

# Effect of Silymarin versus Silymarin and Green Coffee Extract on Thioacetamide Induced Liver Injury in Adult Male Albino Rats (Histological and Immunohistochemical Study)

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Article

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## ABSTRACT

**Background:** Thioacetamide (TAA) is a potent hepatotoxic agent used to induce liver toxicity in experimental animal models. Protective role of silymarin in liver intoxication was proved. Coffee is the most popular beverage used in the world. Consumption of green coffee bean extract has many benefits on health in general, and liver in particular.

**Aim of work:** To evaluate the potential role of adding green coffee extract to silymarin in improving thioacetamide induced hepatic injury in rats.

**Materials and methods:** Thirty six albino rats were divided into 4 groups; group I (control group), group II (thioacetamide group), group III (Silymarin group) and Group IV (Silymarin and green coffee extract group). Biochemical analysis for liver function, antioxidant enzyme assay were performed. Liver sections were stained using hematoxylin and eosin, Masson trichrome stains and immunohistochemical expression for caspase-3. Morphometric and statistical analysis were done.

**Results:** Liver functions of group II showed significant deterioration which slightly improved in group III and tend to be normal in group IV. Antioxidant enzyme levels of group II and group III were significantly lower than that of group I; group III and group IV levels were significantly higher than that of group II. Liver sections of group II showed massive dilated congested portal vein, bile duct proliferation, and mononuclear cellular infiltrates. Group III showed slightly dilated congested portal vein in some areas while appeared normal in other areas. In group IV portal area appeared almostly normal. Marked increased collagen deposition was detected in group II, decreased in group III and markedly decreased in group IV. Immunohistochemical expression of caspase-3 was increased in group II, lower in group III and significantly low in group IV.

**In conclusion:** Adding green coffee extract to silymarin potentiate the therapeutic effect of silymarin in improving experimentally induced hepatic injury by thioacetamide in rats.

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**Key Words:** Green coffee extract, liver, silymarin, thioacetamide.

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## INTRODUCTION

Liver is a vital organ that plays a vital role in human physiology like metabolism of macromolecules and synthesis of useful components. Exposure to chemicals such as thioacetamide (TAA), alcohol, D-galactosamine, environmental pollutants and the abuse of drugs asparacetamol or rifampicin can damage hepatocytes leading to hepatotoxicity and liver fibrosis<sup>[1&2]</sup>. Liver fibrosis is a major health problem resulting in a significant morbidity and mortality. Up-to-date, there is no standard treatment for fibrosis because of its complex pathogenesis<sup>[3]</sup>. Acute liver injury, fibrosis or cirrhosis can be experimentally induced by various chemicals, such as CCl<sub>4</sub>, thioacetamide (TAA), and ethionine<sup>[4&5]</sup>.

TAA is a potent hepatotoxic agent and is widely used to induce liver toxicity in experimental animal models. It causes acute liver toxicity by interfering with the transfer of

RNA to cytoplasm from the nucleus, which leads to injury to the membrane. Basically, microsomal CYP2E1 converts TAA through two steps to "TAA-s-oxide or sulfoxide" and then to a bioactive metabolite TAA-S, S-dioxide which acts as a direct hepatotoxicant and causes centrilobular necrosis<sup>[6]</sup>.

The rate of morbidity and mortality due to hepatotoxicity or liver dysfunction is raising, which makes it a major health problem throughout the world posing a big challenge to health-care professionals, drug regulatory agencies, and pharmaceutical industry to find an adequate, suitable treatment<sup>[7]</sup>.

Thus, experimental models of toxic liver injury induced by TAA may be useful to evaluate the pharmacological effects of candidate hepatoprotectants, including Silymarin, coffee and caffeine products. The use of natural products in the prevention and treatment of liver diseases

has gained considerable popularity<sup>[8]</sup>.

Silymarin extract contains approximately 65% to 80% flavonolignans (silybin A, silybin B, isosilybin A, isosilybin B, silychristin and silydianin), a small proportion of flavonoids, fatty acids and polyphenolic compounds, which possess a range of metabolic regulatory effects<sup>[9]</sup>. The hepatoprotective properties of Silymarin in liver intoxication have been previously described<sup>[10]</sup>.

Coffee is the most popular beverage used worldwide. Consumption of coffee has many benefits on health in general, and liver health in particular. It is an important source of bioactive phytochemicals including; methylxanthines (e.g. caffeine), amino acids, antioxidants like chlorogenic acid (CGA), tocopherols and polyphenols<sup>[11,12 & 13]</sup>.

To avoid loss of the beneficial compounds of coffee, it can be used as green coffee bean extract (GCBE). It is made up of unroasted coffee beans and contains higher amounts of bioactive phytochemicals than that for the usual roasted coffee that is currently used. It has biological effects mostly related to their antioxidant and anti-inflammatory activities<sup>[14 & 15]</sup>.

A lot of interest has been generated in the overall beneficial effects of coffee consumption that plays a protective role against various diseases of modern society<sup>[16 & 17]</sup>. Moreover, a number of studies have suggested the effect of coffee constituents on liver function, development and progression of liver diseases<sup>[18]</sup>. Therefore, the aim of this study was to evaluate the potential role of adding green coffee extract to silymarin in improving thioacetamide induced hepatic injury in rats.

## MATERIALS AND METHODS

### Chemicals:

Thioacetamide was purchased from Sigma Chemical Company (St. Louis, MO, USA).

Pure sample of silymarin was obtained from Sigma Chemicals, USA. Silymarin was dissolved in distilled water, then administered orally to the animals by gastric intubation using a force feeding needle.

GCBE was purchased in the form of Svetol, from Holland & Barrett, Cardiff; UK.

### Animals:

Thirty six adult Wistar male albino rats (2-3 months) weighing 200-230 gm. were purchased from the Animal House Colony of the National Research Center, Dokki, Cairo, Egypt. They were kept under controlled conditions, standard humidity, and a temperature of 22±2°C in plastic cages. The study was carried out in Faculty of Medicine, Zagazig University according to the guidelines for animal research issued by the National Institute of Health, and approved by Animal Ethics Committee, Zagazig University, Zagazig, Egypt.

### Experimental design:

Rats were randomly divided into 4 groups (9 rats/group);

**Group I** served as the control group where rats received normal saline by intraperitoneal injection twice/week for 6 weeks<sup>[19]</sup>.

**Group II** (thioacetamide treated group); rats were injected thioacetamide (100 mg/kg body weight intraperitoneally) twice/week for 6 weeks<sup>[19]</sup>.

**Group III** rats were injected thioacetamide in the same way as group II, then they were received Silymarin (100 mg/kg/day) orally by gastric gavage for 6 weeks<sup>[19]</sup>.

**Group IV** rats were injected thioacetamide in the same way as group II, then they were received Silymarin (100 mg/kg/day) by gastric gavage together with GCBE (200 mg/kg/day) orally for 6 weeks<sup>[20 & 21]</sup>.

At the end of experiment, liver tissue was dissected. Each harvested liver was divided into two halves; one for homogenate and the other for histological and immunohistochemical study.

### Biochemical study:

#### Biochemical analysis for assessment of liver function

Blood samples were collected just before sacrifice from the tail veins for assessment of liver function. Serum alanine transaminase (ALT), and aspartate transaminase (AST) activities were determined using kits provided by Elitech (France). They were measured by spectrophotometer<sup>[22]</sup>.

#### Antioxidant enzymes assays<sup>[23]</sup>:

One half of the livers were used for estimation of Superoxide Dismutase (SOD) and Glutathione levels (GSH) was performed in the Department of Biochemistry, Faculty of Medicine, Zagazig University.

#### Total Superoxide Dismutase (SOD) activity:

SOD activity (antioxidant biomarker) was measured in the liver homogenate using a commercial chemical colorimetric assay kit (Biodiagnostic, Cairo, Egypt).

#### Glutathione levels (GSH):

Reduced GSH was determined in the liver homogenates using a commercial chemical kit (Biodiagnostic, Cairo, Egypt).

### Histological methods:

#### (a) Histological study

Liver tissue was excised and fixed in 10% buffered formalin saline. Then, they were processed to obtain 5µm-thick paraffin sections. Sections were then stained with hematoxylin and eosin (H&E) stain<sup>[24]</sup> and Masson trichrome stain<sup>[25]</sup>.

### **(b) Immunohistochemical study**

Immunohistochemical expression of caspase-3 was performed using streptavidin–biotin complex immune-peroxidase system. Serial sections of paraffin-embedded specimens were deparaffinized on charged slides. sections were then processed according to the method described in<sup>[26]</sup>.

### **Morphometric Analysis:**

The diameter of central vein was measured from photos of X 400 magnification using digimizer 4.3.2. image analysis software (MedCalc Software bvba, Belgium). The area percentage of collagen fibers and area percentage of immune reaction to caspase 3 were measured using the Fiji Image J (1.51n, NIH, USA) program<sup>[27]</sup>.

### **Statistical analysis:**

Statistical analysis was performed using the IBM SPSS 18.0 software. One-way analysis of variance (ANOVA) was used, followed by Posthoc least significant difference (LSD) test to evaluate the differences between the groups. For all comparison  $P < 0.05$  were considered as significant difference<sup>[28]</sup>.

## **RESULTS**

### **Biochemical results**

#### **Effect of GCBE on liver function tests:**

There was a significant difference between groups using one-way ANOVA test ( $P = 0.001^*$ ). Using LSD test, ALT levels of thioacetamide group and Silymarin treated group were significantly higher than that of control group. ALT level of Silymarin treated group and Silymarin & GCBE treated group were significantly lower than that of thioacetamide group. There was a significant difference in ALT levels from Group IV (Silymarin and GCBE treated group) and Group III (Silymarin treated group) (Table 1 & Bar chart 1).

There was a highly significant difference between groups using one-way ANOVA test ( $P < 0.001^*$ ). Using LSD test, AST levels of thioacetamide group and Silymarin treated group were significantly higher than that of control group. AST level of Silymarin treated group and Silymarin & GCBE treated group were significantly lower than that of thioacetamide group. There was a significant difference in AST levels from Silymarin & GCBE treated group and Silymarin treated group (Table 1 & Bar chart 2).

#### **Effect of GCBE on GSH level and SOD activity:**

There was a significant difference between groups using one-way ANOVA test ( $P < 0.001^*$ ). Using LSD test, GSH and SOD levels of thioacetamide group and Silymarin treated group were significantly lower than that of control group. GSH and SOD levels of Silymarin treated group and Silymarin & GCBE treated group were significantly higher than that of thioacetamide group. There was a significant difference in GSH and SOD levels between Silymarin &

GCBE treated group and Silymarin treated group (Table 2 & Bar charts 3, 4).

### **Histological results**

Control group sections stained with H & E showed normal polygonal classic hepatic lobules with tightly packed cords of hepatocytes radiating from the central vein. Portal area was seen at the periphery of the lobule (Fig. 1A). Hepatocytes of the same group contained vesicular nuclei and acidophilic cytoplasm, some of them were binucleated. Blood sinusoid in between hepatocyte cords and their lining endothelium were seen (Fig. 1B). TTA treated group liver sections showed massive dilated congested portal vein, with area of homogenous eosinophilic material, bile duct proliferation, hepatocytes had deep acidophilic cytoplasm with small dark nuclei and mononuclear cellular infiltrates were seen (Fig. 1C). Dilated congested central vein was detected (Fig. 1D). Liver sections of Silymarin treated group revealed many hepatocytes with vesicular nuclei and acidophilic cytoplasm (Fig. 1E, F & G). Portal area showed mononuclear cellular infiltrates, slightly dilated congested portal vein in some areas as in (Fig. 1E) while appeared normal in other areas with normal appearance of branches of hepatic artery and bile duct were seen as in (Fig. 1F), dilated congested central vein was still detected in some areas of this group (Fig. 1G). In Silymarin and GCBE treated group portal area appeared almostly normal with normal appearance of branches of portal vein, hepatic artery and bile duct apart from minimal cellular infiltration were seen, hepatocytes appeared with vesicular nuclei and acidophilic cytoplasm (Fig. 1H). Minimal congestion in central vein was observed in this group (Fig. 1I).

Masson trichrome stained sections of the control group revealed few collagen fibers around central vein and portal area (Fig. 2A & B). Marked increased collagen deposition around central vein and in between hepatocytes (Fig. 2C) and in portal area (Fig. 2D) were detected in TTA treated group. Few collagen fibers were found in Silymarin treated group around central vein (Fig. 2E) and in portal area (Fig. 2F). Minimal amount of collagen deposition was seen in Silymarin & GCBE treated group around central vein (Fig. 2G) and in portal area (Fig. 2H).

### **Immunohistochemical results:**

Examination of Caspase 3 immunostained liver sections of control group revealed negative caspase-3 immunoreaction in cytoplasm of hepatocytes (Fig. 3A). liver sections of TTA treated group showed strong positive immunoreaction for caspase -3 in cytoplasm of most of hepatocytes (Fig. 3B), however Silymarin treated group revealed mild positive caspase-3 immunoreaction in some hepatocytes (Fig. 3C). Silymarin and GCBE treated group showed few areas of weak positive caspase-3 immunoreaction in cytoplasm of hepatocytes (Fig. 3D).

### **Morphometric and statistical results:**

#### **I. Long and short diameters of central vein ( $\mu\text{m}$ )**

There was a highly significant difference between groups using one-way ANOVA test ( $P < 0.001^*$ ). Using LSD test, long diameter of central vein of thioacetamide group and Silymarin treated group were significantly higher than that of control group. Long diameters of central vein of Silymarin treated group and Silymarin & GCBE treated group were significantly lower than that of thioacetamide group. There was a significant difference in long diameters of central vein from Silymarin and GCBE treated group and Silymarin treated group (Table 3 & Bar chart 5A).

There was a highly significant difference between groups using one-way ANOVA test ( $P < 0.001^*$ ). Using LSD test, short diameters of central vein of thioacetamide group and Silymarin treated group were significantly higher than that of control group. Short diameters of central vein of Silymarin treated group and Silymarin & GCBE treated group were significantly lower than that of thioacetamide group. There was a significant difference in short diameters of central vein from Silymarin and GCBE treated group and Silymarin treated group (Table 3 & Bar chart 5B).

**II. The area percentage of collagen fibers:**

There was a highly significant difference between

groups using one-way ANOVA test ( $P < 0.001^*$ ). Using LSD test, the area percentage of collagen fibers of thioacetamide group and Silymarin treated group were significantly higher than that of control group. The area percentage of collagen fibers of Silymarin treated group and Silymarin and GCBE treated group were significantly lower than that of thioacetamide group. There was a significant difference in the area percentage of collagen fibers from Silymarin & GCBE treated group and Silymarin treated group (Table 4 & Bar chart 6).

**III. The area percentage of immunoreaction to caspase 3**

There was a highly significant difference between groups using one-way ANOVA test ( $P < 0.001^*$ ). Using LSD test, the area percentage of immunoreaction to caspase 3 of thioacetamide group and Silymarin treated group were significantly higher than that of control group. The area percentage of immune reaction to caspase 3 of Silymarin treated group and Silymarin & GCBE treated group were significantly lower than that of thioacetamide group. There was a significant difference in the area percentage of immunoreaction to caspase 3 from Silymarin & GCBE treated group and Silymarin treated group (Table 5 & Bar chart 7).

**Table 1:** The effect of Silymarin alone and combination of Silymarin and GCBE on the liver function tests.

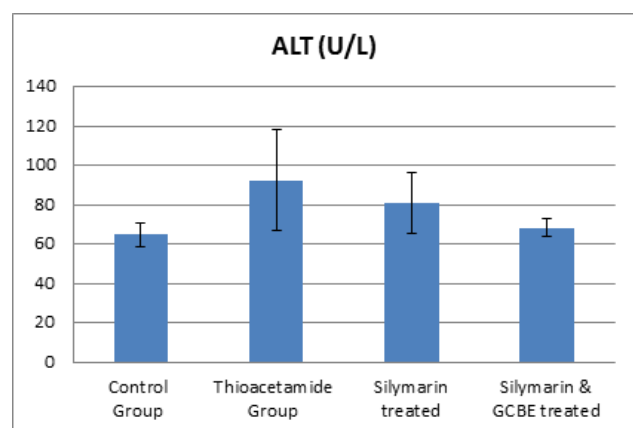
Groups	Control Group	Thioacetamide Group	Silymarin treated	Silymarin & GCBE treated	P value
ALT (U/L)	64.9 ± 6.01	92.4 <sup>a</sup> ± 25.6	80.82 <sup>ab</sup> ± 15.8	68.13 <sup>bc</sup> ± 4.5	0.001*
AST (U/L)	279.3 ± 28.9	470.4 <sup>a</sup> ± 94.1	376.1 <sup>ab</sup> ± 99.6	297.9 <sup>bc</sup> ± 52.5	<0.001*

- Data represent mean ± SD <0.05 was considered significant n=9.

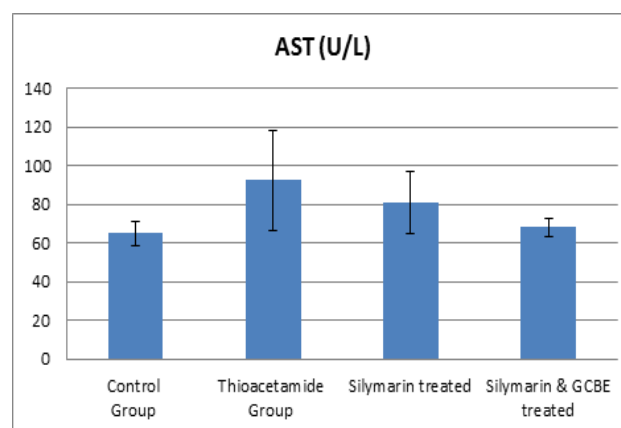
a: Significant difference from the control group.

b: Significant difference from the thioacetamide untreated group.

c: Significant difference from the Silymarin treated group.



**Bar chart 1:** Comparison between mean values of ALT level among the studied groups (N=9,  $P = 0.001^*$ ).



**Bar chart 2:** Comparison between mean values of AST level among the studied groups (N=9,  $P < 0.001^*$ ).

**Table 2:** The effect of Silymarin alone and combination of Silymarin and GCBE on levels of GSH and SOD activity in the liver homogenates of rats.

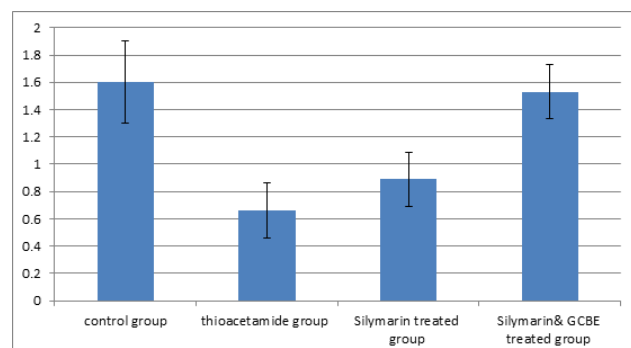
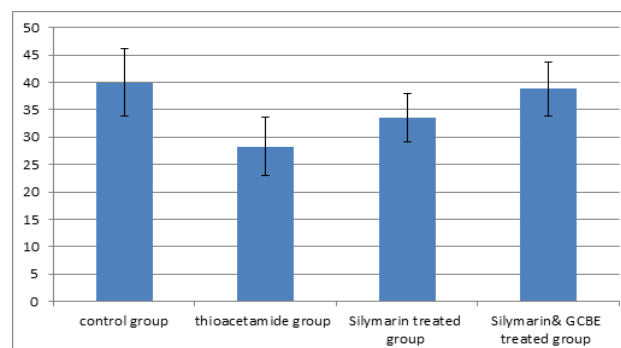
	Control Group	Thioacetamide Group	Silymarin treated group	Silymarin &GCBE treated group	<i>P</i> value
GSH ( $\mu\text{mol/g}$ )	$1.60 \pm 0.3$	$0.66^a \pm 0.2$	$0.89^{ab} \pm 0.2$	$1.53^{bc} \pm 0.2$	0.001*
SOD (U/mg protein)	$40 \pm 6.1$	$28.3^a \pm 5.3$	$33.6^{ab} \pm 4.4$	$38.8^{bc} \pm 4.9$	<0.001*

- Data represent mean $\pm$ SD <0.05 was considered significant n=9.

a: Significant difference from the control group.

b: Significant difference from the thioacetamide untreated group.

c: Significant difference from the silymarin treated group.

**Bar chart 3:** Comparison between mean values of GSH ( $\mu\text{mol/g}$ ) among the studied groups (N=9,  $P < 0.001^*$ ).**Bar chart 4:** Comparison between mean values of SOD (U/mg protein) among the studied groups (N=9,  $P < 0.001^*$ ).**Table 3:** The effect of Silymarin alone and combination of Silymarin and GCBE on the long and short diameters of central vein ( $\mu\text{m}$ ).

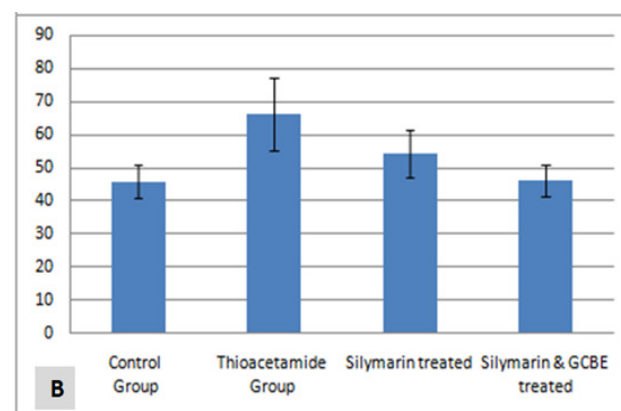
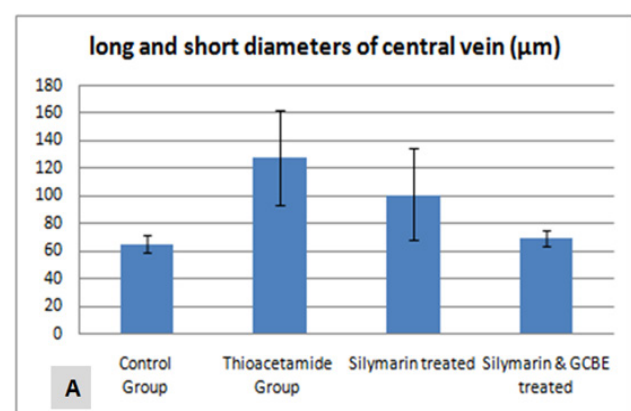
	Control Group	Thioacetamide Group	Silymarin treated group	Silymarin &GCBE treated group	<i>P</i> value
Long diameter of central vein	$65.75 \pm 6.6$	$127.98 \pm 34.6a$	$101.1 \pm 33.2ab$	$69.4 \pm 5.5bc$	0.001*
Short diameter of central vein	$45.9 \pm 4.99$	$66.2 \pm 10.8a$	$54.5 \pm 7.2ab$	$46.4 \pm 4.8 bc$	<0.001*

- Data represent mean $\pm$ SD <0.05 was considered significant n=9.

a: Significant difference from the control group.

b: Significant difference from the thioacetamide untreated group.

c: Significant difference from the silymarin treated group.

**Bar chart 5:** Comparison between mean values of long (A) and short (B) diameters of central vein ( $\mu\text{m}$ ) among the studied groups (N=9,  $P < 0.001^*$ ).

**Table 4:** The effect of Silymarin alone and combination of Silymarin and GCBE on the area percentage of collagen fibers

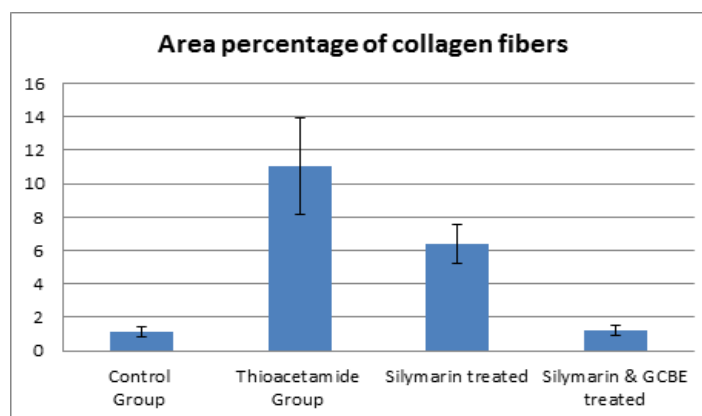
	Control Group	Thioacetamide Group	Silymarin treated group	Silymarin &GCBE treated group	<i>P value</i>
area percentage of collagen fibers	1.1 ± 0.3	11.04 ± 2.9a	6.4 ± 1.2ab	1.19 ± 0.3bc	<0.001*

- Data represent mean±SD <0.05 was considered significant n=9.

a: Significant difference from the control group.

b: Significant difference from the thioacetamide untreated group.

c: Significant difference from the Silymarin treated group.



**Bar chart 6:** Comparison between mean values of area percentage of collagen fibers among the studied groups.

**Table 5:** The effect of Silymarin alone and combination of Silymarin and GCBE on the area percentage of caspase 3 immune reaction:

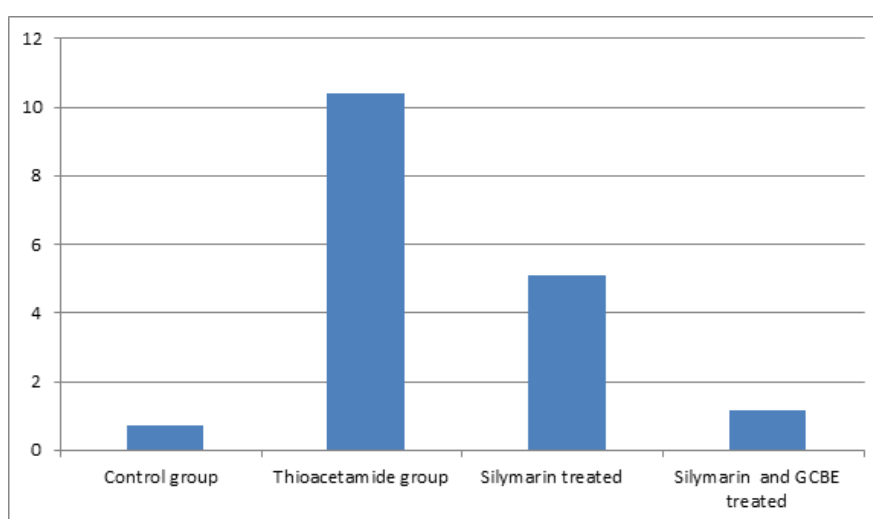
	Control Group	Thioacetamide Group	Silymarin treated group	Silymarin &GCBE treated group	<i>P value</i>
Area percentage of caspase 3 immune reaction	0.73 ± 0.14	10.4 ± 1.4a	5.1 ± 1.3ab	1.15 ± 0.42bc	<0.001*

- Data represent mean±SD <0.05 was considered significant n=9.

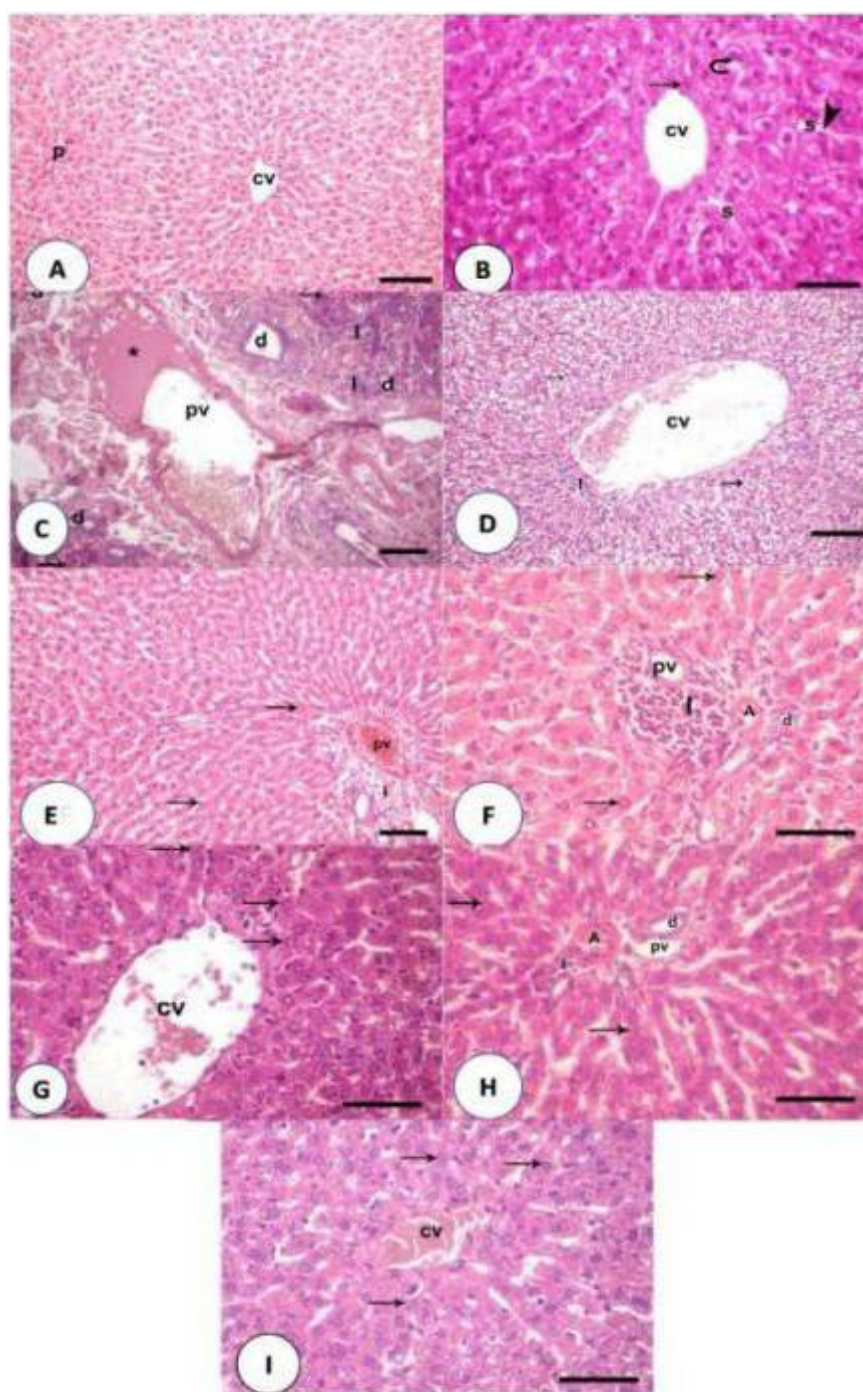
a: Significant difference from the control group.

b: Significant difference from the thioacetamide untreated group.

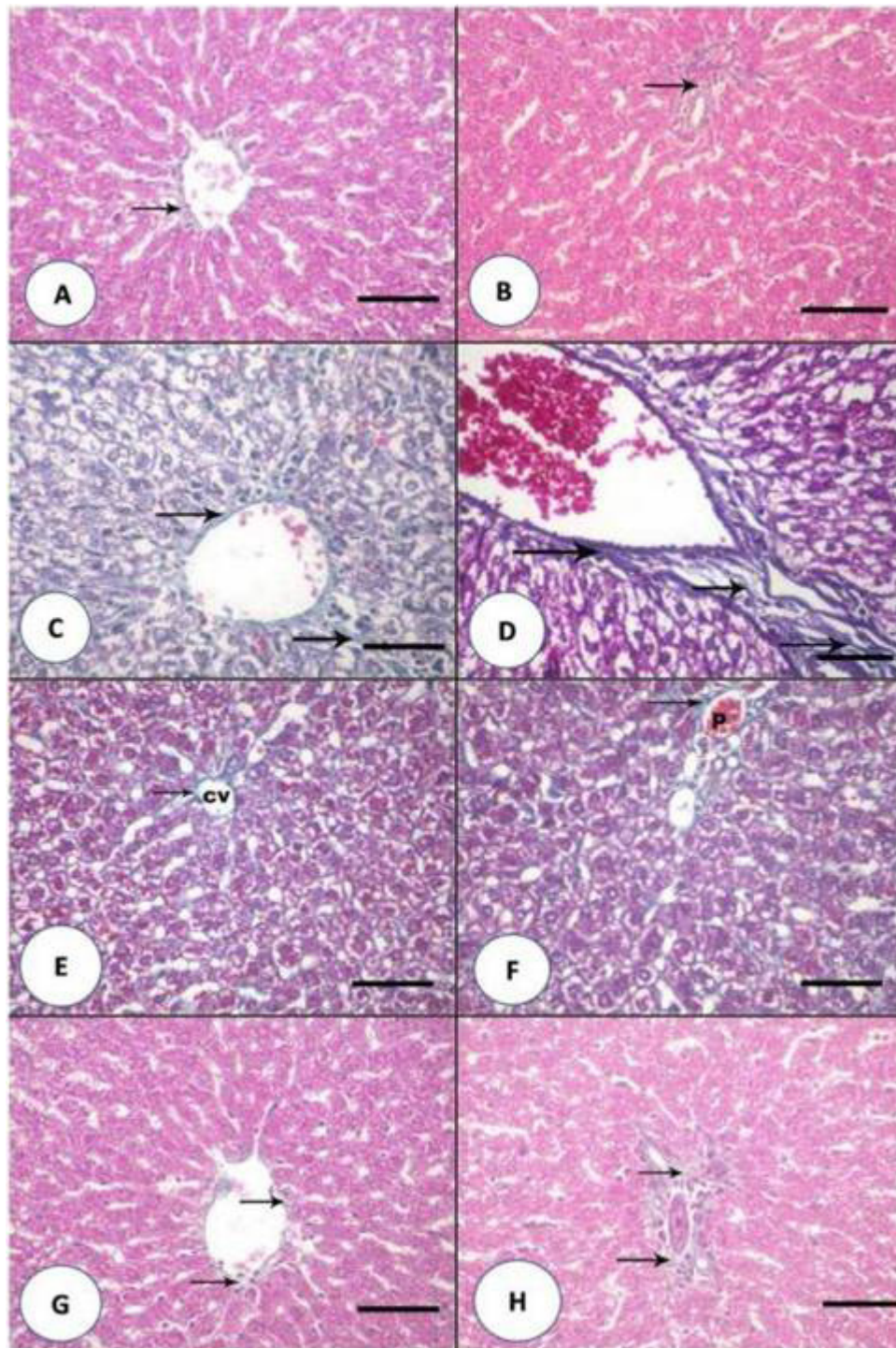
c: Significant difference from the Silymarin treated group.



**Bar chart 7:** Comparison between mean values of area percentage of caspase 3 immune reaction among the studied groups.

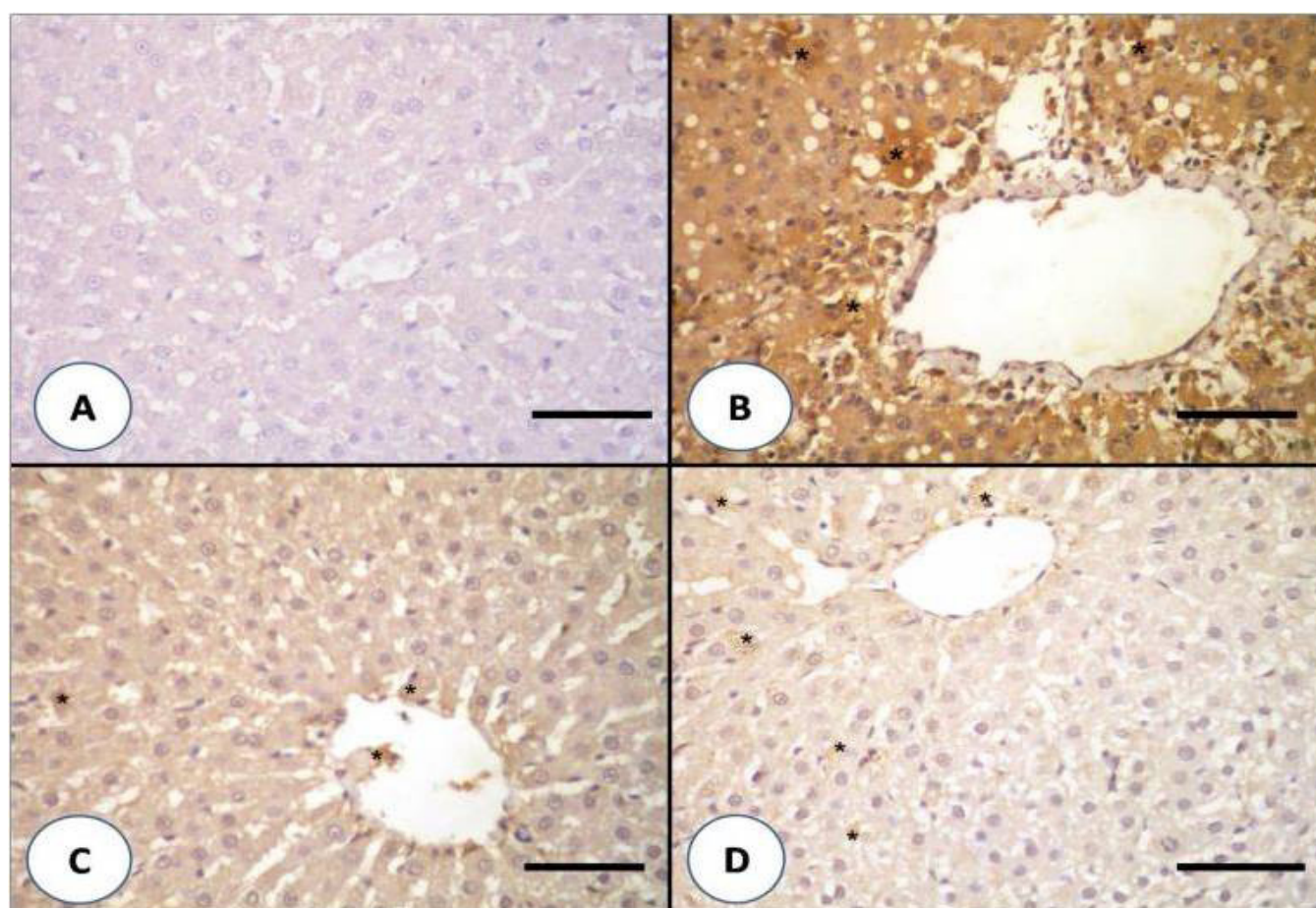


**Fig.1:** Haematoxylin and Eosin stained liver sections A: control group shows normal polygonal classic hepatic lobules with tightly packed cords of hepatocytes are radiating from the central vein (cv), portal area (P) is seen at the periphery of the lobule. B: the higher magnification shows hepatocytes with vesicular nuclei and acidophilic cytoplasm (arrow), some hepatocytes are binucleated (curved arrow). Blood sinusoids (S) inbetweenhepatocytic cords and their lining endothelium (arrows head) are seen. TTA treated group shows C: massive dilated congested portal vein (pv), with area of homogenous oesinophilic material (asterisk), bile duct proliferation (d), hepatocytes have deep acidophilic cytoplasm with small dark nuclei (arrow) and mononuclear cellular infiltrates (I) are seen. D: Dilated congested central vein (cv) is seen. Hepatocytes have vacuolated cytoplasm with small dark nuclei (arrows) and pericentral cellular infiltrates (I) are seen. In Silymarin treated group E & F many hepatocytes appear with vesicular nuclei and acidophilic cytoplasm (arrows), portal area shows mononuclear cellular infiltrates (I), slightly dilated congested portal vein (pv) in some areas as in (E) while appeared normal in other areas as in (F), normal appearance of branches of hepatic artery (A) and bile duct (d) are seen in (F). G: dilated congested central vein (cv) is still detected in some areas, hepatocytes appear with vesicular nuclei and acidophilic cytoplasm (arrows). In Silymarin & Green coffee treated group H: portal area appear almostly normal with normal appearance of branches of portal vein (pv), hepatic artery (A) and bile duct (d) apart from minimal cellular infiltration(I) are seen, hepatocytes appear with vesicular nuclei and acidophilic cytoplasm (arrows). I: minimal congestion in central vein (cv) is observed & most of hepatocytes appear with vesicular nuclei and acidophilic cytoplasm (arrows). (H&E: A x 200; scale bar 30  $\mu$ m, B x400; scale bar 50  $\mu$ m, C, x 200; scale bar 50  $\mu$ m, D,E x 200; scale bar 40  $\mu$ m,F,G,H,I x 40; scale bar 40  $\mu$ m)



**Fig.2:** Masson trichrome stained sections showing A&B: few collagen fibers around central vein (A) and portal area (B) in control group (arrows), C&D: marked increase in collagen deposition around central vein and in between hepatocytes (C) and in portal area (D) in TTA treated group, In E&F Few collagen fibers can be seen in Silymarin treated group around central vein (E) and portal area (F) .In G&H show minimal amount of collagen deposition around central vein (G) and in portal area (H) in Silymarin & Green coffee treated group. (Masson trichrome x400; scale bar 40µm)





**Fig.3:** Caspase 3 immunostained liver sections showing: control group (A): negative caspase-3 immunoreaction in cytoplasm of hepatocytes TTA treated group (B): Strong positive immunoreaction for caspase -3 (asterisks) in cytoplasm of most of hepatocytes. Silymarin treated group (c): Mild positive caspase-3 (asterisks) immunoreaction in some hepatocytes. Silymarin& Green coffee treated group (D): Few areas of weak positive caspase-3 immunoreaction (asterisks) in cytoplasm of hepatocytes. (Immunoperoxidase technique for caspase3  $\times 400$ , Scale bar; 40 $\mu$ m)

## DISCUSSION

Liver fibrosis is a major health problem<sup>[29]</sup>. The present study evaluated the potential role of silymarin and green coffee extract to guard against thioacetamide induced hepatic injury and fibrosis in rats.

In the current study, ALT and AST levels of thioacetamide group and silymarin treated group were significantly higher than that of the control group. ALT and AST levels of silymarin treated group and Silymarin&GCBE treated group were significantly lower than that of thioacetamide group. There was a significant difference in ALT and AST levels between silymarin treated group and silymarin& GCBE treated group.

A significant increase in the level of serum transaminases is considered as a biomarker of cellular leakage and damage of the cell membrane of liver. ALT is a sensitive and important biomarker of liver toxicity. The serum level of ALT activity directly linked to the damage to the hepatocytes. The serum level of AST is another important biomarker of liver functions. TAA intoxicated rats showed significant alterations in the level of serum biomarker enzymes of liver, kidneys, and lipid profile<sup>[30]</sup>.

A single dose of TAA causes necrosis along with increased level of serum transaminases and bilirubin concentrations in rats<sup>[31&32]</sup>.

Several antioxidant agents, including Silymarin, vitamins (C and E), and melatonin have been reported to reduce induced hepatic toxicity<sup>[33]</sup>. Silymarin is a flavonoid extracted from the milk thistle *Silybum marianum*. Although Silymarin has been described to possess antioxidant, immunomodulatory, antiproliferative, antifibrotic, and antiviral activities<sup>[34]</sup>, its mechanisms of action still have not been well established<sup>[35]</sup>.

Wadhawan *et al.*<sup>[12]</sup> reported that consumption of coffee has been shown to benefit health in general, and liver health in particular. The clinical evidence of benefit of coffee consumption in Hepatitis B and C, as well as nonalcoholic fatty liver disease and alcoholic liver disease, has also been presented. Coffee consumption is associated with improvement in liver enzymes (ALT, and AST), especially in individuals with risk for liver disease. Coffee intake more than 2 cups per day in patients with preexisting liver disease has been shown to be associated with lower incidence of fibrosis and cirrhosis, lower hepatocellular carcinoma rates, as well as decreased mortality.

In the present work, GSH and SOD levels of thioacetamide group and silymarin treated group were significantly lower than that of the control group. GSH and SOD levels of silymarin treated group and silymarin & GCBE treated group were significantly higher than that of thioacetamide group. There was a significant difference in GSH and SOD levels between silymarin & GCBE treated group and silymarin treated group.

TAA is a potent hepatic toxicant, and carcinogenic agent in rats. TAA administration at a dose of 100 mg/kg is reported to cause hepatorenal toxicity. The increased oxidative stress is considered as the main cause of TAA-induced hepatotoxicity. Also, chronic exposure of TAA may cause liver cirrhosis in rats. The mechanism of hepatorenal toxicity of TAA is due to interference of the RNA movement to cytoplasm from the nucleus, which results in injury to the membrane<sup>[6&36]</sup>.

Silymarin protects against liver injury caused by ethanol administration. The effect may be related to alleviating lipid peroxidation and inhibiting the expression of NFkB. It can prevent lipid peroxidation, inhibit low-density lipoprotein oxidation and scavenge reactive oxygen species ROS<sup>[35&37]</sup>. Moreover, it has anti-inflammatory effects which may relate its ability to inhibit NFkB, which contributes to the production of proinflammatory mediators such as IL-1 and IL-6, TNF- $\alpha$ , and interferon (IFN)- $\gamma$ <sup>[38]</sup>.

Green coffee extract is a source of dietary antioxidants that may improve the quality of life. It is rich in phenolic compounds (e.g. chlorogenic acid, caffeic acid and caffeoyl tryptophan), cafestol, kahweol, nicotinic acid and caffeine, the antioxidant and antimutagenic effects of which have been demonstrated in vitro. Antioxidants prevent free radical tissue damage by reducing or eliminating reactive oxygen species<sup>[39]</sup>.

In the current study, histological examination of H & E stained liver sections of TTA treated group showed massive dilated congested portal vein, with area of homogenous eosinophilic material and dilated congested central vein. Bile duct proliferation, hepatocytes had deep acidophilic cytoplasm with small dark nuclei and mononuclear cellular infiltrates were seen.

Experimental studies have shown that repeated TAA administration induces liver damage, and fibrosis that was associated with hepatocyte death and activation of Kupffer cells (KC) and hepatic stellate cells. TAA is mainly responsible for hepatorenal toxicity. It causes an increase in the concentration of intracellular calcium, alteration in cell permeability, karyomegaly with increased nuclear volume, and mitochondrial inhibition which results in hepatic cell death<sup>[40]</sup>.

Liver sections of Silymarin treated group revealed many hepatocytes with vesicular nuclei and acidophilic cytoplasm, the portal area showed mononuclear cellular infiltrates, slightly dilated congested portal vein in some areas while appeared normal in other areas with normal

appearance of branches of hepatic artery and bile duct were seen.

The protective effect of Silymarin was attributed to its antioxidant and free radical scavenging properties<sup>[41]</sup>.

In Silymarin & Green coffee treated group portal area appeared almost normal with normal appearance of branches of portal vein, hepatic artery and bile duct apart from minimal cellular infiltration were seen, hepatocytes appeared with vesicular nuclei and acidophilic cytoplasm. Minimal congestion in central vein was observed in this group.

Caffeine present in green coffee provided an anti-fibrogenic, anti-inflammatory, and antioxidant effect that was associated with recovery of hepatic histological and functional alterations from TAA-induced hepatotoxicity<sup>[41]</sup>.

In the present work, marked increase in collagen fiber deposition around central vein, in-between hepatocytes and in the portal area was detected in Masson trichrome stained sections of TTA treated group. Few collagen fibers around central vein and in portal area in Silymarin treated group was detected. Minimal amount of collagen deposition was seen in Silymarin & Green coffee treated group.

TAA induced liver toxicity with elevated liver enzymes and histological alterations, fatty changes, apoptosis, and fibrosis evidenced by increased immunohistochemical reaction to matrix metalloproteinase-9 (MMP-9) and collagen type IV in hepatocytes. Also, the levels of pro-inflammatory cytokines (TNF- $\alpha$ , IL-1 $\beta$ , and IL-6) in serum were significantly elevated. Co-treatment with caffeine and TAA restored normal liver structure and function<sup>[42]</sup>.

Receiving conventional and decaffeinated coffee and caffeine might be linked to the reduction in collagen deposition and fibrosis. Caffeine strongly down-modulates expression of TGF- $\beta$ -induced connective tissue growth factor (CTGF) in the hepatocytes by stimulating the degradation of SMAD 2 (a TGF- $\beta$  effector), inhibition of SMAD3 phosphorylation and up-regulation of peroxisome proliferator-activated receptor gamma (PPAR- $\gamma$ )<sup>[43]</sup>.

Examination of Caspase 3 immunostained liver sections of TTA treated group showed strong positive immunoreaction for caspase -3 in cytoplasm of most of hepatocytes, however Silymarin treated group revealed mild positive caspase-3 immunoreaction in some hepatocytes. Silymarin & Green coffee treated group showed few areas of weak positive caspase-3 immunoreaction in cytoplasm of hepatocytes.

Several studies revealed that the administration of Silymarin with CCl<sub>4</sub> ameliorated the levels of Bax, Bcl2 and ALT which were disturbed after treatment of rats with CCl<sub>4</sub><sup>[44&45]</sup>. It can prevent lipid peroxidation, inhibit LDL oxidation and scavenge reactive oxygen species (ROS)<sup>[37]</sup>. Moreover, it has anti-inflammatory effects which may relate its ability to inhibit the transcription factor NFkB,

which contributes to the production of proinflammatory mediators such as interleukin (IL)-1 and IL -6, TNF- $\alpha$ , lymphotoxin, granulocyte macrophage, colony-stimulating factor (GM-CSF) and interferon (IFN $\gamma$ )-c<sup>[38]</sup>.

Some GCE constituents, such as chlorogenic acid is the major active ingredient found in a variety of fruits, dietary vegetables and many traditional Chinese medicines<sup>[46, 47 and 48]</sup>. CGA has been reported to possess antibacterial, antioxidant, and anti-carcinogenic properties. CGA has also been reported to be a potent polyphenolic antioxidant as it reduces the levels of the apoptosis related proteins, including caspase-3 and Bax<sup>[49]</sup>.

### CONCLUSION AND RECOMMENDATIONS

The results of this experimental study strengthened and provided additional insight to the important role of adding green coffee extract to silymarin in ameliorating TTA induced liver injury in albino rat model. Future studies should be carried out to delineate the mechanisms underlying their action.

### CONFLICTS OF INTEREST

The authors declare there is no conflict of interest regarding the publication of this article.

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The research was personally funded by all authors.

### ABBREVIATIONS

TAA: Thioacetamide

CGA: Chlorogenic acid

Ccl4: Carbon tetrachloride

SOD: Superoxide Dismutase

GCBE: Green coffee Bean extract

ALT: Alanine aminotransferase

AST: Aspartate aminotransferase

GGT: Gamma-glutamyltransferase

GGTP: Gamma-glutamyltranspeptidase

ALP: Alkaline phosphatase

MMP-9: Matrix metalloproteinase-9

ROS: Reactive oxygen species

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الملخص العربي

تأثير السيليمارين مقابل السيليمارين ومستخلص البن الأخضر على إصابة الكبد  
المستحدثة بمادة الثيواسيتاميد في ذكور الجرذان البيضاء البالغة  
(دراسة هستولوجية وهستوكيميائية مناعية)

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**الخلفية:** يعتبر الثيواسيتاميد من العوامل السامة للكبد فهو يستخدم على نطاق واسع لإحداث سمية الكبد في حيوانات التجارب. تم إثبات خصائص السيليمارين الوقائية لسمية الكبد سابقا. تعتبر القهوة أكثر المشروبات شعبية في العالم. استهلاك القهوة له فوائد عديدة على الصحة بشكل عام ، وصحة الكبد على وجه الخصوص، لذلك أجريت الدراسة الحالية لتقييم الدور المحتمل لإضافة مستخلص البن الأخضر إلى سيليمارين في تحسين إصابة الكبد المستحدثة بالثيو أسيتاميد في الجرذان.

**المواد والطرق:** تم تقسيم ستة وثلاثين جرد من ذكور الجرذان البيضاء البالغة إلى أربع مجموعات. المجموعة الأولى (المجموعة الضابطة)، المجموعة الثانية (المجموعة المعالجة بالثيو أسيتاميد) (المجموعة الثالثة) (المجموعة المعالجة بالسيليمارين) والمجموعة الرابعة (المعالجة بالسيليمارين ومستخلص القهوة الخضراء). (في نهاية التجربة أجرى التحليل الكيميائي الحيوي لتقييم وظائف الكبد (مصل ألانين ترانسامينز و اسبرتات ترانسامينز) كما قيست أيضا إنزيمات مضادة للأكسدة ومقاييس الجلوتاثيون و اجريت دراسة هستولوجية باستخدام صبغة الهيماتوكسين والإيوسين و صبغة الماسون ثلاثية اللون و استخدمت صبغة هستوكيميائية مناعية للكشف عن كاسباس 3 و تم إجراء تحاليل مورفومترية للصور وإحصائية للنتائج.

**النتائج:** لقد أظهرت وظائف الكبد لجرذان المجموعة الثانية تدهورا كبيرا في الاستجابة للثيو أسيتاميد بالإضافة إلى التغيرات النسيجية الهامة في فصوص الكبد والمناطق البابية التي تحسنت بشكل طفيف في المجموعة الثالثة وتميل إلى أن تكون طبيعية في جرذان المجموعة الرابعة.

**الخلاصة:** نستخلص من هذا البحث أن إضافة مستخلص البن الأخضر إلى السيليمارين يحسن التأثير العلاجي للسيليمارين في علاج الإصابة الكبدية المستحدثة تجريبياً بواسطة الثيواسيتاميد في الجرذان.