SILYMARIN AMELIORATES DIETHYLNITROSAMINE-INDUCED LIVER FIBROSIS IN WISTAR RATS

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ABSTRACT

Aim: the aim of this study was to compare the impact of silymarin on the liver fibrosis induced by diethylnitrosamine (DEN) between both sexes of Wistar rats and proposing possible mechanisms.

Main Methods: twenty-four Wistar male and twenty-four Wistar female rats were randomly assigned into 8 groups according to their sex (n=6) for administration of vehicle, DEN, silymarin or both DEN and silymarin for 8 weeks. At the end of the experiment, the traditional rat body and liver weight parameters, liver injury biomarkers (serum ALT, AST, ALP, and total bilirubin) were measured. Furthermore, hematological parameters, lipid profiles (TC, LDL-C, HDL-C, and TG) and oxidative stress biomarkers (TBARS, SOD, CAT, and GSH) were determined. Also, the inflammatory biomarkers in liver tissue homogenate (TNF-α, TGF-β) were evaluated. Histopathological subjective scoring system graded the damage markers of liver tissue. Expression of NF-kB was measured immunohistochemically.

Results: Markedly diminished DEN induced liver fibrosis markers in female groups while worsened in male groups. Silymarin regimen improved liver functions and fibrosis markers. Additionally, it counteracted DEN-induced oxidative stress, lipid peroxidation and inflammations, silymarin provided these ameliorative effects either in males or females rats.

Conclusion: Silymarin plays an ameliorative role of DEN-induced liver fibrosis in male and female rats via reducing oxidative stress and inflammations.

Keywords: DEN, liver fibrosis, silymarin, inflammations, and oxidative stress.

Introduction

liver is identified as the largest solid organ in the human body, its weight is between 1,200–1,500 g, making up around 1/50th of an adult's total body weight **(Dooley et al., 2018)** Around 2 million people globally pass away from liver disease each year, with one million of those fatalities coming from cirrhosis complications, one million from viral hepatitis and hepatocellular carcinoma (HCC) **(Mokdad et al., 2014)**. Following liver injury, extracellular matrix is deposited coinciding with processes of wound healing and regeneration; nevertheless, repeated injury due to immune diseases, fast food, alcohol, environmental toxins, or chronic viral hepatitis (HBV, HCV, etc.) may result in a progressive fibrotic response that is characterized by an excessive collagen accumulation. As normal liver regeneration becomes increasingly limited by hepatic fibrosis, the risk of liver failure rises. Additionally, liver fibrosis provides an environment that is conducive to the development of liver cancer through poorly understood mechanisms **(Affo et al., 2017)**.

Liver hepatic stellate cells (HSCs), the primary collagen-producing cells in the liver are the driving cause of hepatic fibrosis. In a physiological condition, quiescent HSCs act as the body's primary location for retinyl ester storage, which is apparent as fat droplets in the cytoplasm. Damage-associated molecular patterns and pathogen-associated molecular patterns generated by injured hepatocytes cause liver injury and cause quiescent HSCs that store retinoid droplets to trans-differentiate into myofibroblast-like that is highly fibrogenic, via enhanced transforming growth factor beta 1 (TGF-β1)signaling, proliferative and migratory, via platelet-derived growth factor receptor beta signaling, contractile, via activation of endothelin 1 (ET-1) signaling; and pro-inflammatory, via interaction with monocyte-derived macrophages **(Tsuchida & Friedman, 2017).**

N-nitroso compounds risk for cancer was first made widely known in 1937,by reporting that dimethylnitrosamine (DMN) might be one of the potential causes that induce liver injury in human **(FREUND, 1937).** Many other N-nitroso compounds, notably diethylnitrosamine (DEN), have been linked to hepatotoxic and cancerous consequences **(Druckrey et al., 1967)**. Inducing hepatic tumors in animals through continuous oral feeding or parenteral administration of DEN in high dosages is remarkably effective **(Magee & Barnes, 1956)**. A number of studies have shown that an important factor in the pathogenesis of hepatocarcinoma is the excessive generation of ROS during the hepatic metabolization of DEN. HCC can develop as a result of intracellular signaling pathways disruption, oxidative damage to DNA, lipids, and proteins by ROS **(Poli et al., 2004)**.It was proven that liver homogenates can metabolize DEN and other nitrosamines into their corresponding aldehydes and chemically reactive alkylating species**(Challis and Rayman, 1973; Magee & Barnes, 1956)** and DNA**(Lin & Hollenberg, 2001)**,and forms specific DNA adducts**(Carlson et al., 2017; Asamoto et al., 1991)**.However, age, sex, species, and strain have a major impact on the metabolic dealkylating effect of DEN**(Liu et al., 2013)**.

In the recent two decades, there has been a lot of attention paid to the therapeutic use of natural ingredients **(Gillessen & Schmidt, 2020)**. Since ancient times, milk thistle (Silybum marianum) has been used safely as herbal remedy to treat liver diseases. In

preclinical research, silymarin, a milk thistle bioactive extract, has demonstrated antioxidant and hepatoprotective activities**(Van Pelt et al., 2003; Dehmlow, et al., 1996)**. Silymarin exhibit the capacity to protect mammals livers against liver toxicity induced by ethanol**(Zhang et al., 2013),** carbon tetrachloride **(Yadav et al., 2008)**, and acetaminophen **(Avizeh et al., 2009)**. This study aimed at investigating the potential protective role of silymarin on DEN-induced hepatic fibrosis in rats and establishing the possible protective mechanisms. In addition DEN and /or silymarin's effects were compared in male and female rats.

Materials and methods:

Chemicals, reagents, and kits:

DEN was purchased from Sigma-Aldrich Co. (St. Louis, MO, USA) (CAS No: 55-18-5, MDL No: MFCD00013890). Silymarin was purchased from (Rottapharm|MadausEgypt). The Egyptian International Pharmaceutical Industries Company (EPICO) (Cairo, Egypt) was the source of thiopental sodium. Isotonic saline was bought from El-Nasr Pharmaceutical Chemicals Company (Abou-Zaabal, Egypt). Povidone-iodine was purchased from the El-Nile for Pharmaceuticals & Chemical Industries Company (Cairo, Egypt). The rat kits for ALT, AST, ALP, and total bilirubin were purchased from Bio-Med Company (Cairo, Egypt). Rat's ELISA kits for TNF-α, TGF-β 1, TBARS, SOD, CAT, and GSH were purchased from Bio-diagnostic Co. The serum lipid profile levels of total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), and triglycerides (TG) were purchased from Bio-diagnostic Co. for research kits, Egypt. Santa Cruz Biotechnology Company's (Sino thinker. sk9000, U.S). (SCBT) sc-8008 was the source of anti-NF-B p65 antibody (F-6) (Texas, USA). All other chemicals were purchased as high grade from commercial vendors.

Pre-experimental preparations:

Adult Wistar albino rats weighing between 150 and 170 g were purchased from the animal house at the El-Nile Company for Pharmaceuticals & Chemical Industries. Rats were housed six in a cage for two weeks under regulated housing settings (room temperature 25± 2°C , humidity 50 to 70 %, and 12/ 12 dark/ light cycle) while being fed a regular diet and having unlimited access to food and drink.

Experimental design

The investigation was conducted on rats for 8 weeks, with silymarin administration beginning one week prior to the DEN-induced liver fibrosis (LF) and continuing throughout the DEN treatment course. A total of 48 rats (24 males and 24 females) were divided into eight groups (n=6) randomly as follows:

1) Male-Control (n=6): Rats were treated with vehicle (0.2ml) orally once daily over the experiment.
2) Male+Silymarin (n=6): Rats, treated with silymarin (50 mg/kg) orally once daily over the experiment.

3) Male-DEN (n=6): Rats were treated with DEN (100 µl/L) free access in drinking water.
4) Male-DEN+Silymarin (n=6): Rats were co-administered with DEN and silymarin as previous regimen.
5) Female-Control (n=6): Rats were treated with vehicle (0.2ml) orally once daily over the experiment.
6) Female-Silymarin (n=6): Rats were administered silymarin (50 mg/kg) orally once daily over the experiment.
7) Female-DEN (n=6): Rats were treated with DEN (100 µl/L) free access in drinking water.
8) Female-DEN+Silymarin (n=6): Rats were co-administered with DEN and silymarin as previous regimen.

The model of DEN-induced LF in rats was produced by continuous oral dosing of DEN in drinking water at 100 µl/L (Fathy et al., 2017) for 8 weeks, the appropriate dosing of silymarin was given in a dose of (50 mg/kg/day, P.O) (Pradeep et al., 2007). However, this regimen was based on our pilot study data.

Experimental ending:

Following general anesthesia with thiopental (50 mg/kg, i.p.) and retro-orbital plexus hemorrhage, the blood samples were collected from each rat at the conclusion of the treatment session in accordance with (Sharma et al., 2014). The extracted samples were allowed to clot for 15–30 minutes at room temperature, after which the sera were separated by centrifugation at 5000 rpm for 30 minutes in a refrigerator-cooled centrifuge. Once the isolated sera had been processed for biochemical analysis, they were kept at -20°C. Rats were executed by using the cervical dislocation technique in conformity with guidelines for standard animal euthanasia methods created in 2010 by the Canadian Council on Animal Care **(Canadian Council on Animal Care, 2010).**

Liver function and lipid profile biomarkers:

The quantitative colorimetric analysis for serum levels of the conventional liver function biomarkers (ALT, AST, ALP, and total bilirubin) and lipid profile (triglycerides, TG; total cholesterol, TC; high-density lipoprotein cholesterol, HDL-C; low-density lipoprotein cholesterol, LDL-C) was conducted using the automated clinical chemistry analyzer (Spinlab) photometer after calibration according to supplier company instructions for kits.

Hematological Parameters:

The erythrocyte count, total and differential leukocyte count, platelet count, hematocrite percentage, and hemoglobin concentration were measured in the blood by Using a CBC analyzer.

Histopathology:

Rat liver sections were removed from the animals as soon as they were sacrificed in order to be dissected for histopathological evaluation of liver tissue damage markers.

The livers were then fixed in 10% neutral formalin for two days, dehydrated in ethanol concentrations that increased, cleared in xylene, embedded in paraffin, and sectioned at a thickness of 4-5 µm. Hematoxylin and eosin (H&E) was used to stain the sections in accordance with **(Lefkowitch, 2006)**.The METAVIR scoring system was used to determine the extent of liver fibrosis **(Staub et al., 2009)**.The evaluation of the collagen expression in liver tissues was carried out in accordance with the Masson's trichrome staining **(Lefkowitch, 2006)**. The stained slides were inspected under a light microscope (Olympus BX43) and photomicrographs of various liver sections were taken using a digital camera (Olympus DP27) by a pathologist who was blinded to the treatment procedure.

Immunohistochemistry:

Sections of liver tissue were fixed with formalin and embedded in paraffin underwent heat-induced epitope retrieval technique (50°C for up to 60 minutes).Afterwards, a high-sensitivity visualization system was used to dilute and auto-stain the concentrated primary antibody (Anti-NF-B p65),(Envision™ FLEX, High pH, and Link system in the autostainer Link 48 where the software was pre-programmed in accordance with the manufacturer's instructions provided in the concentrated primary antibody package insert. A light microscope was used to view the sectioned slides and evaluate the expression of NF-*k*B. For each slide, six pictures were collected (x100). Using a (Olympus BX43) light microscope, photos were taken using the linked (Olympus DP27) camera and (CellSens Olympus software) and the positive immune staining was expressed as a percentage of the stained area.

ELISA assay:

Tissue levels of Tumor Necrosis Factor- α subtype (TNF-α), Transforming Growth Factor- β (TGF-β), Thiobarbituric Acid Reactive Substance (TBARS), Superoxide Dismutase (SOD), Catalase (CAT), and Reduced Glutathione (GSH) in liver tissue homogenates were measured in accordance with the standard manufacturer's instructions included in ELISA kits.

Statistical analysis

The statistical analysis was carried out using One-way analysis of variance (ANOVA) followed by post hoc Tukey-Kramer test for multiple comparisons between groups. All date was presented as means ± standard deviations. Differences between means were considered significant At P 0.05. The experiments were carried out at least twice using the PRISM (5.0 VERSION) program.

Results:

Silymarin prevents weight loss and reduces the relative liver weight ratio (RLWR) caused by DEN:

Body weight in the DEN-treated groups was considerably lower than in comparison to the control groups (Male-Control and Female-Control).Male-DEN, Male-

DEN+Silymarin, Female-DEN, and Female-DEN+Silymarin groups all had significantly higher RLWR values than the control groups. Moreover, Male-DEN group's RLWR was significantly greater than that of Female-DEN group. The Male-DEN+Silymarin and Female-DEN+Silymarin groups had a significant regression in RLWR values when compared to Male-DEN group. All differences between control groups were non significant **(Table.1).**

Table.1: Effect of DEN and/or silymarin on liver and body weight male and female rats

Groups	Final body wt. (g)	Gain in body wt. (g)	Liver wt. (g)	RLWR%
Male-Control	217.0 ± 9.53	35.00 ±9.47	6.34 ± 0.29	2.93 ± 0.20
Female-Control	214.2± 27.83	28.00± 5.73	6.137± 0.84	2.88±0.35
Male + Silymarin	194±21.02	35.33± 13.74	5.37 ±0.61	2.78 ±0.21
Female +Silymarin	196.3 ± 15.38	29.00 ± 20.06	5.90 ± 0.43	3 ± 0.27
Male-DEN	169.8±7.11[a]	-33.33 ± 14.78 [a]	16.54 ±2.26 [a]	10.58 ±0.93 [a]
Female –DEN	162.5 ± 27.03[a]	-5.500±20.87[a,b]	9.18± 0.67[a,b]	5.822± 1.262[a,b]
Male-DEN+Silymarin	172.2 ± 5.40[a]	1.67± 8.04[a,b]	10.16 ± 1.37[a,b]	6.19 ± 0.82[a,b]
Female - DEN+Silymarin	159.6 ± 10.00[a]	-4.5± 7.82[a,b]	10.62 ± 1.618[a,b]	6.69 ± 1.19[a,b]

Data were presented as means ± SD and statistically analyzed by one-way (ANOVA) test, followed by post hoc Tukey-Kramer test for multiple comparisons between groups. LF: liver fibrosis, DEN: diethylnitrosamine, wt: Weight, RLWR%: Relative Liver Weight Ratio

a:significant from control, b:significant from Male-DEN, c:significant from Female-DEN.

Silymarin influence on liver function markers:

In DEN insulted groups, especially those who were not protected by silymarin, we found a significant substantial increase in AST, ALT, ALP, and TBIL compared to the control group (s).In contrast to Male-DEN and Female-DEN groups, Male-DEN+Silymarin and Female-DEN+Silymarin groups showed a statistically significant decreased AST, ALT, ALP, and TBIL. There was no statistically significant difference in the previously mentioned biomarkers comparing the two last groups **(Fig.1).**

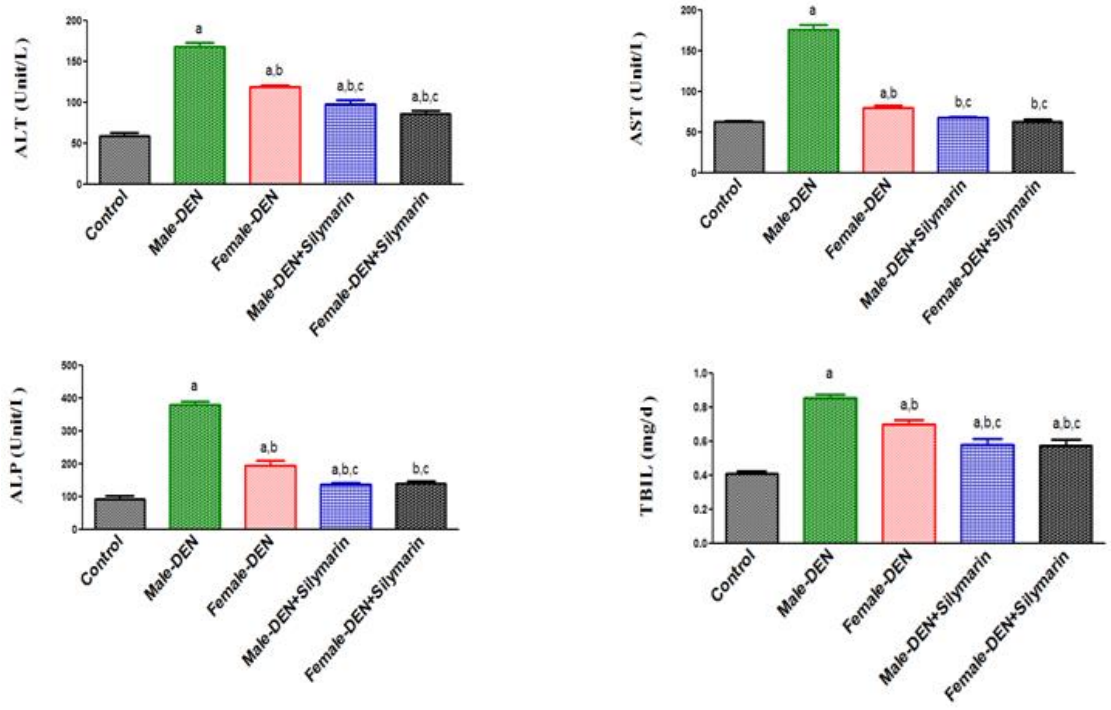


Fig.1: Effect of DEN and/or silymarin on liver functions in male and female rats, Data were presented as means ± SD and statistically analyzed by one-way (ANOVA) test, followed by post hoc Tukey-Kramer test for multiple comparisons between groups. LF: liver fibrosis, DEN: diethylnitrosamine, ALT: Alanine Aminotransferase, AST: Aspartate Aminotrasferase, ALP: Alkaline Phosphatase, TBIL: Total Bilirubin.

a:significant from control, b:significant from Male-DEN, c:significant from Female-DEN.

The impact of silymarin on the hematological parameters in DEN-induced LF

There was no difference between male and female control groups. Conversely, the DEN-treated male rats exhibited higher abnormal Red Blood Cells count, Hemoglobin (Hb) concentration, hematocrit (Hct) percentage, platelet count, and WBC count when compared to the control groups and female-DEN rats. Silymarin groups which administered DEN showed significant increase in all measured hematological parameters when compared to the group treated with DEN alone **(Table.2).**

Table.2: effect of silymarin on hematological parameters of DEN-induced LF in male and female rats

Groups	RBC count (10^6 corpuscle/mm^3)	Hb concentration (g/dL)	Hct (%)	Platelet count (10^3 platelet/mm^3)	WBC Count (10^3 cell/mm^3)
Male-Control	8.721±0.06	15.91 ±0.17	47.42 ±0.41	936.8 ±4.69	9.69±0.15
Female-Control	8.700± 0.04	15.56 ±0.13	46.88 ±0.16	931.2 ± 3.56	9.64± 0.06
Male+Silymarin	8.69±0.09	15.94 ±0.42	47.37 ±0.26	935.4 ±4.32	9.67±0.06
Female+Silymarin	8.630± 0.04	15.55 ± 0.14	47.04 ± 0.63	932.9 ±4.31	9.6±0.03
Male-DEN	6.610± 0.08^{a}	11 ±0.24^{a}	28.81 ± 0.45 ^{a}	526± 19.03 ^{a}	7± 0.24^{a}
Female-DEN	7.312± 0.04^{a,b}	13.04 ± 0.65^{a,b}	34.26 ± 2.93^{a,b}	623.1 ± 46.04 ^{a,b}	7.93± 0.26^{a,b}
Male-DEN+Silymarin	7.671±0.11^{a,b,c}	14.28 ± 0.49^{a,b,c}	39.86 ± 2.24 $^{a,b.c}$	692.8 ± 3.675^{a,b,c}	8.25± 0.34^{b,c}
Female-DEN+Silymarin	7.777± 0.19 ^{a,b,c}	14.51 ± 0.48^{b,c}	40.65 ±0.60^{a,b,c}	689.9 ± 10.27 ^{a,b,c}	8.53± 0.24^{b,c}

Data were presented as means ± SD and statistically analyzed by one-way (ANOVA) test, followed by post hoc Tukey-Kramer test for multiple comparisons between groups. LF: liver fibrosis, DEN: diethylnitrosamine, RBC, red blood corpuscle: Hb, hemoglobin; Hct: hematocrit; WBC: white blood cell count.

a:significant from control, b:significant from Male-DEN, c:significant from Female-DEN.

Table.3: Effect of DEN and /or silymarin on serum Lipid Profiles in male and female rats Data were presented as means ± SD and statistically analyzed by one-way (ANOVA) test,

Groups	LDL (mg/dl)	HDL (mg/dl)	T.Cholesterol (mg/dl)	Triglycerides (mg/dl)
Male-Control	27.72±5.435	58.87±15.84	102.1 ±9.644	78.90 ±2.60
Female-Control	30.68± 8.766	60.08±14.78	101.1 ±3.97	74.99 ± 15.10
Male + Silymarin	21.77±6.171	62.21±19.23	96.85 ±12.62	69.84 ±17.89
Female +Silymarin	24.27± 7.389	61.46± 22.91	103.4 ± 27.64	69.94 ±16.15
Male-DEN	83.37± 22.67^{a}	20.12 ± 6.154 ^{a}	191.1 ± 8.463^{a}	142.4± 5.13^{a}
Female –DEN	57.23± 15.46^{a,b}	40.01± 4.730^{a,b}	158.0 ± 15.33^{a,b}	120.8± 11.37^{a,b}
Male-DEN+Silymarin	56.73±12.44^{a,b}	57.42 ± 8.417 ^{b,c,}	122.5± 15.36 $^{b.c}$	91.08± 7.23^{b,c}
Female -DEN+Silymarin	40.21±8.147	66.48 ± 12.37 ^{b,c}	123.7 ± 24.7 ^{b,c}	76± 10.18 ^{b,c}

followed by post hoc Tukey-Kramer test for multiple comparisons between groups. LF: liver fibrosis, DEN: diethylnitrosamine, TG, triglycerides; TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol.

a:significant from control, b:significant from Male-DEN, c:significant from Female-DEN.

The DEN administered groups recorded a significant increase in serum levels of LDL-C, total cholesterol, and triglycerides with concomitant decrease in HDL-C ($p<0.05$) when compared to control groups. While silymarin-DEN administered groups recorded a significant decrease in LDL-C, total cholesterol, and triglycerides with concomitant increase in HDL-C when compared to the corresponding DEN-only administered groups ($p<0.05$) **(Table3).**

Lipid peroxidation and oxidative stress biomarkers:

The liver tissue levels of TBARS, SOD, CAT, and GSH are illustrated in **(Fig. 2).** Significantly Elevated TBARS and decreased SOD, CAT, and GSH levels was observed particularly in male rats when compared to females. Moreover, silymarin-treated rats revealed a significant reduction in levels of TBARS, increase in levels of SOD, CAT, and GSH when compared with their corresponding DEN-treated groups **(Fig.2).**

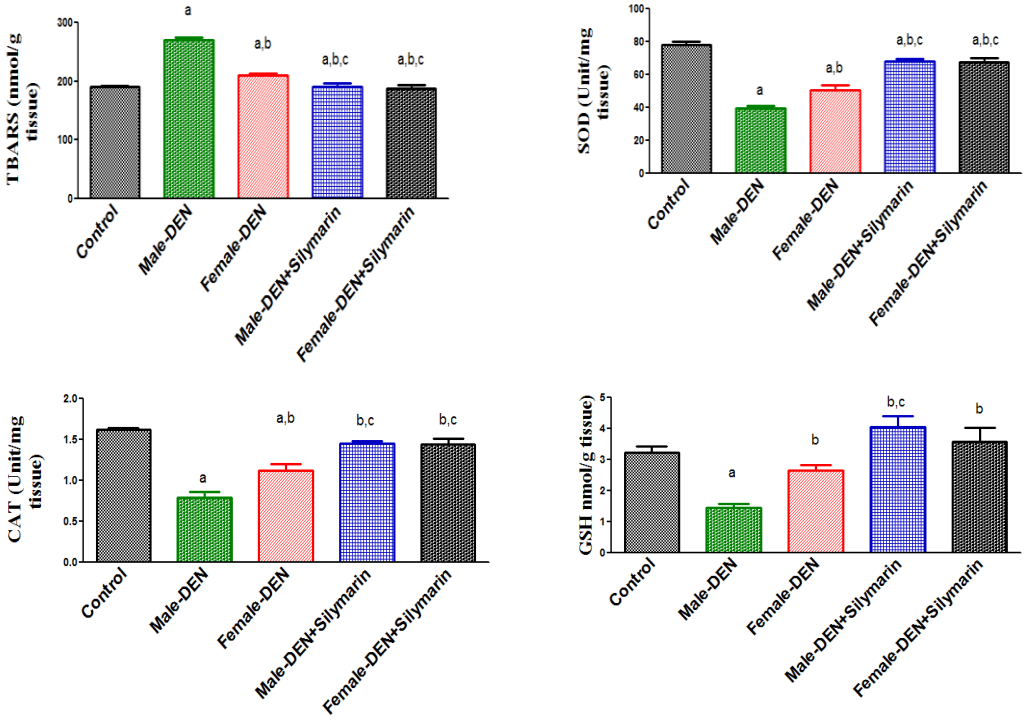


Fig.2: Effect of DEN and /or silymarin on oxidative stress biomarkers in male and female rats, Data were presented as means ± SD and statistically analyzed by one-way (ANOVA) test, followed by post hoc Tukey-Kramer test for multiple comparisons between groups. LF: liver fibrosis, DEN: diethylnitrosamine,TBARS: Thiobarbituric Acid Reactive Substance, SOD: Superoxide Dismutase, CAT: Catalase, GSH: Reduced Glutathione.

a:significant from control, b:significant from Male-DEN, c:significant from Female-DEN

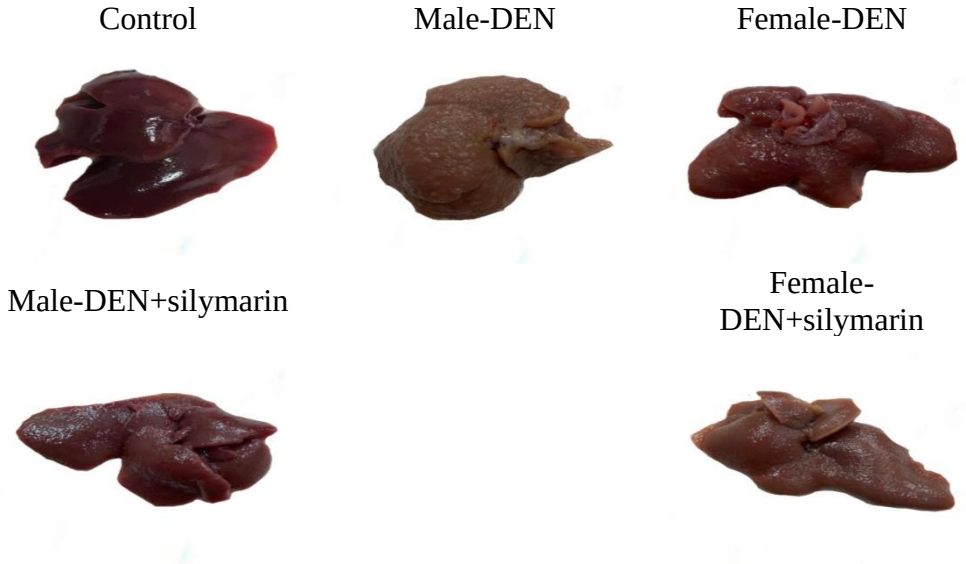
Rat liver histopathological abnormalities were decreased by silymarin:

Liver tissues from control groups showed normal smooth surfaces with an average portal tracts and portal veins. They also showed average hepatocytes in peri-portal area,

and average central veins and hepatocytes arranged in single-cell cords with normal intervening blood sinusoids. Conversely, the liver tissues showed an increase of liver damage in DEN-treated groups **(Fig.3).**Female-DEN group showed expanded portal tract with mild portal and peri-portal inflammatory infiltrate, mildly dilated congested portal veins, scattered apoptosis and incomplete nodule formation, while Male-DEN group livers showed markedly expanded portal tracts with mild portal and peri-portal inflammatory infiltrate, complete nodule formation, and hepatocytes with marked ballooning, markedly pleomorphic nuclei and marked bile stasis. Male-DEN+Silymarin and Female-DEN+Silymarin livers showed mildly expanded portal tracts with mild portal inflammatory infiltrate, average portal veins and hepatocytes with mildly vacuolated cytoplasm, few complete nodule formation, and average central veins with scattered apoptotic hepatocytes in peri-venular area from the previous data. It is obvious that silymarin treated group demonstrated marked protection against histopathological abnormalities caused by DEN **(Fig.3).**

Silymarin inhibited the DEN-induced pathologic collagen deposition in hepatic tissues:

Control liver groups revealed an average collagen distribution around central veins and in portal tracts, no fibrosis was noticed. The Female-DEN group livers showed excess collagen in portal tracts, with incomplete nodule formation which reveal moderate fibrosis. While Male-DEN group livers showed the highest collagen distribution in portal tracts with concomitant complete nodule formation. Marked significant reduction in hepatic fibrosis was detected in Male-DEN+Silymarin and Female-DEN+Silymarin groups in LF area percentage with moderate collagen distribution in portal tracts and incomplete nodule formation **(Figs.3)**.



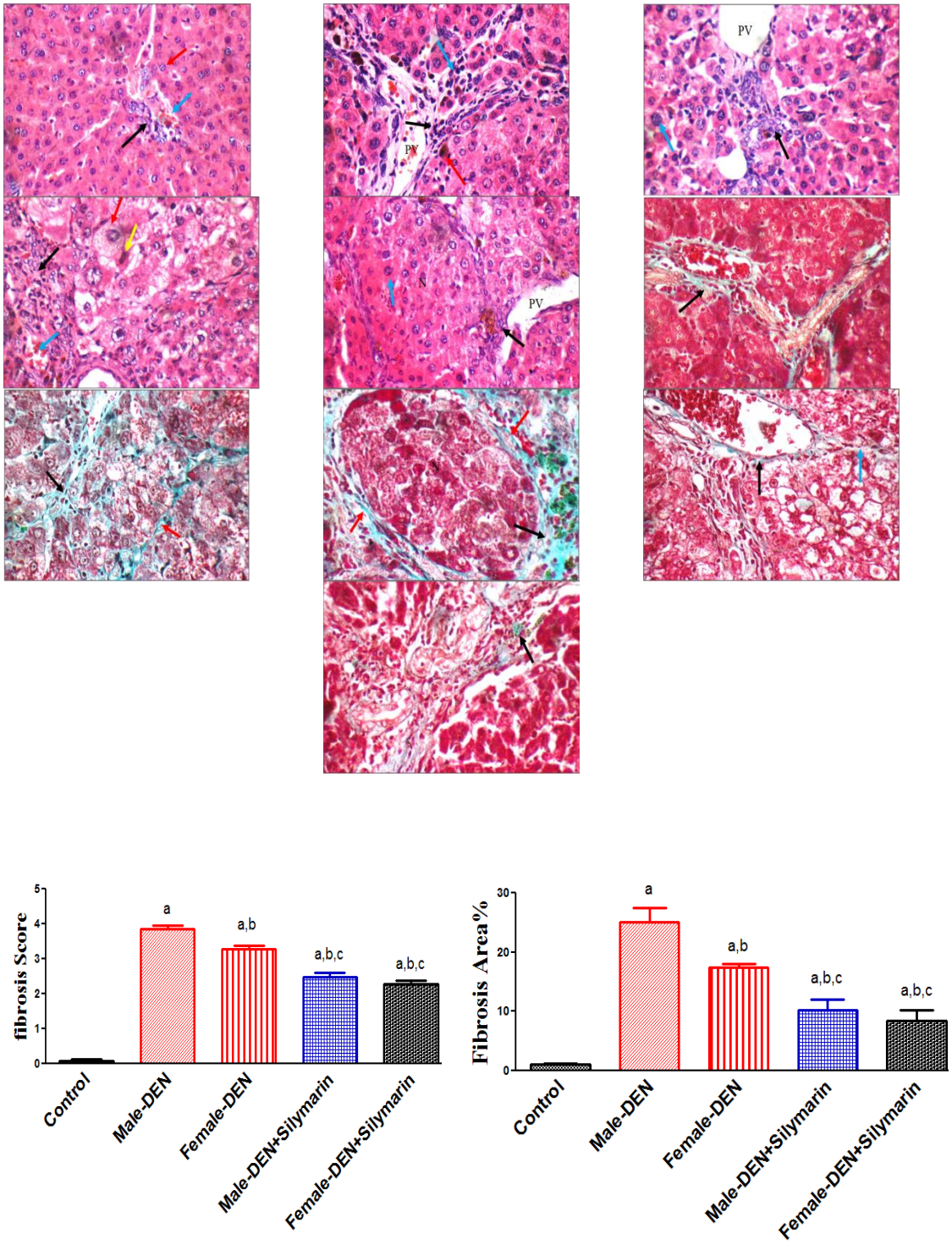


Fig.3: Effect of DEN and /or silymarin on liver morphology, fibrosis scoring (Heamtoxylin&Eosin H&E Staining),and fibrosis %Area (Masson's trichrome) in male and female rats, showing normal morphology, normal hepatocytes, and normal collagen deposition in control group, while Male-DEN group showing morphological abnormalities in architecture, markedly expanded portal inflammatory infiltrate, complete nodule formation, and hepatocytes with marked ballooning, and high deposition of collagen, Female-DEN and Silymarin-protected groups (compared with Male-DEN

group) shows less morphological abnormalities, mildly expanded portal tracts with mild portal inflammatory infiltrate, and moderate collagen deposition. Data were presented as means ± SD and statistically analyzed by one-way (ANOVA) test, followed by post hoc Tukey-Kramer test for multiple comparisons between groups. LF: liver fibrosis, DEN: diethylnitrosamine.

The impact of silymarin in diminishing DEN-induced cytokine boost in liver tissues:

According to the ELISA analysis, with the expression of TNF-α and TGF-β were significantly higher in DEN-insulted groups, when compared with control groups. The opposite was noticed, when comparing Male-DEN+Silymarin and Female-DEN+Silymarin groups with DEN-only treated groups **(Table.4)**.

Table.4: Effect of DEN and /or silymarin on inflammatory mediators in male and female rats

Groups	TNF-α (pg/mg)	TGF-β (pg/mg)
Male-Control	85.88±9.27	39.91±5.51
Female-Control	84.89±7.96	40.11±3.67
Male + Silymarin	87.42±10.12	38.76±5.29
Female +Silymarin	86.56±11.24	40.50±7.55
Male-DEN	192.8±18.9 ^{a}	200.8±15.1 ^{a}
Female-DEN	166.2±19.13^{a,b}	150±15.25^{a,b}
Male-DEN+Silymarin	133.4±8.39 ^{a,b,c}	62.69 ±11^{a,b,c}
Female-DEN+Silymarin	137.3±11.75 ^{a,b,c}	67.28 ±10.42^{a,b,c}

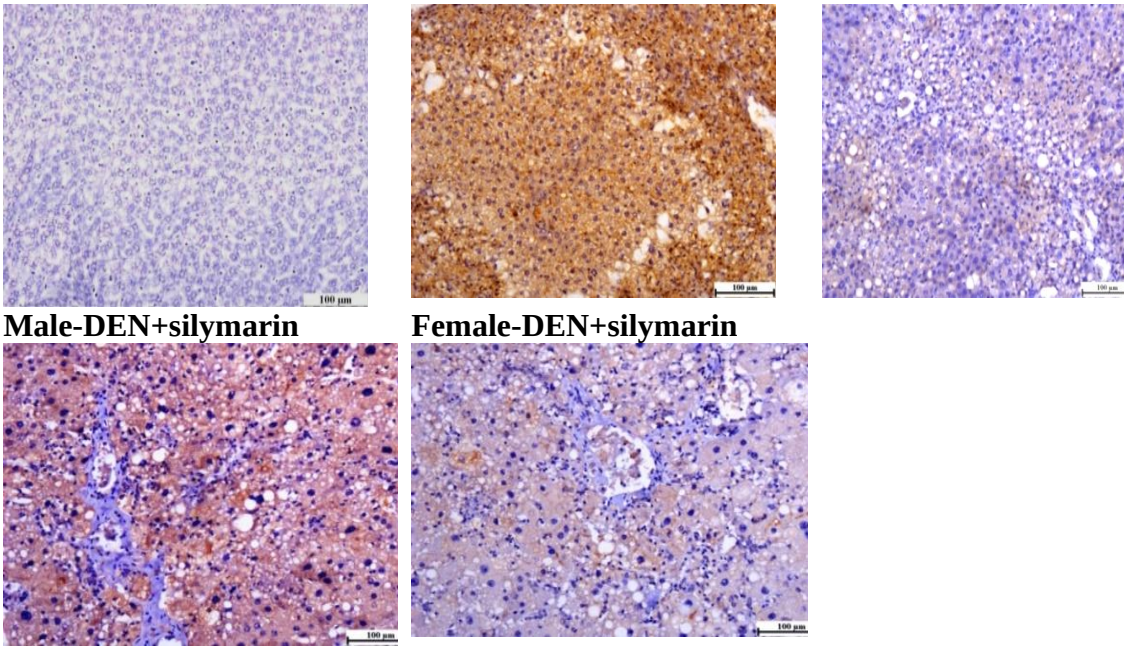
Data were presented as means ± SD and statistically analyzed by one-way (ANOVA) test, followed by post hoc Tukey-Kramer test for multiple comparisons between groups. LF: liver fibrosis, DEN: diethylnitrosamine, TNF-α: Tumor Necrosis Factor- α subtype, TGF-β: Transforming Growth Factor- β subtype.

a: significant from control, b: significant from Male-DEN, c: significant from Female-DEN.

The impact of silymarin in inflammatory/profibrotic mediator NF-(P-65) protein expressions in liver tissue as measured by immunohistochemical staining:

The measurement of NF-(p-65) expression in control groups demonstrated average expression, comparing to the Male-DEN+Silymarin and Female-DEN+Silymarin groups which revealed moderate expression in the hepatic parenchyma of the Male-DEN group displayed the highest level of NF-(p-65) expression. Lower NF-(p-65) levels have been found in the nucleus and cytoplasm of the Female-DEN and silymarin-administered group's affected hepatocytes. When NF-(p-65) area% expression was statistically evaluated, the control group's expression was significantly lower than that of the other experimental groups. However, no statistically significant difference (p0.05) between the Male-DEN+Silymarin and Female-DEN+Silymarin groups was found. Finally, NF-(p-65) expression in the Male-DEN group was highly significant compared to that of the other experimental groups (**Fig.5)**.

Control **Male-DEN** **Female-DEN**



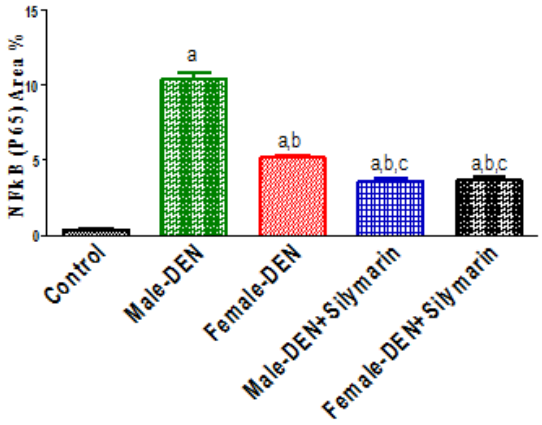


Fig.5: Effect of DEN and /or silymarin on the expression of NF-kB (p-65) in male and female rat's hepatocytes, Data were presented as means ± SD and statistically analyzed by one-way (ANOVA) test, followed by post hoc Tukey-Kramer test for multiple comparisons between groups. LF: liver fibrosis, DEN: diethylnitrosamine.

a:significant from control, b:significant from Male-DEN, c:significant from Female-DEN

Discussion

Liver fibrosis leads to HCC as a fate of chronic liver inflammation passing by liver cirrhosis. During fibrgenesis, extracellular matrix (ECM) proteins, primarily cross-linked collagens type I and type III, build up in the liver, causing the creation of a fibrous scar to replace injured normal tissue**(Scott, 2003)**.Fibrosis is characterized by a significant accumulation of ECM in and around the diseased tissues. LF exacerbations may lead to organ failure and higher death rates **(Wynn and Ramalingam, 2013)** in

DEN-induced liver injury models leading to HCC, it exhibits a sequential pattern in liver damaging according to distinct staging criteria **(Fathy et al., 2017)**. DEN causes a series of molecular events that leads to oxidative stress and inflammations in the liver **(Mansour et al., 2019)**.Our present study aimed to evaluate the protective role of silymarin in preventing DEN-induced LF and comparing this role in male with female rats. One of the major factors that cause the occurrence of hepatic diseases is gender differences and changes in sex hormone levels **(Kur et al., 2020)**.Thus, we established our model on male and female regimens. Furthermore, the metabolic effect of DEN is significantly influenced by age, species, and strain **(Liu et al., 2013)**.To avoid such variations, we collected data about DEN dose (100 µl/L in drinking water) and silymarin dose (50 mg/kg/day, P.O) from our previous preliminary study.

Generally, male rats more affected by DEN in all parameters than females. LF and nonalcoholic fatty liver diseases (NAFLD) are more common in men and postmenopausal woman**(Shimizu et al., 2007)**. this could be attributed to the strong anti-inflammatory, anti-oxidant, and anti-apoptotic properties of estrogen which prevent the progress of LF as previously explained **(Lu et al., 2004)**.

In the light of our study, DEN slowed the growth of both male and female rats, However, differences between both genders in final body weight were non-significant. our findings about relative liver weight ratio (RLWR) confirmed the previous work which demonstrated the increase in their values in DEN-treated groups **(Mokhtar & Elshafee, 2022;Memon et al., 2020)**, the values of RLWR increased significantly in males against females, this could be explained by the ability of estrogen as anti-inflammatory to prevent ascites and hepatomegaly. The lowest values were observed in silymarin administered groups.

According to our work, silymarin has a preventive effect against LF at the basis of the hematological parameters, and liver functions and histopathology. However, this was compatible with other studies **(Mukhtar et al., 2021;Tsai et al., 2008).** Silymarin provided the same protection in both genders. Thus, there was statistically no difference between the Male-DEN+Silymarin and Female-DEN+Silymarin groups in most parameters. The Male-DEN group, however, had the worst results. These findings are supported by other earlier investigations conducted on both male and female individually **(Ghaffari et al., 2011; Pradeep et al., 2007)**.

In our study, silymarin improved lipid profile and enhanced dramatically lipid peroxidation and oxidative stress in male and female rats with the same efficiency. Reactive oxygen species (ROS) induced oxidative stress has been implicated in liver diseases **(Jadeja et al., 2017)** as we mentioned.DEN can provoke lipid peroxidation leading to its damaging effects via oxidative stress in the liver **(Mansour et al., 2019)**. The term "Oxidative stress" is used to describe the prolonged presence of ROS that are not neutralized by endogenous antioxidants. Oxidative stress is linked to the pathophysiology of a number of liver illnesses, including liver fibrosis**(Pellicoro et al., 2014)**. In several experimental systems, including rat liver microsomes, is was established that silibinin is discovered to be a strong scavenger of ROS, such as hydroxyl and peroxyl anions and hypochlorous acid**(Valenzuela and Guerra, 1986)**. In addition, silibinin

treatment decreased the production of nitric oxide and superoxide anion radicals in isolated Kupffer cells**(Dehmlow et al., 1996)**.

The key factor influencing the development of LF is inflammation, through crosstalk and communication between inflammatory cells, cytokines, and associated signaling pathways **(Zhangdi et al., 2019)**. Our findings revealed that silymarin ameliorated both of the DEN-induced profibrotic/inflammatory mediators TNF-α and TGF-β in male and female rats. This was in the line of other studies **(Ogaly et al., 2022; Jeong et al., 2005)**.

The significance of NF-*κ*B in DEN-induced liver injury is obvious, and it may be a target for the prevention or treatment of liver fibrosis. NF-*κ*B also serves as a crucial link between hepatic injury, fibrosis, and HCC **(Luedde & Schwabe, 2012)**. Our immunohistochemical analysis indicates that the injured hepatocytes of the Male-DEN group had higher levels of the active form of NF- *κ*B (p-65) in their nucleus and cytoplasm than the Female-DEN group. Silymarin reversed the excess nuclear translocation of NF- (p-65) in male and female rats.

The reason behind using silymarin as a protectant from DEN-induced LF in our study its multi-target mechanisms of action. silymarin has long been studied since it has few adverse effects, is simple to administer orally, is inexpensive, and has good curative benefits **(Tamayo et al., 2013)**. Silymarin provides protection to both sexes because it protects hepatocyte by different mechanisms **(Jiang et al., 2022).**According to reports, silymarin works to protect the liver through a variety of processes, including as antioxidant activity, scavenging of free radicals, a rise in cellular glutathione levels, activation of DNA polymerase, and stabilization of the hepatocellular membrane **(ŠIManek et al., 2000)**.

Our study has been succeeded to link the severity and progress of DEN-induced LF with e gender dimorphism by comparing these effects in male and female rats. Accordingly, it opened an avenue for further investigations of the possible molecular mechanism(s) that could be involved in the hepatoprotective effect of estrogen and their cross-links with hepatoprotective mechanisms of silymarin.

Conclusion

In conclusion, DEN-induced liver fibrosis is more aggressive in male rats than females. Additionally, silymarin provides an ameliorative effect against DEN-induced LF in both sexes by improving liver functions , reducing the oxidative stress and inflammations.

Ethical approval

All applicable international, national, and institutional guidelines for the care and use of animals were considered Experiments were conducted according to NIH Guidelines for the Care and Use of Laboratory Animals.

Informed consent

Not applicable.

Conflict of interest

The authors declare that no conflict of interest exists.

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REFERENCES:

Affo, S., Yu, L. X., & Schwabe, R. F. (2017). The role of cancer-associated fibroblasts and fibrosis in liver cancer. *Annual review of pathology*, *12*, 153. https://doi.org/10.1146/annurev-pathol-052016-100322.

Asamoto, M., Mikheev, A. M., Jiang, Y. Z., Wild, C. P., Hall, J., & Montesano, R. (1991). Immunohistochemical detection of DNA alkylation adducts in rat and hamster liver after treatment with dimethylnitrosamine. *Experimental pathology*, *41*(2), 71-78. https://doi.org/10.1016/S0232-1513(11)80004-6.

Avizeh, R., Najafzadeh, H., Jalali, M. R., & Shirali, S. (2010). Evaluation of prophylactic and therapeutic effects of silymarin and N-acetylcysteine in acetaminophen-induced hepatotoxicity in cats. *Journal of veterinary pharmacology and therapeutics*, *33*(1), 95-99. https://doi.org/10.1111/j.1365-2885.2009.01100.x.

Care, Canadian Council on Animal Care. (2010). CCAC guidelines on: euthanasia of animals used in science.

https://www.ccac.ca/Documents/Standards/Guidelines/Euthanasia.pdf%0Ahttp://www.ccac.ca.

Carlson, E. S., Upadhyaya, P., & Hecht, S. S. (2017). A general method for detecting nitrosamide formation in the in vitro metabolism of nitrosamines by cytochrome P450s. *JoVE (Journal of Visualized Experiments)*, (127), e56312. https://doi.org/10.3791/56312.

Challis, B. C., & Rayman, M. P. (1973). Potential alkylating agents from the oxidation of carcinogenic cyclic n-nitrosamines. *British Journal of Cancer*, *28*(1), 84. https://doi.org/10.1038/bjc.1973.101.

Dehmlow, C., Erhard, J., & de Groot, H. E. R. B. E. R. T. (1996). Inhibition of Kupffer cell functions as an explanation for the hepatoprotective properties of silibinin. *Hepatology*, *23*(4), 749-754.

https://doi.org/10.1053/jhep.1996.v23.pm0008666328.

Dooley, James S.; Lok, Anna S. F.; Garcia-Tsao, Guadalupe; Pinzani, M. (2018). *SHERLOCK'S Diseases of the LIVER and Biliary system, Thirteenth Edition*.

Fathy, A. H., Bashandy, M. A., Bashandy, S. A., Mansour, A. M., & Elsadek, B. (2017). Sequential analysis and staging of a diethylnitrosamine-induced hepatocellular carcinoma in male Wistar albino rat model. *Canadian journal of physiology and pharmacology*, *95*(12), 1462-1472. https://doi.org/10.1139/cjpp-2017-0413.

FREUND, H. A. (1937). Clinical manifestations and studies in parenchymatous hepatitis. *Annals of internal medicine*, *10*(8), 1144-1155. https://doi.org/10.7326/0003-4819-10-8-1144.

Ghaffari, A. R., Noshad, H., Ostadi, A., Ghojazadeh, M., & Asadi, P. (2011). The effects of milk thistle on hepatic fibrosis due to methotrexate in rat. *Hepatitis monthly*, *11*(6), 464. PMID: 22087179; PMCID: PMC3212785.

Gillessen, A., & Schmidt, H. H. J. (2020). Silymarin as supportive treatment in liver diseases: A narrative review. *Advances in therapy*, *37*(4), 1279-1301. https://doi.org/10.1007/s12325-020-01251-y.

Druckrey, H., Preussmann, R., Ivankovic, S., & Schmähl, D. (1967). Organotropic carcinogenic effects of 65 various N-nitroso-compounds on BD rats. *Zeitschrift fur Krebsforschung*, *69*(2), 103-201. PMID: 4230610.

Jadeja, R. N., Devkar, R. V., & Nammi, S. (2017). Oxidative stress in liver diseases: pathogenesis, prevention, and therapeutics. *Oxidative medicine and cellular longevity*, *2017*. https://doi.org/10.1155/2017/8341286.

Jeong, K. S., Kim, K. J. (2005). Alterations of mast cells and TGF-beta1 on the silymarin treatment for CCl4-induced hepatic fibrosis. *World J Gastroenterol*, *11*(8), 1141-8. 10.3748/wjg.v11.i8.1141.

Jiang, G., Sun, C., Wang, X., Mei, J., Li, C., Zhan, H., ... & Mao, J. (2022). Hepatoprotective mechanism of Silybum marianum on nonalcoholic fatty liver disease based on network pharmacology and experimental verification. *Bioengineered*, *13*(3), 5216-5235. https://doi.org/10.1080/21655979.2022.2037374.

Kur, P., Kolasa-Wołosiuk, A., Misiakiewicz-Has, K., & Wiszniewska, B. (2020). Sex hormone-dependent physiology and diseases of liver. *International journal of environmental research and public health*, *17*(8), 2620. https://doi.org/10.3390/ijerph17082620.

Lefkowitch, J. H. (2006, August). Special stains in diagnostic liver pathology. In *Seminars in diagnostic pathology* (Vol. 23, No. 3-4, pp. 190-198). WB

Saunders. https://doi.org/10.1053/j.semdp.2006.11.006.

Lin, H., & Hollenberg, P. F. (2001). *N -Nitrosodimethylamine-Mediated Formation of Oxidized and Methylated DNA Bases in a Cytochrome P450 2E1 Expressing Cell Line*. *Chemical Research in Toxicology*, *14(5), 562–566.* https://doi.org/10.1021/tx0001979 562–566.

Liu, Y., Meyer, C., Xu, C., Weng, H., Hellerbrand, C., ten Dijke, P., & Dooley, S. (2013). Animal models of chronic liver diseases. *American Journal of Physiology-Gastrointestinal and Liver Physiology*, *304*(5), G449-G468. https://doi.org/10.1152/ajpgi.00199.2012.

Lu, G., Shimizu, I., Cui, X., Itonaga, M., Tamaki, K., Fukuno, H., ... & Ito, S. (2004). Antioxidant and antiapoptotic activities of idoxifene and estradiol in hepatic fibrosis in rats. *Life Sciences*, *74*(7), 897-907. https://doi.org/10.1016/j.lfs.2003.08.004

Luedde, T., & Schwabe, R. F. (2011). NF-κB in the liver—linking injury, fibrosis and hepatocellular carcinoma. *Nature reviews Gastroenterology & hepatology*, *8*(2), 108-118. https://doi.org/10.1038/nrgastro.2010.213.

Magee, P. N., & Barnes, J. M. (1956). The production of malignant primary hepatic tumours in the rat by feeding dimethylnitrosamine. *British journal of cancer*, *10*(1), 114. https://doi.org/10.1038/bjc.1956.15.

Mansour, D. F., Abdallah, H. M., Ibrahim, B. M., Hegazy, R. R., Esmail, R. S., & Abdel-Salam, L. O. (2019). The carcinogenic agent diethylnitrosamine induces early oxidative stress, inflammation and proliferation in rat liver, stomach and colon: protective effect of ginger extract. *Asian Pacific journal of cancer prevention: APJCP*, *20*(8), 2551.https://doi.org/10.31557/APJCP.2019.20.8.2551.

Memon, A., Pyao, Y., Jung, Y., Lee, J. I., & Lee, W. K. (2020). A modified protocol of diethylnitrosamine administration in mice to model hepatocellular carcinoma. *International journal of molecular sciences*, *21*(15), 5461. https://doi.org/10.3390/ijms21155461.

Mokdad, A. A., Lopez, A. D., Shahraz, S., Lozano, R., Mokdad, A. H., Stanaway, J., ... & Naghavi, M. (2014). Liver cirrhosis mortality in 187 countries between 1980 and 2010: a systematic analysis. *BMC medicine*, *12*(1), 1-24. https://doi.org/10.1186/s12916-014-0145-y.

Mokhtar, A. M., Elshafee, M. F., Mansour, A. M., & El-Sayed, E. S. M. (2022). Ameliorative Effects of Lycopene on Diethylnitrosamine-Induced Hepatocarcinogenesis in Male Rats. *Journal of Food and Nutrition Research*, *10*(4), 261-273. https://doi.org/10.12691/jfnr-10-4-2.

Mukhtar, S., Xiaoxiong, Z., Qamer, S., Saad, M., Mubarik, M. S., Mahmoud, A. H.,

& Mohammed, O. B. (2021). Hepatoprotective activity of silymarin encapsulation against hepatic damage in albino rats. *Saudi Journal of Biological Sciences*, *28*(1), 717-723. https://doi.org/10.1016/j.sjbs.2020.10.063.

Nakatani, T., Roy, G., Fujimoto, N., Asahara, T., & Ito, A. (2001). Sex hormone dependency of diethylnitrosamine-induced liver tumors in mice and chemoprevention by leuprorelin. *Japanese journal of cancer research*, *92*(3), 249-256. https://doi.org/10.1111/j.1349-7006.2001.tb01089.x.

Ogaly, H. A., Aldulmani, S. A. A., Al-zahrani, F. A. M., & Abd-elsalam, R. M. (2022). D-Carvone Attenuates CCl 4 -Induced Liver Fibrosis in Rats by Inhibiting Oxidative Stress and TGF-ß 1/SMAD3 Signaling Pathway. *Biology*.11, 739. https://doi.org/10.3390/biology11050739.

Pellicoro, A., Ramachandran, P., Iredale, J. P., & Fallowfield, J. A. (2014). Liver fibrosis and repair: immune regulation of wound healing in a solid organ. *Nature Reviews Immunology*, *14*(3), 181-194. https://doi.org/10.1038/nri3623.

Poli, G., Leonarduzzi, G., Biasi, F., & Chiarpotto, E. (2004). Oxidative stress and cell signalling. *Current medicinal chemistry*, *11*(9), 1163-1182. https://doi.org/10.2174/0929867043365323.

Pradeep, K., Victor Raj Mohan, C., Gobianand, K., & Karthikeyan, S. (2007). Silymarin: an effective hepatoprotective agent against diethylnitrosamine-induced hepatotoxicity in rats. *Pharmaceutical biology*, *45*(9), 707-714. https://doi.org/10.1080/13880200701575254.

Scott, L. F. (2003). Liver fibrosis–from bench to bedside. *Journal of hepatology*, *38*, 38-53. https://doi.org/10.1016/S.

Sharma, A., Fish, B. L., Moulder, J. E., Medhora, M., Baker, J. E., Mader, M., & Cohen, E. P. (2014). Safety and blood sample volume and quality of a refined retro-orbital bleeding technique in rats using a lateral approach. *Lab animal*, *43*(2), 63-66. https://doi.org/10.1038/laban.432.

Shimizu, I., Kohno, N., Tamaki, K., Shono, M., Huang, H. W., He, J. H., & Yao, D. F. (2007). Female hepatology: favorable role of estrogen in chronic liver disease with hepatitis B virus infection. *World Journal of Gastroenterology: WJG*, *13*(32), 4295. https://doi.org/10.3748/wjg.v13.i32.4295

ŠIManek, V. I. L. Í. M., Kren, V., Ulrichová, J., Vicar, J., & Cvak, L. (2000). Silymarin: what is in the name…? An appeal for a change of editorial policy. *Hepatology*, *32*(2), 442-444. https://doi.org/10.1053/jhep.2000.9770.

Staub, F., Tournoux-Facon, C., Roumy, J., Chaigneau, C., Morichaut-Beauchant, M., Levillain, P., ... & Tasu, J. P. (2009). Liver fibrosis staging with contrast-enhanced ultrasonography: prospective multicenter study compared with METAVIR scoring. *European radiology*, *19*(8), 1991-1997.

https://doi.org/10.1007/s00330-009-1468-5.

Tamayo, C., & Diamond, S. (2007). Review of clinical trials evaluating safety and efficacy of milk thistle (Silybum marianum [L.] Gaertn.). *Integrative cancer therapies*, *6*(2), 146-157. https://doi.org/10.1177/1534735407301942.

Tsai, J. H., Liu, J. Y., Wu, T. T., Ho, P. C., Huang, C. Y., Shyu, J. C., ... & Liu, Y. C. (2008). Effects of silymarin on the resolution of liver fibrosis induced by carbon tetrachloride in rats. *Journal of Viral Hepatitis*, *15*(7), 508-514. https://doi.org/10.1111/j.1365-2893.2008.00971.x.

Tsuchida, T., & Friedman, S. L. (2017). Mechanisms of hepatic stellate cell activation. *Nature reviews Gastroenterology & hepatology*, *14*(7), 397-411. https://doi.org/10.1038/nrgastro.2017.38.

Valenzuela, A., & Guerra, R. (1986). Differential effect of silybin on the Fe2+-ADP and t-butyl hydroperoxide-induced microsomal lipid peroxidation. *Experientia*, *42*(2), 139-141. https://doi.org/10.1007/BF01952435.

Van Pelt, J. F., Verslype, C., Crabbé, T., Zaman, Z., & Fevery, J. (2003). Primary human hepatocytes are protected against prolonged and repeated exposure to ethanol by silibinin-dihemisuccinate. *Alcohol and alcoholism*, *38*(5), 411-414. https://doi.org/10.1093/alcalc/agg099.

Wynn, T. A., & Ramalingam, T. R. (2012). Mechanisms of fibrosis: therapeutic translation for fibrotic disease. *Nature medicine*, *18*(7), 1028-1040. https://doi.org/10.1038/nm.2807.

Yadav, N. P., Pal, A., Shanker, K., Bawankule, D. U., Gupta, A. K., Darokar, M. P., & Khanuja, S. P. (2008). Synergistic effect of silymarin and standardized extract of Phyllanthus amarus against CCl4-induced hepatotoxicity in Rattus norvegicus. *Phytomedicine*, *15*(12), 1053-1061.. https://doi.org/10.1016/j.phymed.2008.08.002.

Zhang, W., Hong, R., & Tian, T. (2013). Silymarin's protective effects and possible mechanisms on alcoholic fatty liver for rats. *Biomolecules & therapeutics*, *21*(4), 264. https://doi.org/10.4062/biomolther.2013.020.

Zhangdi, H. J., Su, S. B., Wang, F., Liang, Z. Y., Yan, Y. D., Qin, S. Y., & Jiang, H. X. (2019). Crosstalk network among multiple inflammatory mediators in liver fibrosis. *World journal of gastroenterology*, *25*(33), 4835. https://doi.org/10.3748/wjg.v25.i33.4835.

سيليمارين يخفف التليف الكبدي المستحث بثنائي إيثيل نيتروزامين في ذكور وإناث الجرذان

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الهدف : استهدفت الدراسة مقارنة بين جنسي جرذان ويستر في تأثير سليمارين على التليف الكبدي المستحث بواسطة ثنائي إيثيل نيتروزامين، وافتراض الآليات الممكنة.

المنهجية الرئيسية : تم تقسيم أربعة وعشرون من الذكور وأربعة وعشرون من إناث الجرذان من نوع ويستر ألبينو في مجموعات بشكل عشوائي إلى ثمان مجموعات وفقا لجنسها (عدد=6) لكي تتلقى إما :الحامل الدوائي، ثنائي إيثيل نيتروزامين، سيليمارين، أو كلا من ثنائي إيثيل نيتروزامين و سيليمارين لمدة ثمانية أسابيع.

في نهاية التجربة، تم حساب المؤشرات التقليدية الخاصة بوزن الجسم والكبد للجرذان وتم قياس مؤشرات تضرر الكبد (وظائف الكبد). كذلك، تم قياس مؤشرات صورة الدم الكاملة وملف الدهون والإجهاد التأكسدي، وتم تقييم مؤشرات الإلتهابات في المخلوط المتجانس للنسيج الكبدي.تم ترتيب علامات العطب للنسيج الكبدي من خلال النظام الإخضاع المقياسي لأمراض الأنسجة.تم قياس التعبير البروتيني للعامل النووي كابا بيتا عن طريق تقنية الصبغ المناعي التشريحي الكيمائي.

نتائج البحث : تضاؤل واضح لعلامات التليف الكبدي المستحث ب داي إيثيل نيتروزامين في مجموعات الإناث، وازيادها سوءا في مجموعات الذكور .قام سيليمارين بتحسين وظائف الكبد وعلامات التليف ، بالإضافة الى أنه عاكس الإجهاد التأكسدي وتأكسد الدهون والإلتهابات المستحثة من ثنائي إيثيل نيتروزامين.حقق سيليمارين هذه التأثيرات التخفيفية سواء في الذكور أو الإناث.

الإستنتاج : سيليمارين يلعب دورا تخفيفيا للتليف الكبدي المستحث بثنائي إيثيل في ذكور وإناث الجرذان عن طريق تقليل الإجهاد التأكسدي والإلتهابات.

الكلمات المفتاحية : ثنائي إيثيل نيتروزامين ، تليف كبدي، سليمارين، التهابات، وإجهاد تأكسدي.