

## Protective Effect of Grape Seed Extract and/or Silymarin Against Thioacetamide-induced Hepatic Fibrosis in Rats

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### Abstract

The aim of the present study was designed to evaluate the hepatoprotective and antioxidant potentials of GSE (100 and 200 mg/kg) and/or silymarin against TAA-induced liver fibrosis in rats.

This study was designed to investigate the protective effect of grape seed extract (GSE) and/or silymarin against thioacetamide (TAA)-induced hepatic fibrosis in Sprague-Dawley rats. Mature male Sprague-Dawley rats were divided into 7 equal groups (8 rats each) and treated as follows: Group 1, kept as control group and orally given saline; groups 2-7 were injected intraperitoneally (i.p.) with TAA (100 mg/Kg) twice weekly for 6 weeks to induce hepatic fibrosis. Group 2, kept as control positive; groups 3-5 were administered daily oral doses of silymarin (50 mg/kg), GSE (100 mg/kg) and GSE (200 mg/kg), respectively. While groups 6-7 were administered combined treatments of silymarin and GSE (100 mg/kg) or GSE (200 mg/kg), respectively. Our results indicated that TAA caused significant elevation of hydroxyproline (Hyp), malondialdehyde (MDA) and nitric oxide (NO) contents in liver homogenate and increased serum levels of: aminotransferases (AST and ALT), alkaline phosphatase (ALP) and total bilirubin. While, TAA-treatment alone significantly decreased serum total protein and reduced glutathione (GSH) content in liver homogenate. Administration of GSE (100 and 200 mg/kg) and/or silymarin attenuated TAA-induced hepatic fibrosis, improved enzymes and reduced the oxidative stress in dose dependant manner. Histopathological study showed disruption of the hepatic architecture and collagen fibers deposition in the portal tract of TAA-injected group. Concomitant treatment with GSE (100 and 200 mg/kg) and/or silymarin significantly improved histopathological structure of liver tissue in variable degrees. In conclusion, the combined effect of GSE (200 mg/kg) with silymarin (50 mg/kg) had powerful hepatoprotective effect than any other studied doses.

**Keywords:** Grape seed extract; Liver fibrosis; Rat

### Introduction

Hepatic fibrosis, defined by excessive accumulation of extracellular matrix (ECM) and resultant loss of pliability and liver function, is the result of wound-healing responses triggered by either acute or chronic liver injury [1]. Hepatic stellate cells (HSCs) are the main ECM-producing cells in the injured liver [2].

Thioacetamide (TAA) is a thiono-sulfur containing compound. It has been used as a fungicide, organic solvent, accelerator in the vulcanization of rubber, and as a stabilizer of motor oil [3]. Treatment of rodents with TAA is known to lead to liver fibrosis and eventually cirrhosis, with the morphological and biochemical changes resembling that of the human disease [4]. The toxicity of TAA results from its bioactivation by a mixed-function oxidase system, particularly by CYP2E1 and flavin adenine dinucleotide (FAD) monooxygenases [5]. Metabolic activation of TAA then leads to the formation of reactive metabolites that are represented by radicals derived from TAA-S-oxide and by reactive oxygen species (ROS) generated as intermediates [6]. It was documented that reactive metabolites can covalently bind to cellular macromolecules and/or induce oxidative stress [7].

Grape seed extract (GSE) is a natural extract from the seed of *Vitis vinifera*. It is a rich source of flavonoids, proanthocyanidins oligomers. Proanthocyanidins are a class of phenolic compounds that take the forms of oligomers or polymers of polyhydroxy falvan- 3-ol units, such as (+)-catechin, (-)-epicatechin [8]. These flavonoids exert many health promoting effects including the ability to increase intracellular vitamin C levels, decrease capillary permeability and fragility and scavenge oxidants and free radicals [9,10]. Although these bioactive components of grape mainly exist in grape skin and seeds, grape skin and seeds are usually discarded in regular dietary intake and the winery and grape juice industry. These wastes contain bioactive components with potent antioxidant and free radical scavenging activity. Grape seed extract proanthocyanidins has antibacterial, and anti-inflammatory effect, they have been reported to inhibit lipid peroxidation [11-14].

Silymarin is a mixture of flavonolignans extracted from "milk thistle" *Silybum marianum* [15]. It is a mixture of flavonoid isomers such as silibinin, isosilibinin, silidianin, and silichristin. Silymarin is used primarily to treat various liver diseases and dysfunctions including alcoholic cirrhosis, hepatitis (due to viral infections or drug-induced), as well as hepatic problems related to diabetes [16]. Silymarin has free radical scavenging properties and its ability to enhance endogenous anti-oxidant defense systems in vivo [17].

Silymarin has been shown to reduce liver fibrosis up to 30–35%, and in few cases it has reversed the liver fibrosis [18]. Silymarin showed anti-inflammatory and anti-metastatic activities; it has protective effect against toxicity of chemotherapy and radiotherapy [19].

## Materials and Methods

### Chemicals

TAA was obtained from El-Gomhouria Company for drug and chemicals, Egypt, silymarin was obtained from Sigma-Aldrich, USA; GSE was obtained from Arab Gelatin Pharmaceutical Products Company, Egypt. All other chemicals, used throughout the experiment, were of the highest analytical grade available. Kits used to measurement of serum aspartate transaminase (AST), alanine transaminase (ALT); alkaline phosphatase (ALP); total protein; total bilirubin levels; hepatic malondialdehyde (MDA); nitric oxide (NO) and hepatic reduced glutathione (GSH) were purchased from Biodiagnostic, Inc., (Egypt). Kit used for measurement of hepatic hydroxyproline (Hyp) was purchased from Glory Science Co., Ltd.

### Animals

Adult male Sprague- Dawley rats weighing 150-200 gm were obtained from the animal house at the National Research Center (Giza, Egypt), and fed a standard laboratory diet and tap water ad libitum. Experimental animals were housed in an air-conditioned room at 22–25 °C with a 12-h light/dark cycle. All animals received humane care and the study protocols were in compliance with institutional guidelines for the use of laboratory animals.

### Experimental design

After an acclimatization period of one week, sixty-four healthy male Sprague- Dawley rats were randomly assigned to seven groups of eight rats per group and that were treated as follows:

**Group (1):** Received normal saline orally, daily and injected intraperitoneally (i.p.) with sterile distilled water twice a week for 6 weeks (normal control group);

**Groups (2-7):** Rats injected with TAA (100 mg/kg, i.p.), according to Hamed et al. [20], dissolved in distilled water, twice a week for 6 weeks; and the following treatments were given daily oral doses in concomitant with TAA for six weeks: Group (2): Kept as positive control, and received normal saline; Group (3) given silymarin (50 mg/kg) according to Wills and Asha [21]; Group (4) administered GSE (100 mg/kg) according to Pallares et al. [22]; Group (5) received GSE (200 mg/kg) according to Yousef et al. [23]; Group (6) treated daily with GSE (100 mg/kg) and silymarin (50 mg/kg); and Group (7) received GSE (200 mg/kg) and Silymarin (50 mg/kg).

### Collection of blood samples

At the end of the experimental period; blood samples were withdrawn from the retro-orbital vein of each animal, under light anesthesia by diethyl ether, according to the method described by Cocchetto and Bjornsson [24]. Blood was allowed to coagulate and then centrifuged at 3000 rpm for 15 min. The obtained serum was used to estimate the levels of AST; ALT activities according to the method of Reitman and Frankel [25]; ALP was determined according to the method of Belfield and Goldberg [26]; total bilirubin was

analyzed by the method of Walter and Gerade [27]; and total protein was estimated by the method of Gornal et al. [28].

### Preparation of liver homogenate

Immediately after blood sampling, animals were sacrificed by cervical dislocation under ether anesthesia and livers were collected for biochemical and histopathological examinations. Liver tissues were rapidly removed, washed in ice-cooled saline, plotted dry and weighed. A weighed part of each liver was homogenized, using a homogenizer (Medical instruments, MPW-120, Poland), with ice-cooled saline (0.9% NaCl) to prepare 20% w/v homogenate. The homogenate was then centrifuged at 4000 rpm for 5 min. at 4°C using a cooling centrifuge to remove cell debris (Laborzentrifugen, 2k15, Sigma, Germany). The aliquot was divided into four parts; the 1st part was used for the assessment of lipid peroxidation (LPO) as MDA according to the methods described by Ohkawa et al.; the 2nd part was used for the determination of the level of NO by the method of Montgomery and Dymock; the 3rd part was used to estimate GSH by the method of Beutler et al.; and the 4th part was used to estimate Hyp according to the instructions of the manufacturer, using glory science ELISA kit [29-31].

### Histopathological examinations

The specimens from the liver were taken and fixed immediately in 10% neutral buffered formalin, processed for light microscopy to get (5µm) paraffin sections and stained with: Hematoxylin & Eosin (H & E) to verify histological details and Masson's trichrome staining (MTs) to demonstrate the collagen fibers as described by Bancroft and Gamble [32].

### Statistical analysis

The degree in variability of results was expressed as means ± standard error of means (SEM). Data were evaluated by one-way analysis of variance (ANOVA) followed by Tukey's multiple comparisons test. The level of significance was accepted at  $P < 0.05$ .

## Results

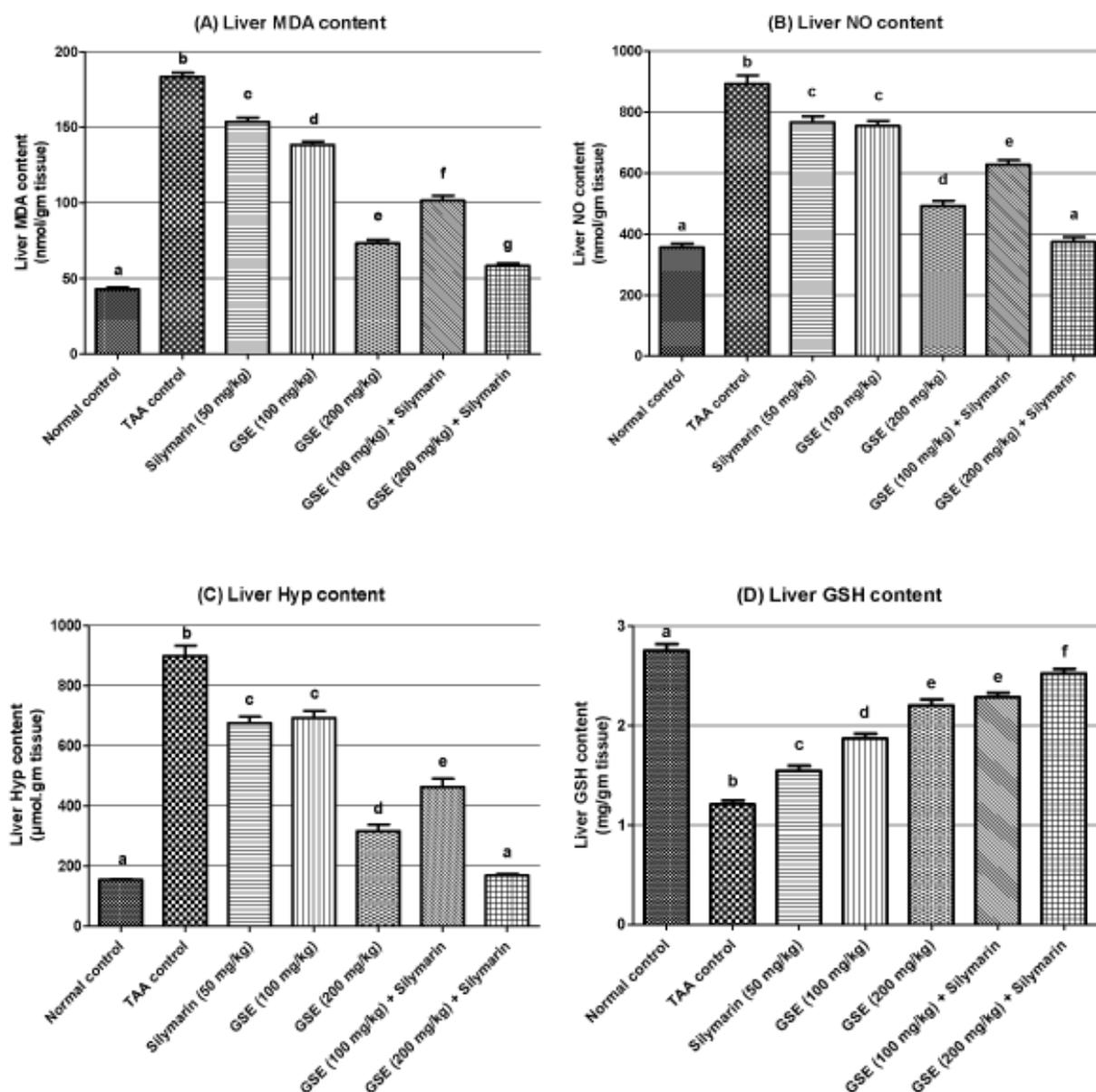
### Effect of GSE (100 and 200 mg/kg) and/or silymarin (50 mg/kg) on serum liver function tests in TAA-induced hepatic fibrosis in rats

Injection of TAA (100 mg/kg, i.p.) resulted in a considerable hepatic injury as assessed by a significant elevations in serum AST, ALT, ALP and total bilirubin by 636.39 %, 574.11%, 175.35%, and 438.67%, respectively as compared to normal control values (Table 1). Treatment with silymarin (50 mg/kg) exerted a significant decrease in serum AST, ALT, ALP, and total bilirubin levels by 53.49%, 34.9%, 37.48%, and 43.59%, respectively as compared to TAA control group. Administration of GSE (100 and 200 mg/kg) significantly reduced TAA-induced elevations by 42.09%, and 68.15% for AST; 25.45%, and 60.07% for ALT; 27.99%, and 49.68% for ALP; and 34.26%, and 61.33% for total bilirubin, respectively in dose-dependent manner, compared to TAA control group. Meanwhile, administration of silymarin (50 mg/kg) combined with either, GSE (100 mg/kg) or GSE (200 mg/kg), significantly decreased elevated serum AST by 66%, and 84.02%; ALT by 49.62%, and 70.91%; ALP by 47.54%, and 57.10%, and

total bilirubin by 54.46%, and 69.13%, respectively, compared to TAA control group.

Results showed that TAA injection significantly decreased serum total protein level by 38.95%, compared to normal control group (Table 1). A significant elevation in serum total protein level was observed after treatment with silymarin (50 mg/kg) by 17.49%, as compared to TAA control group. Meanwhile, GSE (100 and 200

mg/kg) showed a significant elevation in serum total protein level as compared to TAA control group by 20.37%, and 37.14%, respectively. Administration of silymarin (50 mg/kg) combined with either, GSE (100 mg/kg) or GSE (200 mg/kg), significantly increased serum total protein level by 35.06%, and 51.83%, respectively, compared to TAA control group (Figure 1).



**Figure 1:** The effect of oral administration of GSE (100 and 200 mg/kg) and/or silymarin (50 mg/kg) on liver MDA (A), NO (B), hydroxyproline (Hyp) (C), and reduced GSH (D) contents in TAA-induced hepatic fibrosis in rats: (Means  $\pm$  SEM, n = 8 rats/group), ANOVA – one way (groups with different letters are significantly different at P < 0.05). MDA, Malondialdehyde; NO, Nitric oxide; Hyp, Hydroxyproline; GSH, Reduced glutathione; TAA, Thioacetamide; GSE, Grape seed extract.

Groups\Parameters	AST(U/ml)	ALT(U/ml)	ALP(IU/L)	Total bilirubin(mg/dl)	Total Protein(gm/dl)
Normal control (Saline)	34 ± 0.74 <sup>a</sup>	28 ± 0.82 <sup>a</sup>	77.47 ± 1.09 <sup>a</sup>	0.32 ± 0.01 <sup>a</sup>	7.04 ± 0.17 <sup>a</sup>
TAA (100 mg/Kg), i.p.	250.38 ± 2.11 <sup>b</sup>	188.75 ± 2.13 <sup>b</sup>	213.32 ± 2.03 <sup>b</sup>	1.71 ± 0.03 <sup>b</sup>	4.29 ± 0.14 <sup>b</sup>
Silymarin (50 mg/Kg)	116.44 ± 1.62 <sup>c</sup>	122.88 ± 1.59 <sup>c</sup>	133.38 ± 1.74 <sup>c</sup>	0.96 ± 0.02 <sup>c</sup>	5.05 ± 0.07 <sup>c</sup>
GSE (100 mg/Kg)	145 ± 1.73 <sup>d</sup>	140.71 ± 2.07 <sup>d</sup>	153.62 ± 1.85 <sup>d</sup>	1.12 ± 0.02 <sup>d</sup>	5.17 ± 0.06 <sup>c</sup>
GSE (200 mg/Kg)	79.75 ± 1.05 <sup>e</sup>	75.38 ± 1.07 <sup>e</sup>	107.34 ± 1.41 <sup>e</sup>	0.66 ± 0.02 <sup>e</sup>	5.89 ± 0.11 <sup>d</sup>
GSE (100 mg/Kg) + Silymarin	85.13 ± 1.14 <sup>e</sup>	95.08 ± 1.38 <sup>f</sup>	111.92 ± 1.63 <sup>e</sup>	0.78 ± 0.02 <sup>f</sup>	5.8 ± 0.08 <sup>d</sup>
GSE (200 mg/Kg) + Silymarin	40 ± 0.74 <sup>a</sup>	54.92 ± 0.99 <sup>g</sup>	91.39 ± 1.24 <sup>f</sup>	0.53 ± 0.01 <sup>g</sup>	6.52 ± 0.15 <sup>e</sup>

ANOVA one way, within each raw, means the different superscript letters are significantly different between groups at P< 0.05; Data are expressed as Mean ± SEM (n=8 rats).

**Table 1:** Effect of GSE (100 and 200 mg/kg) and/or silymarin (50 mg/kg) on serum liver function tests in TAA-induced hepatic fibrosis in rats.

### Effect of GSE (100 and 200 mg/kg) and/or silymarin (50 mg/kg) on liver MDA, NO, Hyp, and GSH contents in TAA-induced hepatic fibrosis in rats

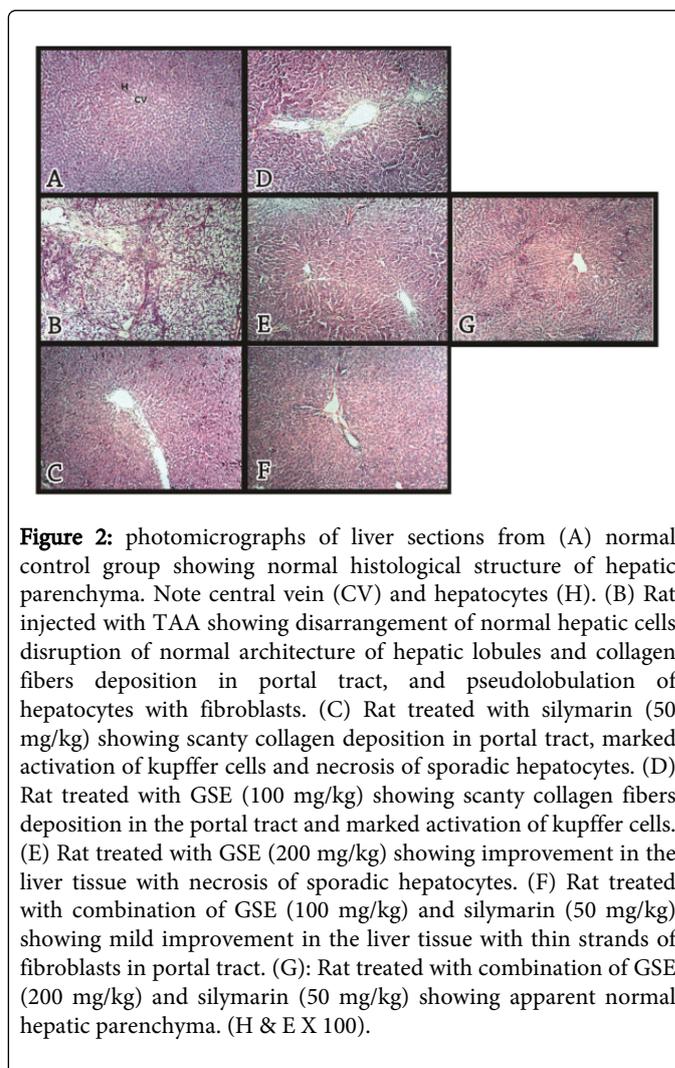
TAA injection caused a significant elevation in MDA, NO, and Hyp values as well as significant depletion in GSH value (Figure 1). Injection of TAA significantly elevated liver MDA, NO, and Hyp contents by 325.65%, 150.57%, and 481.96%, respectively, as compared to normal control values. Treatment with silymarin (50 mg/kg), significantly decreased liver MDA, NO, and Hyp contents by 16.22%, 14.12%, and 24.89%, respectively, compared to TAA control group. Treatment with GSE (100 and 200 mg/kg) showed a significant decrease in elevated liver MDA by 24.56%, and 59.98% (Figure 1A); NO by 15.45%, and 44.82% (Figure 1B), and Hyp by 22.97%, and 64.84% (Figure 1C), compared to TAA control group, respectively. Administration of silymarin (50 mg/kg) combined with either, GSE (100 mg/kg) or GSE (200 mg/kg), significantly decreased elevated liver MDA, NO, and Hyp contents by 44.63%, and 68.18% for MDA (Figure 1A); 29.68%, and 58.02% for NO (Figure 1B); and 48.56%, and 81.3% for Hyp (Figure 1C), respectively, compared to TAA control group.

Results showed that TAA significantly decreased liver GSH content by 56.13%, as compared to normal control group. Treatment with silymarin (50 mg/kg), significantly increased the hepatic concentration of GSH by 27.93%, compared to TAA control group. Moreover, treatment with GSE (100 and 200 mg/kg) caused a significant increase in GSH level by 54.83%, and 82.41%, respectively, compared to TAA control value. Administration of silymarin (50 mg/kg) combined with either, GSE (100 mg/kg) or GSE (200 mg/kg), significantly increased liver GSH content by 89.31%, and 108.97%, respectively, compared to TAA control group (Figure 1D).

### Histopathological studies

The histological examination of the liver of control rats revealed normal architecture of hepatic lobules with hepatocytes radiating from the central veins, with narrow sinusoids and prominent nucleus (Figure 2A).

Light microscopic examination of the TAA control rats showed disruption of normal architecture of hepatic lobules and collagen fibers deposition in portal tract, and pseudolobulation of hepatocytes with fibroblasts (Figure 2B).



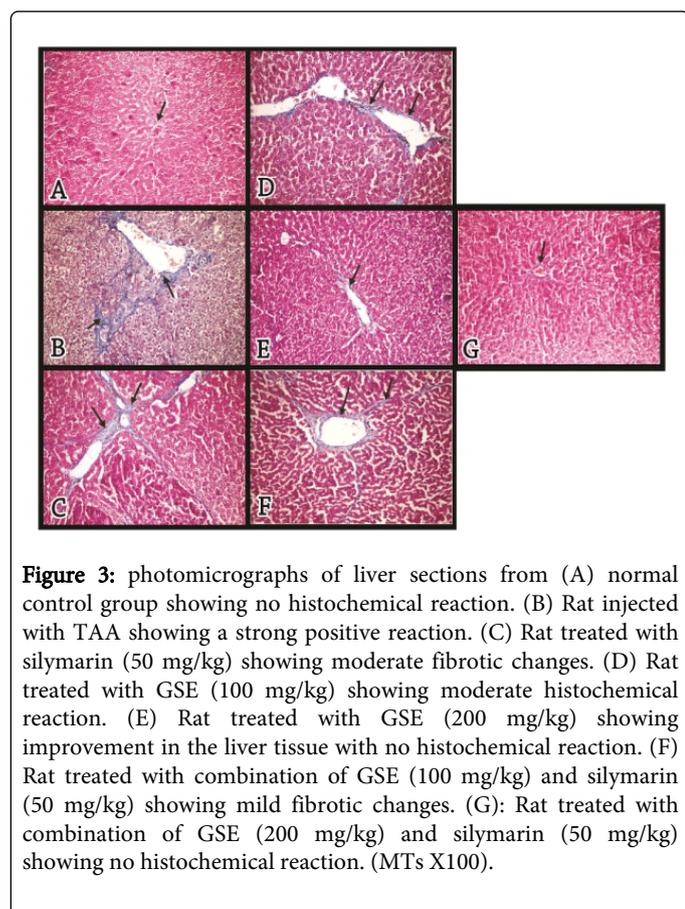
**Figure 2:** photomicrographs of liver sections from (A) normal control group showing normal histological structure of hepatic parenchyma. Note central vein (CV) and hepatocytes (H). (B) Rat injected with TAA showing disarrangement of normal hepatic cells disruption of normal architecture of hepatic lobules and collagen fibers deposition in portal tract, and pseudolobulation of hepatocytes with fibroblasts. (C) Rat treated with silymarin (50 mg/kg) showing scanty collagen deposition in portal tract, marked activation of kupffer cells and necrosis of sporadic hepatocytes. (D) Rat treated with GSE (100 mg/kg) showing scanty collagen fibers deposition in the portal tract and marked activation of kupffer cells. (E) Rat treated with GSE (200 mg/kg) showing improvement in the liver tissue with necrosis of sporadic hepatocytes. (F) Rat treated with combination of GSE (100 mg/kg) and silymarin (50 mg/kg) showing mild improvement in the liver tissue with thin strands of fibroblasts in portal tract. (G): Rat treated with combination of GSE (200 mg/kg) and silymarin (50 mg/kg) showing apparent normal hepatic parenchyma. (H & E X 100).

Treatment with silymarin (50 mg/kg), showed scanty collagen deposition in portal tract, marked activation of kupffer cells and necrosis of sporadic hepatocytes (Figure 2C).

Treatment with GSE (100 mg/kg) showed scanty collagen fibers deposition in the portal tract and marked activation of kupffer cells (Figure 2D).

Treatment with GSE, (200 mg/kg), showed necrosis of sporadic hepatocytes (Figure 2E). Administration of combination of GSE (100 mg/kg) and silymarin (50 mg/kg) showed thin strands of fibroblasts in portal tract (Figure 2F).

In group treated with GSE (200 mg/kg) and silymarin 50 mg/kg combination, the liver sections exhibited apparent normal hepatic parenchyma (Figure 2G).



**Figure 3:** photomicrographs of liver sections from (A) normal control group showing no histochemical reaction. (B) Rat injected with TAA showing a strong positive reaction. (C) Rat treated with silymarin (50 mg/kg) showing moderate fibrotic changes. (D) Rat treated with GSE (100 mg/kg) showing moderate histochemical reaction. (E) Rat treated with GSE (200 mg/kg) showing improvement in the liver tissue with no histochemical reaction. (F) Rat treated with combination of GSE (100 mg/kg) and silymarin (50 mg/kg) showing mild fibrotic changes. (G): Rat treated with combination of GSE (200 mg/kg) and silymarin (50 mg/kg) showing no histochemical reaction. (MTs X100).

### Histochemical studies

The histochemical examination of the liver sections of normal control rats revealed no histochemical reaction (Figure 3A).

Examination of the TAA control rats showed a strong positive reaction which indicate markedly increased accumulation of collagen that was deposited (Figure 3B).

Silymarin (50 mg/kg) treatment showed moderate fibrotic changes (Figure 3C).

Treatment with GSE (100 mg/kg) showed moderate histochemical reaction (moderate fibrotic changes) (Figure 3D).

GSE (200 mg/kg) administration showed no histochemical reaction (Figure 3E).

Administration of combination of GSE (100 mg/kg) and silymarin (50 mg/kg) showed mild fibrotic changes which indicated ameliorated

fibrosis formation with only a few tiny, short bundles of collagen (Figure 3F).

Treatment with combination of GSE (200 mg/kg) and silymarin (50 mg/kg) showed no histochemical reaction (Figure 3G).

### Discussion

The present study shows that TAA-treatment alone had abundant hepatotoxic effect, clearly demonstrated by the elevation in liver enzyme markers, TB, Hyb., MDA and NO levels in hepatic homogenate. As well as, significant decrease in serum TP and hepatic -GSH content.

TAA bioactivation is mediated by microsomal cytochrome P450E1 and/or flavin-containing monooxygenase systems to form TAA sulfoxide involving TAA-S,S-dioxide, which caused lipid peroxidation at the plasma membrane level [33]. The S-oxide metabolite (TASO<sub>2</sub>) covalently binds to liver macromolecules forming acetylimidolysine derivatives that are responsible for hepatotoxic effects [34]. TAA induces calcium (Ca<sup>2+</sup>) mobilization from its intracellular stores [35]. Both ROS and Ca<sup>2+</sup> were determined to activate multiple mechanisms related to cell damage or proliferation [36]. The increased ROS formation and disruption of calcium homeostasis increased the permeability and disrupt the mitochondrial inner membrane and inhibit its respiration [37].

In accordance with our results, total protein significantly decreased as a result of TAA-induced liver toxicity this may be due to defect in RNA synthesis [38] and disturbances in carbohydrate, protein, lipid metabolisms due to acute liver injury induced by TAA [39].

Moreover, TAA injection significantly elevated liver MDA content while, significantly decreased liver GSH content. This may be due to chronic injection with TAA, a hepatotoxin containing thiono-sulfur compound, which induce hepatic damage and fibrosis by generation of ROS and suppressed antioxidant defense mechanism [40,41]. Meanwhile, TAA injection, showed a significant elevation in liver NO content. This may be due to the formation of NO, which increases in liver disease where L-arginine-NO pathway is activated [39,42].

Finally, TAA treatment caused a significant increase in hepatic Hyp content, which supported by histopathological and histochemical observations that showed the presence of fibrosis and numerous connective tissue strands [43]. Hepatic fibrosis is the progressive accumulation of ECM in hepatic tissue resulted from unbalance status of ECM production and degradation [44]. The oxidative stress activates HSCs, induces the secretion of growth factors and profibrogenic cytokines that stimulates collagen synthesis [45].

The present study showed that treatment with GSE (100 and 200 mg/kg) significantly reduced serum AST, ALT, ALP, and TB levels while, significantly increased serum TP level in a dose-dependent manner, which attributed to GSE prevent the leakage of intracellular enzymes [46].

Moreover, GSE (100 and 200 mg/kg) significantly reduced liver MDA and NO contents and increased liver GSH content in a dose-dependent manner [47].

GSE contains polyphenolic compounds such as procyanidins and proanthocyanidins that have powerful free radical scavenging effect [48,49]. Previous study demonstrated that GSE prevent hepatic fibrosis and dysfunction caused by chronic arsenic administration in rats [50].

Meanwhile, GSE significantly reduced liver Hyp content in a dose-dependent manner, our finding was supported by significant improvement in histopathological and histochemical examinations, this may be attributed to GSE inhibited HSCs activation, which subsequently resulted in suppressed mRNA level of the  $\alpha_1$ -I-collagen and decreased collagen accumulation [51].

In the present study, treatment with silymarin (50 mg/kg) significantly decreased serum AST, ALT, ALP, and total bilirubin levels while, significantly elevated serum total protein level. These may attributed to protection of liver cells directly through stabilizing the cell membrane by preventing liver glutathione depletion and inhibiting lipid peroxidation [52]. The pharmacological properties of silymarin involve regulating cell membrane permeability and integrity, inhibiting leukotriene, scavenging reactive oxygen species [53]. Abdel-Salam et al. reported that the silymarin (22 mg/kg) decreased leakage of hepatocellular enzymes ALT and AST into the plasma, decreased serum levels of ALP and lessened the development of liver necrosis and fibrosis caused by carbon tetrachloride (CCl<sub>4</sub>) [54].

Moreover, silymarin significantly decreased liver MDA and NO contents while, significantly increased liver GSH content, compared to TAA control group. This due to silymarin has antioxidant and scavenging free radicals (ROS) activities, thus protecting against oxidative stress. It augments the non-enzymatic and enzymatic antioxidant defense systems of cells involving GSH, superoxide dismutase and catalase. It can protect the liver, brain, heart and other vital organs from oxidative damage for its ability to prevent lipid peroxidation and replenishing the reduced glutathione levels. Furthermore, silibinin exhibits membrane protective properties and it may protect blood constituents from oxidative damage [55].

Silymarin administration significantly declined liver Hyp content induced by TAA –treatment and there were significant improvement in the histopathological and histochemical hepatic architecture. It was found that silymarin inhibit stellate hepatocytes transformation to myofibroblasts, which is responsible for collagen deposition in CCl<sub>4</sub> -induced hepatic fibrosis [56,57].

Combined administration of GSE and silymarin effectively attenuate TAA-induced hepatic fibrosis in dose dependant manner and significantly improved the tested biochemical parameters, decreased hepatic Hpy content, prevent the oxidative stress and restoring GSH level in experimental hepatic fibrosis.

Our results indicated the potential and beneficial role of GSE and silymarin combinations in preventing oxidative stress-mediated damage and strengthening antioxidant defense mechanism increased antioxidant status of animals.

## Conclusion

It is suggested that GSE and silymarin combination had synergistic effect as antifibrotic therapy than single treatment with silymarin or GSE alone and GSE (200 mg/Kg) combination with silymarin had a good resultant effect.

## Conflict of interest

The authors declared no conflicts of interest.

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