAGGCAACGA	XM_032898971.1
	RE	CAGTCTCCTCCTGCTGTCGAT	
TLR4	FD	CCGTCACCACATACTGCCTTTA	NM_001109668.1
	RE	GCAGTTTGGACTATTGAAATACGAAA	

10. Examination of the histopathology

Samples collected from the livers of rats from different groups underwent standardized processing to create paraffin sections following a twenty-four-hour fixation in a 10% concentration of neutral buffered formalin. Subsequently, the specimens were rinsed with distilled water, dehydrated using ethanol solutions, and clarified with xylene. Subsequent to this, sections measuring four to five micrometers in thickness were generated from the paraffin blocks. Following the placement of the tissue sections on glass slides, deparaffinization was carried out, followed by staining with hematoxylin and eosin (H&E). An experienced evaluator conducted all histopathological examinations, maintaining blinding throughout the sample identification process to prevent bias.

The assessment of histological impairments was conducted utilizing the METAVIR test, which categorizes the extent of fibrosis and includes various indicators of liver damage such as bile duct proliferation, steatosis, portal vein dilatation, and fibrosis. These lesions were classified as follows: F0 = absence of lesions (-), F1 = presence of mild lesions (+), F2 = presence of moderate lesions (++), and F3 = presence of severe lesions (+++).

11. Statistical analysis

Prior to proceeding with the statistical analysis, the normality assumption was validated using the Shapiro-Wilk test. The results are expressed in the form of means ± standard error. Subsequently, the data underwent analysis through one-way ANOVA, followed by the Tukey-Kramer post hoc test. The statistical analysis and graphical representations were carried out using GraphPad Prism software (version 9, USA). A significance level of P<0.05 was chosen for all statistical tests.

RESULTS

Impact of silymarin on the serum levels of AST, ALT, and albumin in rats receiving TAA

As markers of liver damage, serum ALT, AST, and albumin were routinely measured. Rats given TAA injections showed a substantial increase in serum AST and ALT activities, whereas serum albumin was reduced in comparison to the non-treated control group. Silymarin administration significantly reduced the levels of AST and ALT and significantly elevated the albumin level as compared to the hepatotoxic group (**Fig.1 a, b, c**).

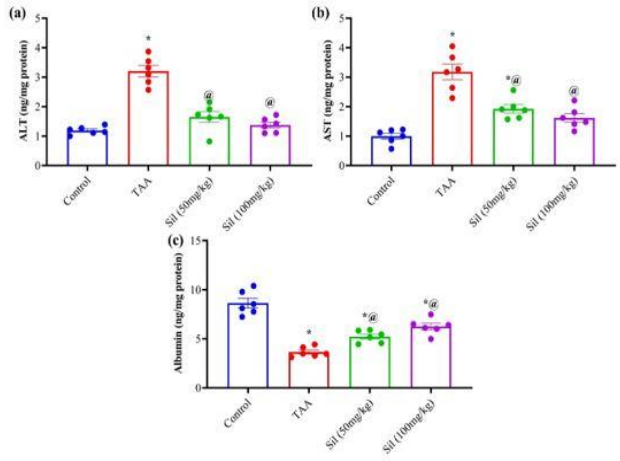

Fig.1: The impact of silymarin administration on the liver function indicators (a) ALT, (b) AST, and (c) albumin in rats subjected to thioacetamide-induced intoxication. Data are presented as mean ± SE. * vs. normal control group, @ vs. TAA group, # vs. Silymarin groups at p<0.05.

Impact of silymarin on oxidation stress in rats receiving TAA

Figure 2 (a, b, c) clearly showed how TAA had a negative impact on the liver's antioxidant defenses and significantly increased the lipid peroxidation product MDA in the liver tissue. According to the table, silymarin was able to restore the liver's antioxidant status by markedly elevating both the liver's SOD activity and GSH levels. Malondialdehyde levels were markedly reduced by silymarin to normal levels compared to the hepatotoxic group.

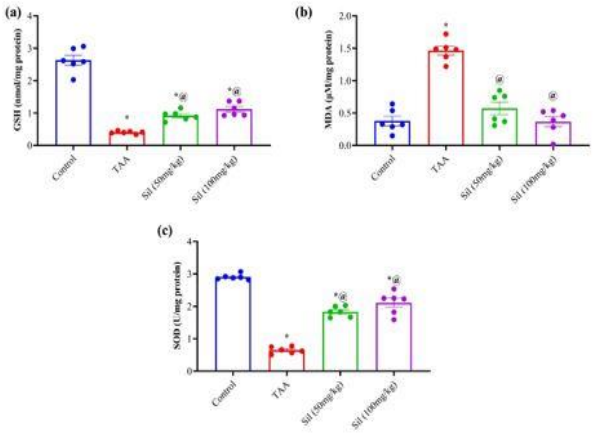

Fig. 2: The impact of silymarin administration on the liver oxidative stress indicators (a) GSH, (b) MDA, and (c) SOD in rats subjected to thioacetamide-induced intoxication. Data are presented as mean ± SE. * vs. normal control group, @ vs. TAA group, # vs. Silymarin groups at p<0.05.

Impact of silymarin on liver's profibrotic and inflammatory marker content in rats receiving TAA

In comparison to the negative control group, TAA dramatically elevated the levels of TLR4, TNF-α, IL-6, TGF β1, NF- κB, and p-NF-κB in the rats' livers (**Fig. 3 a-f)**. Treatment with silymarin significantly and dose-dependently decreased the levels of aforementioned parameters in the rat livers compared to the TAA-treated group. Silymarin at a dose of 100 mg/kg revealed the best effects in inhibition of inflammatory response.

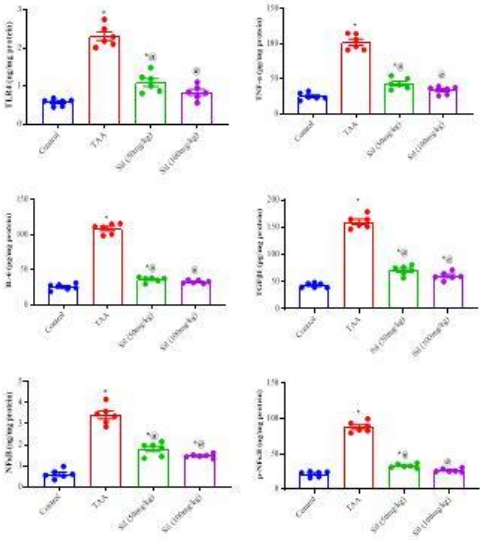

Fig. 3: The impact of silymarin administration on the liver profibrotic and inflammatory indicators (a) TLR 4, (b) TNF-α, (c) Il-6, (d) TGF1β-1, (e) NF-κB, and (f) p-NF-κB in rats subjected to thioacetamide-induced intoxication. Data are presented as mean ± SE. * vs. normal control group, @ vs. TAA group, # vs. Silymarin groups at $p<0.05$.

Impact of silymarin on the liver levels of Nrf2 and HO-1 in rats receiving TAA

Hepatic Nrf2 and HO-1 levels significantly decreased in the TAA group compared to the negative control group **(Figs. 4 a, b)**. Rats given both dosages of silymarin, as opposed to the TAA group, exhibited a marked rise in Nrf2 and HO-1 levels.

Impact of silymarin on genes expression of Nrf2, TLR4, AKT, and PI3K in rats receiving TAA

In hepatic tissue, the relative expression of the mRNAs for Nrf2 **(Fig.5d)** showed that TAA significantly downregulated their expression; meanwhile, the mRNAs for TLR4, Akt, and PI3K expressions were significantly upregulated when compared to the control groups **(Fig. 5 a, b, c)**. Silymarin administrations could up regulate Nrf2 expression and down regulate PI3K, Akt, and TLR4 expressions, compared with the TAA group.

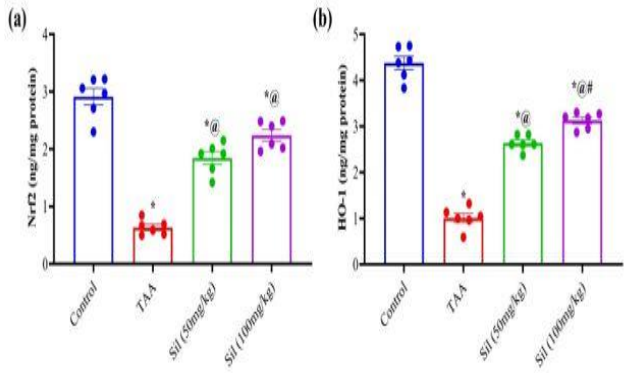

Fig.4: The impact of silymarin administration on the liver antioxidant proteins (a) Nrf2 and (b) HO-1 in rats subjected to thioacetamide-induced intoxication. Data are presented as mean ± SE. * vs. normal control group, @ vs. TAA group, # vs. Silymarin groups at $p<0.05$.

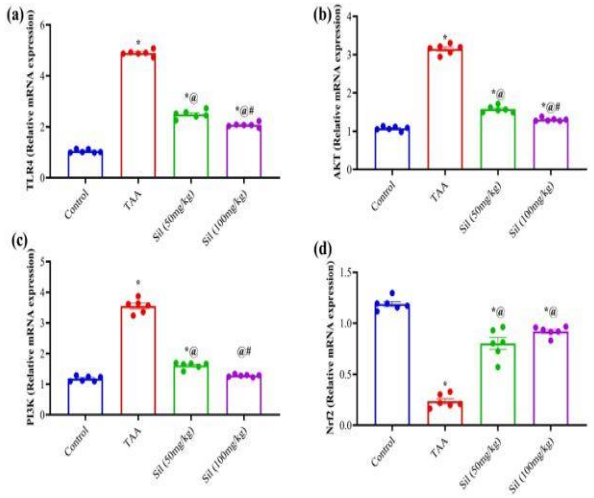

Fig. 5: The impact of silymarin administration on the liver mRNA expression (a) TLR4, and (b) AKT, (c) PI3K, and (d) Nrf2 in rats subjected to thioacetamide-induced intoxication. Data are presented as mean ± SE. * vs. normal control group, @ vs. TAA group, # vs. Silymarin groups at $p<0.05$.

Impact of silymarin on liver histopathology in rats intoxicated with TAA

Liver specimens from the control group rats exhibited a normal histological arrangement of central veins, portal regions, and hepatic cell cords **(Fig. 6).** Conversely, liver specimens from the TAA group rats demonstrated a profound fibrotic response. The hepatic capsule displayed distinct corrugation, accompanied by the absence of typical hepatic lobulation. Fibrous proliferation extensively enlarged the portal triads, characterized by mononuclear inflammatory cell infiltration, proliferation of cholangiolar epithelium leading to multiple newly formed bile ductules, and vascular congestion

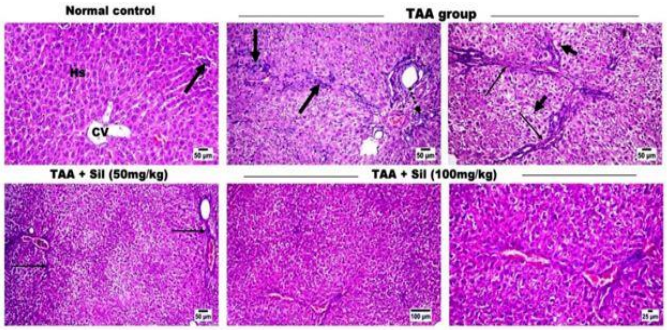

Fig. 6: Impact of silymarin on liver histopathology in rats intoxicated with TAA

Table 2: Showing scoring of the histological lesions observed.

Item	Control group	TAA group	TAA+Sil (50mg/kg)	TAA+Sil (100mg/kg)
Fibrosis	-	+++*	+@	+@
Bile duct proliferation	-	+++*	++*	+@
Steatosis	-	+++*	++*	+@
Dilatation of portal vein	-	+++*	+@	+@

Data are presented as mean ± SE. * vs. normal control group, @ vs. TAA group, # vs. Silymarin groups at $p<0.05$.

METAVIR score

Severity of liver fibrosis were graded as (- equal no lesion), (+++ equal severe fibrosis including nodules, sepat, and portal tract fibrosis), (++ equal moderate fibrosis including sepat and portal tract fibrosis), (+ mild portal tract fibrosis) **(Table 3).**

Table 3: showing grading of histological fibrosis using the METAVIR score.

score	Histological character	Normal control group	TAA group	TAA+Sil (50mg/kg)	TAA+Sil (100/kg)
		The number of animals showed the lesion per group			
F0	No fibrosis	6	-	-	-
F1	Portal fibrosis	-	-	2	5
F2	Portal fibrosis with few septa	-	-	4	1
F3	Portal fibrosis with numerous septa	-	6	-	-
F4	Cirrhosis	-	-	-	-

DISCUSSION

Silymarin, a group of flavonoids found in milk thistle, has been extensively studied and shown protective activity in liver diseases **(Gillessen and Schmidt 2020).** However, silymarin has limited bioavailability due to poor solubility and absorption. This means a large portion of the ingested silymarin isn't absorbed by the body. Effervescent formulations offer a potential solution by enhancing silymarin's dissolution in the gastrointestinal tract, leading to increased absorption. Our study showed that the small dose and doubled dose of silymarin have a very similar potency of reversing the liver toxicity induced by TAA, reflecting the potent bioavailable efficacy of the small dose of the effervescent formulation. A model that can give inference to the potent bioavailable effect in treating pathological oxidative burden of chemotherapeutic drugs. The liver's biotransformation of TAA into TAA sulfoxide and thioacetamide-S, S-dioxide makes TAA a powerful centrilobular hepatotoxicant. Due to their ability to alter lipid and protein structure, these reactive metabolites are extremely harmful to hepatocytes **(Hajovsky *et al.*, 2012; Ayoub *et al.*, 2022; Elbaset *et al.*, 2022; Abdel-Rahman *et al.*, 2023)**.

Accordingly, our study found that TAA rats had elevated levels of hepatic injury markers and disrupted hepatic lobules, both of which suggested injured hepatic cells. The current study's findings, along with those of earlier studies, showed that TAA can cause liver oxidative damage that is shown as lipid peroxidation and impairment of the liver's antioxidant defense mechanisms **(Ramadan *et al.*, 2018; Abd El-Rahman and Fayed, 2019)**. According to reports, silymarin exhibits protective effects across a variety of tissues and exhibits antioxidant activity **(Vargas-Mendoza *et al.*, 2014)**. Treatment with silymarin significantly reduced the levels of MDA, an indication of oxidative stress. In contrast, after silymarin administration, the activities of SOD and GSH in the liver tissues of hepatotoxic rats were significantly elevated.

TAA has the capacity to induce the release of several hepatotoxic cytokines from immune cells **(Jiao *et al.*, 2022)** i.e., TNF-α, IFN-γ, and IL-6. Through the TLRs signal pathway, these cytokines further contribute to the development of hepatocyte injury by stimulating and activating immune cells **(Xu *et al.*, 2013)**. A class of receptors known as toll-like receptors (TLRs) is implicated in inflammation and innate immunity. TLRs play a role in liver fibrosis, which is brought on by a variety of common liver infections, including parasite, viral, and toxin-induced hepatitis **(Cengiz *et al.*, 2015)**. Kupffer cells, hepatic stellate cells, hepatocytes, sinusoidal endothelial cells, biliary epithelial cells, and

hepatic dendritic cells are only a few of the liver cells that express TLRs broadly **(Xu *et al.*, 2011)**, which are crucial for the health of the liver **(Kim *et al.*, 2010)**.

Many therapeutic medications that prevent liver injury may interact with the TLR4 signaling system, according to previous studies that suggest that TLR-mediated signals are considered to be involved in practically all liver illnesses **(Zhai *et al.*, 2016).** When the TLR4 signal pathway is activated, a series of things happen, such as the nuclear factor kappa B (NF-κB) p65 moving into the nucleus, which increases the production and release of inflammatory cytokines (TNF-α, TGFβ1, and IL-6) **(Lawrence 2009)**. Further, we inspected the TLR4 expression. We show that we found that effervescent silymarin formulation in lower dose administration after TAA toxicity was more potent than the doubled dose in the anti-inflammatory properties due to its significant suppression of TLR4 expression and blockage of the NF-κB signaling pathway. Our findings demonstrated that low-dose effervescent silymarin suppressed the generation of inflammatory cytokines, which are involved in TLR4-NF- κB signaling.

We thoroughly examined alternative potential mechanisms and found that effervescent silymarin formulation in lower dose administration after TAA toxicity with potency as the doubled dose altered the expression of many different genes in both up- and down-regulation. The cellular kinase PI3K produces the intracellular second messenger phospholipid PIP3 by upstream activating other signaling molecules like protein kinase B "Akt" (Troutman ***et al.*, 2012)**. Recently, a number of biological processes, including inflammation and cell differentiation, have been connected to the PI3K/Akt pathway **(Mohamed and Hafez 2019)**. Although the significance of PI3K/Akt in inflammation is debatable **(Troutman *et al.*, 2012),** our results, along with others, suggested that this pathway has a positive effect on inflammation in the liver **(Hazeki *et al.*, 2007; Kidd *et al.*, 2008)**. Silymarin downregulated the expression of the PI3K/Akt gene in hepatic tissue.

The hepatic tissue's normal pattern and antioxidant state were successfully restored by the low-dose effervescent silymarin. It was shown that an essential mechanism in normal liver cells to counteract oxidative stress is mediated by nuclear factor erythroid 2-related factor (Nrf2) and its antagonist kelch-like ECH-associated protein 1 (Keap1) **(Wang *et al.*, 2016)**. The antioxidant response element (ARE), which modifies detoxification and controls the production of antioxidant genes, is thought to be primarily directed by Nrf2 **(Raslan *et al.*, 2021)**. In a healthy state, Keap1 and Nrf2 interact, and Nrf2 remains in the cytoplasm. Oxidative stress causes Nrf2 to separate from Keap1 and migrate to the nucleus, where it binds to ARE and activates downstream particular genes, such as quinone oxidoreductase 1 (NQO1), heme oxygenase (HO-1), and glutathione, which work to combat oxidative stress. Therefore, treatments that might activate Nrf2 may be helpful in reducing liver damage caused by oxidative stress. The current study concentrated on lower-dose effervescent silymarin's capacity to upregulate the antioxidant genes HO-1 and Nrf2 in comparison to controls and to the doubled-dose treatments. Rasha *et al*., who attributed silymarin's protective effect against TAA-induced acute liver damage to its induction for the Nrf2/HO-1 pathway, provide strong support for our findings **(Hussein *et al.*, 2021)**. Another investigation examined silymarin's potential to shield rats from acute kidney damage brought on by paraquat by inhibiting Nrf2, which reduced oxidative stress and cellular damage **(Jia *et al.*, 2022).**

Silymarin effervescence at a relatively low dose (50 mg/kg body weight) was potent and had a strong bioavailable effect when comparing the treated TAA group to the naive control, regarding the normalization of Nrf2/HO-1, PI3K/Akt, and TLR4/NF-κB values of the TAA silymarin-treated group to almost normal control levels. Compared to non-formulated silymarin (200 mg/kg body weight) in previous reports **(Tsai *et al.*, 2008).**

It's worth mentioning that it has been reported that albino rabbits were used in in vivo experiments to quantify the pharmacokinetics of both the enhanced effervescent formulation with 200 mg of silymarin and the regular standard dosage form. After oral administration, the plasma concentration of silymarin was measured. The formulation's Cmax increased by 180%. For the formulation, the area under the curve was 310% greater **(Ahmad *et al.*, 2023).**

Regarding the clinical relevance of using silymarin as a protective adjunct in chemotherapeutic treatments, silymarin may have the ability to modulate the xenobiotic system by affecting the activity of metabolizing phase I and phase II enzymes, leading to the protection of healthy cells from the insults of chemotherapeutic drugs. Additionally, silymarin and its primary bioactive ingredients block organic anion transporters (OAT) and ATP-binding cassettes (ABC), which helps to fight possible chemoresistance. Silymarin and its derivatives have two main roles: they prevent cancer cells from progressing through various stages, which forces them to evolve toward cell death, and they accumulate cancer cells in a particular stage of the cell cycle, which enables a particular anticancer agent to target a larger quantity of tumor cells **(Delmas *et al.*, 2020).**

CONCLUSION

Silymarin effervescent low dose was potent and had a strong bioavailable effect compared to the doubled dose in reversing oxidative hepatotoxicity via its antioxidant and anti-inflammatory effects attributed to the upregulation of Nrf2/HO-1, downregulation of PI3K/Akt, and TLR4/NFκB pathways. Further, the present study highlights effervescent silymarin as a potential formulation giving protection against chemotherapy-related toxicities in a clinical setting.

Conflicts of interest

The authors declare that there is no conflict of interest regarding the research data and tools used in this study.

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