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Research Article

Comparison of the oral bioavailability of Egyptian silymarin extract with Chinese silymarin Phytosome® drug on liver fibrosis model

M. A. Elhaak[1], Engy M. A. Elfetoh[2], Ehab Tousson[3] and Afrah F. Salama[2].

[1]Botany Department, [2]Biochemistry Section, Department of Chemistry,

[3]Department of Zoology, Faculty of Science, Tanta University, Egypt.

ABSTRACT

Silymarin–Phytosome® (Chinese silymarin) with growing popularity as food supplement is considered to be the most widespread in the medical field due to its high capacity of protection for liver damage during liver fibrosis. We investigated the possibility of using the Egyptian *Silybum marianum* extract, a flavonoid mixture, instead of the Silymarin–Phytosome®. A total of 72 female *Albino* rats were divided into six groups; G_1 (Control), G_2 (Ethanol), G_3 (Chinese silymarin), G_4 (Ethanol + Chinese silymarin), G_5 (Egyptian silymarin extract), G_6 (Ethanol + Egyptian silymarin extract). The present results indicated that the Silymarin–Phytosome® could protect the liver during liver fibrosis but less than the Egyptian silymarin capacity. Serum liver enzymes activities (ALT, AST and GGT) were significantly decreased in G_6 compared to those in G_2 and G_4. Serum total protein and the body weight gain were significantly increased in G_6 as compared to both previous treatments. Liver sections in alcoholic rat co-treated with the Chinese silymarin revealed moderate vacuolation between cells. However, liver sections in alcoholic rat co-treated with the Egyptian silymarin extract showed few granular hepatocytes with little congestion in portal tract and enhancement to normal one. The results concluded a promising improvement in liver functions and its histological changes by the Egyptian plant extract in comparison with the Chinese silymarin, in case of liver fibrosis.

Keywords: *Silybum marianum*, Silymarin– Phytosome®, Liver fibrosis.

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INTRODUCTION

Myofibroblasts are stromal cells mainly involved in tissue repair. These cells present contractile properties and play a major role in extracellular matrix deposition and remodeling. In liver, myofibroblasts are found in two critical situations. First, during fetal liver development, especially in portal tracts, myofibroblasts surround vessels and bile ducts during their maturation. After complete development of the liver, myofibroblasts disappear and are replaced in portal tracts by portal fibroblasts. Second, during liver injury, myofibroblasts re-appear principally deriving from the activation of local stromal cells such as portal fibroblasts and hepatic stellate cells or can sometimes emerge by an epithelial-mesenchymal transition process [1]. Hepatitis C and alcohol are the most widespread causes of liver disease worldwide. Approximately 80% of patients with a history of hepatitis C and alcohol abuse develop chronic liver injury [2]. Thus Liver fibrosis is the excessive accumulation of extracellular matrix proteins including collagen that occurs in most types of chronic liver damage [3]. Liver damage ranged from acute hepatitis to hepatocellular carcinoma, through apoptosis, necrosis, inflammation, immune response, fibrosis, ischemia due to altering gene expression and regeneration [4]. The cellular and

molecular mechanisms of liver fibrosis have greatly advanced. Activated hepatic stellate cells, portal fibroblasts and myofibroblasts of bone marrow origin have been identified as major collagen-producing cells in the injured liver. These cells are activated by fibrogenic cytokines such as TGF-β1, angiotensin II, and leptin [5].

Liver is a primary site of ethanol metabolism. Ethanol is metabolized to acetaldehyde by two major enzyme systems, alcohol dehydrogenase (ADH) and microsomal ethanol-oxidizing system (MEOS), cytochrome P4502E1 (CYP2E1). CYP2E1, in addition, generates both stable, nontoxic and highly unstable (*i.e.*, reactive) harmful molecules, called free radicals. Free radicals include superoxide (O_2), hydrogen peroxide (H_2O_2) and hydroxyl radicals (OH^-). An excess of oxygen radicals causes oxidative stress, thereby attacking vital cell components, fat and protein constituents of the cell wall and the nucleus (DNA). These reactive oxygen species (ROS) may contribute to the development of fatty liver and fibrosis [6]. Ethanol consumption increases the intestinal permeability of endotoxin. The endotoxin mediated inflammatory signaling plays a major role in alcoholic liver fibrosis [7]. These substances included highly reactive molecules that could damage vital cell components through oxidation [8].

Many herbal, medicinal and pharmaceutical plants and their extracts were widely studied by many researches to treat liver fibrosis. *Silybum marianum* (Milk thistle) plant got a bright reputation in relieve of the liver fibrosis and that might be for the potent silymarin mixture. Mechanism of action for silymarin conducted mainly to the antioxidant, anti-inflammatory, antifibrotic and antilipdemic roles. The extract of the seeds of *Silybum marianum* has been used for centuries to treat liver disorders [9].

Silymarin is considered as a mixture of flavonolignan compounds isolated from the seeds of *Silybum marianum* plant. The most effective constituents are silibinin, isosilibinin, silicristin and silidianin [10]. These compounds type and quantity differed by the natural environments where the plant is growing [11].

Therefore the present study aimed to compare the chemical and medicinal effect of naturally growing *Silybum marianum* plant in Egypt extract (Egyptian silymarin extract) with the Chinese silymarin (Silymarin–Phytosome®) which considered the commercial drug, as well as capacity of protection of liver damage during liver fibrosis, via estimation of biochemical markers in addition to the histopathological changes.

MATERIALS AND METHODS

Sample preparation and extraction process

Plant was identified by the taxonomist of Botany Department of Tanta University. Seeds of *Silybum marianum* were fine separated from the plant fruits, dried in the shade, chopped and extracted with methanol. Solvents were removed under reduced pressure to obtain the dried extract (Egyptian silymarin extract), according to Salama *et al.* [12]. An aqueous suspension contain the dried extract was prepared and administered to the rats orally. Silymarin–Phytosome® (Chinese medication) as standard protection was administered to the rats orally and obtained from (Sedico Company). Ethanol was obtained from Elgomhoria Company, Egypt.

Animal's experimental design

The experiments were performed on 72 female *Albino* rats weighing 120 g (± 20 g) obtained from Faculty of Veterinary, Cairo University, Egypt. The rats were housed in the laboratory for 1 week before the experimental work and maintained on the standard diet and water available in the animal research house of Zoology Department Faculty of Science, Tanta University. The temperature in the animal room was maintained at 23 ± 2 °C with a relative humidity of 55 ± 5% and at a 12:12 h light–dark cycle. The experimental protocol was approved by Local Ethics Committee and Animals Research. The rats were randomly and equally divided into six groups (12 animals each).

Group 1 (G_1): Control group is the rats access to free food and water *ad libidum*, and intragastrically added with saline solution (1 ml/100 g B.W) for 8 weeks.

Group 2 (G_2): Fibrosis group in which rats treated with ethanol (10%, vol. /vol.) as the sole source of drinking for 8 weeks for induction of liver fibrosis according to [13].

Group 3 (G_3): Chinese silymarin group in which the rats left at normal life as G_1. Each rat received 200 mg Chinese silymarin /kg body weight/ day for 4 weeks by oral gavage (From the beginning of the 5th week till the 8th weeks) [4].

Group 4 (G_4): Rats received ethanol for 4 weeks as in G_2 (for induction of fibrosis), the treatment started from the beginning of the 5th week till the 8th weeks, in which each rat received 200 mg Chinese silymarin /kg body weight/ day by oral gavage with continuing drinking ethanol.

Group 5 (G_5): Egyptian silymarin extract group in which the rats left at normal life as G_3. Each rat received 200 mg a crude extract of *Silybum marianum* /kg body weight/ day for 4 weeks by oral gavage (From the beginning of the 5th week till the 8th weeks) [4].

Group 6 (G_6): Like G_4, but rats were treated with Egyptian silymarin extract instead of Chinese silymarin.

At the end of the experimental period and after an overnight fast the rats were euthanized for collecting the blood samples, liver tissues.

Histological investigation:

The liver was immediately removed and fixed in 10% neutral-buffered formalin for 24 hours. The fixed specimens were then dehydrated, cleared and embedded in paraffin. Serial sections of 5-mm thick were cut by means of rotary microtome (Litz, Wetzlar, Germany). Sections were processed for haematoxylin and eosin staining [14]. All stained slides were viewed using Olympus microscope and images were captured by a digital camera (Cannon 620). Brightness and contrast were adjusted using Adobe Photoshop software (Version 4.0.1; Adobe Systems, Mountain View, California).

Blood collection:

Blood samples from each rat were collected from the eyes by retro-orbital puncture from orbital plexus using blood capillary tubes. Blood was incubated at room temperature for 10 minutes, and then centrifuged at 3000 r.p.m for 10 min and the sera were collected; serum separated and kept in clean stopper plastic vials at –80°C until analysis of serum parameters.

Estimation of liver function markers:

Aspartate aminotransferase (AST), Alanine aminotransferase (ALT) and Gamma glutamyl transferase (γGT) enzymes activities in serum were measured using Spectrum Co. kits according to Murray el al. [15]; Szasz and Persijn [16], respectively. The total protein content in serum was assayed by the colorimetric method using Diamond Co. kits according to Gornall [17].

Statistical analysis:

The data obtained in the experiment were expressed in terms of mean ± SEM. Statistical significance of data variations were assessed by one way analysis of variance (ANOVA) followed by a comparison between different groups using "Tukey-Kramer" multiple comparison t-test, using Graph pad Instate software [18].

RESULTS

Food intake, body weight gain and relative organ weight:

Table (1) showed that the food intake by Fibrosis rat group ($G_{2)}$ decreased significantly ($p<0.01$) than that by the control group (G_1). The food intake was improved by the rest groups and increased significantly ($p<0.001$) than G_2. On the other hand, food intake by G_5 decreased significantly ($p<0.001$) than by G_4.

The body weight gain of G_2 decreased significantly ($p<0.001$) than all groups. Also, G_3 decreased significantly ($p<0.001$) than groups G_4 and G_6, and also G_5 decreased significantly ($p<0.001$) than both groups G_4 and G_6 (Table 1).

Relative to liver relative organ weight, G_2 decreased significantly ($p<0.001$) than all groups (Table 1).

Liver function test:

Results of liver enzymes activities (ALT, AST and GGT) clarified the very strong effect of ethanol on the liver in induction of fibrosis where the data showed a significant ($p<0.001$) increase in levels of ALT, AST and GGT in G_2 than that of all groups. On the other hand; G_3 and G_5 showed a significant ($p<0.001$) decrease in enzymes activities than the other groups treated with ethanol alone or with the treatment. Also, the level of AST, GGT of G_5 and G_3 decreased significantly than that of $G_{1,}$ ($p<0.001$) and ($p<0.01$) respectively (Table 1).

ALT, AST and GGT enzymes activities showed highly significant ($p<0.001$) increase in G_4 than that of G_3. Also, AST enzyme activity of G_6 increased significantly ($p<0.05$) than G_3. GGT enzyme activity of G_5 decreased significantly ($p<0.001$) than G_3. At the same time, all enzymes activities of G_5 decreased significantly ($p<0.001$) than G_4. On the other hand, AST and GGT enzymes activities of G_6 decreased significantly ($p<0.05$) than $G_{4.}$ While, G_6 increased significantly ($p<0.001$) than G_5 in ALT and GGT enzymes activities and also, increased significantly ($p<0.01$) in AST enzyme activity (Table 1).

Serum total protein of G_2, G_4 and G_6 decreased significantly than that of the control $G_{1,}$ ($p<0.001$) and ($p<0.01$) respectively. While, serum total protein of G_3 and G_6 increased significantly than that of $G_{2,}$ ($p<0.001$) and ($p<0.01$) respectively. On the other hand, serum total protein of G_4 decreased significantly ($p<0.001$) than G_3 while, G_5 increased significantly ($p<0.001$) than G_4 (Table 1).

Feeding efficiency:

The relationship between rats body weight and the food intake under the different the liver fibrosis treatments (Figure 1) was significant with a binomial regression equation (R=0.944*). The relationship showed an increasing trend of gain in weight with increasing food intake due to the applied treatment with a marked gain by both silymarin compounds especially from the extracted plant.

The feeding efficiency for rats as unit body weight gain by unit food intake under the different treatments (Figure 2) indicated great inhibition in rat feeding and utilization of food in gain in body weight

due to the induced fibrosis in group G_2. The rats in group G_3 and G_5 had slightly lower feeding efficiency in comparison with those of control group. On the opposite G_4 and G_6 groups acquired a remarkable high feeding efficiency with slight high value for G_6 groups. This enhancement in feeding was important in relive the effect of fibrosis of liver.

Histological examination of liver tissue:

Histological examination of the liver sections in control, Chinese silymarin and Egyptian silymarin extract group's revealed normal structures of the hepatocytes which extend from a central vein to the periphery of the hepatic lobules at which the portal tracts appears (Figure 3A, 3D and 3F). Liver sections in Fibrosis group rats showed variable histopathological changes. These changes were severe fat accumulation, mild inflammation, degeneration with focal area, central zonal necrosis, severe dilated CV engorged with edema and focal hemorrhage (Figure 3B and 3C). Also, liver sections in Fibrosis group rat treated with the Chinese silymarin revealed moderate vacuolation between cells in which occurred fatty degeneration of liver cells with focal sporadic necrotic cells (Figure 3E). However, liver sections in fibrosis group rat treated with the Egyptian silymarin extract showed few granular hepatocytes with little congestion in portal tract and enhancement to normal one in which the number of vacuoles was decreased; generally there was an improvement in liver tissue cells (Figure 3G and 3H).

DISCUSSION

The rate of food intake was significantly decreased in G_2 (Fibrosis group) compared with that of G_1 (Control group). While, groups fed on the Chinese silymarin and the Egyptian silymarin extract alleviated the disorder effect occurred during liver fibrosis leading to significant increase in the rate of food intake and the effect was greater by the Egyptian silymarin extract than the Chinese silymarin. This is may be because both silymarin help in the elimination of toxins directly from the intestines and perhaps because they prevent fat accumulation in the liver. The results appreciate the Egyptian silymarin extract to be used instead of the Chinese silymarin for resuming normal rate of food intake by rats. These observations are similar to the data reported by Shaker *et al.* [4].

The body weight gain showed marked decrease in G_2 which are compatible with the data obtained by Smith *et al.* [19] who proved that liver fibrosis affect body weight gain and body fat levels. Liver fibrosis has profound effect on nutritional status which may cause primary malnutrition by displacing other nutrients in the diet of high energy content or because of associated medical disorders. Secondary malnutrition results due to maldigestion or malabsorption of nutrients caused by gastrointestinal complications [20]. Significant improvement in body weight gain was observed with Chinese silymarin and Egyptian silymarin extract treatment. The improvement in body weight gain in Egyptian silymarin extract may be due to their inhibitory effect on CYP2E1 in liver microsomes [21]. The relationship between rats body weight and the food intake under the different treatments of liver fibrosis was significant with a binomial regression equation (R=0.944*). This relationship signified an increasing trend of gain in weight with increasing food intake due to the applied treatment with a marked gain by both silymarin compounds especially from the Egyptian silymarin extract.

The fibrosis rats in group G_2 suffered from feeding efficiency compare to those under the different medication treatments. The rats in group G_3 and G_5 had slightly lower feeding efficiency in comparison with those of control group. On the opposite rats treated with either Chinese silymarin or Egyptian silymarin extract of G_4 and G_6 groups acquired a remarkable high feeding efficiency with slight high value for G_6 groups. This enhancement in feeding was important in relive the effect of fibrosis of liver.

The result of the present study showed a reduction in relative liver weights in rats treated with ethanol in comparison with those of the control. Treatment with the Chinese silymarin or the Egyptian silymarin extract was beneficial in increasing the liver weight or repairing the damaged in liver as compared to not treated fibrosis group. Tedesco *et al.* [22] reported that the silymarin extract increased the elimination of ethanol directly from the intestines without absorption and the silymarin consumption prevents fat accumulation in the liver.

When the liver gets damaged after consumption of ethanol, leakage of cellular enzymes into the plasma was induced [23]. Increased levels of serum enzymes such as (ALT), (AST) and (GGT) increased permeability and necrosis of hepatocytes were observed in liver damage rats treated with ethanol [24]. Serum GGT is a sensitive marker enzyme widely used as a laboratory test for the diagnosis of hepatic fibrosis [25]. In the present study, administration of the Chinese silymarin and the Egyptian silymarin extract alleviated the observed increase in the serum enzymes ALT, AST and GGT activities when compared to both the ethanol and the control group. The Egyptian silymarin extract decreased the AST and GGT levels better than the Chinese silymarin. Shaker *et al.* [4] reported that the Egyptian silymarin extract and the used medical Chinese silymarin decreased enzymes activities. The stabilization of

these enzymes by the Egyptian silymarin extract is a clear indication of the improvement of the functional status of the liver. This result agrees with the previous works done by Saller *et al.* [26] and Cordero-Pérez *et al.* [27] where they found silymarin has protected liver against injury from various other hepatotoxicants (Carbon tetrachloride and paracetamol) as indicated by lowering the elevation on ALT, AST levels.

Also, Ahmed *et al.* [28] confirmed the hepatoprotective effect of silymarin in a study on the effect of silymarin against cisplatin. Silymarin significantly restored the change of ALT due to its antioxidant effect and its ability to act as a free radical scavenger, thereby protecting membrane permeability. The liver enzymes (AST and GGT) activities were decreased by both Egyptian silymarin extract and Chinese silymarin treatments but reached to maximum reduction with the Egyptian silymarin extract in comparison with ethanol treatment which showed high differences.

The Chinese silymarin and the Egyptian silymarin extract led to normal value of total bilirubin compared to the control group which was also reported by Ashtiani *et al.* [29] who found positive effect of silymarin in prevention of ethanol miss use consequences. The treatment with the Egyptian silymarin extract showed highly reduction on the total bilirubin value as compared with the Chinese silymarin treatment.

On the other hand, the administration of ethanol for two months resulted in a decrease of serum total protein and albumin concentrations as found by Reddy *et al.* [30] who reported that the decrease in the plasma total protein in rats treated with ethanol is due to free radical production by alcohol. Ethanol inhibited the secretion of protein from the liver.

Lang *et al.* [31] mentioned that chronic consumption of ethanol have slightly decreased the rates of all body protein synthesis and breakdown. Likewise albumin concentration was found to be lowered by Abraham *et al.* [32] in the rat plasma in the absence of hepatocellular necrosis.

However, treatment with the Chinese and the Egyptian silymarin extract increased the serum total protein and albumin when compared to the ethanol group. These results were in agreement with those obtained also by Hessien *et al.* [33] The hepatoprotective effect offered by the Egyptian silymarin extract was significantly greater than by the Chinese silymarin. The Egyptian silymarin extract increased the serum total protein and albumin to their maximum values. This again appreciated using of the Egyptian silymarin extract instead of the Chinese medication (Chinese silymarin).

In the current study, we found that the consumption of ethanol for 8 weeks induced many histopathological changes in the liver of rats include proliferation of fibrous connective tissue around the hepatic lobules, fatty changes of hepatocytes, thickening of hepatic capsule, infiltration of inflammatory cells and coagulative necrosis of hepatocytes. These results were in agreement with those of Kono *et al.* [34]

Our histological study confirms our biochemical result where the Egyptian silymarin extract improve the liver tissues better than the Chinese silymarin. Shaker *et al.* [4] and El-Shafeey *et al.* [35] evaluated the protective role of the Egyptian silymarin extract against the toxicity of ethanol and the histological changes in the liver. Some improvements especially for the Egyptian silymarin have been shown in the protective groups as dilatation in the hepatic sinusoids associated with inflammatory cells infiltration and diffuse kupffer cells proliferation in between the degenerated hepatocytes.

CONCLUSION

The Egyptian silymarin extract could be extended for the isolation and structure determination of hepatoprotective principle. Silymarin is considered as a mixture of flavonolignan compounds isolated from the seeds of *Silybum marianum* plant. These compounds type and quantity differed by the natural environments where the plant was collected. This confirms the increased number and amounts of compounds in the Egyptian silymarin extract as compared with the Chinese one. Silymarin caused marked alteration in some biochemical parameters induced oxidative damage. Results proved the efficiency of the Egyptian silymarin extract in comparison with the Chinese silymarin in treatment of liver fibrosis.

A**bbreviation:** *ALD, alcoholic liver disease; AST, aspartate aminotransferase; ALT, alanine aminotransferase; γGT, gamma glutamyl transferase; SEM, standard error deviation; CYP2E1, cytochrome P450 2E1.*

Table 1
Food intake (g/rat/day), body weight gain and relative liver weights (g/100 g body weight), liver function tests

Groups / Parameters	G_1	G_2	G_3	G_4	G_5	G_6
Food intake	13.4 ± 0.37 [b]	11.3 ± 0.45 [bd]	14.4 ± 0.58 [d]	15.8 ± 0.3 [bdl]	14.1 ± 0.33 [dl]	15.7 ± 0.35 [bd]
Body weight gain	20.5 ± 3.0 [a]	2.0 ± 1.25 [ad]	19.0 ± 1.60 [dg]	42.2 ± 3.3 [adgj]	20.5 ± 2.90 [djm]	43.0 ± 3.2 [adgm]
Liver weight	3.6 ± 0.14 [a]	2.4 ± 0.08 [ad]	3.5 ± 0.09 [d]	3.5 ± 0.1 [d]	3.4 ± 0.09 [d]	3.3 ± 0.06 [d]
SGPT (ALT)	22 ± 2.1[a]	50 ± 2.2[ad]	20 ± 1.6[dg]	34 ± 0.9[adgj]	21 ± 1.5[djm]	35 ± 2.5[adgm]
SGOT (AST)	34 ± 2.9[ab]	85 ± 3.9[ad]	21 ± 1.7[bdgi]	41± 2.1[dgjl]	17 ± 0.6[adjn]	31 ± 1.2[diln]
GGT	25.5 ± 0.78[ab]	61.5 ±1.27[ad]	30 ± 0.58[bdg]	39.6 ± 0.70[adgjl]	18.3 ± 0.49[adgjm]	35.8 ± 1.23[aglm]
Serum total protein	6.1 ± 0.11[a]	4.1 ± 0.1[ade]	5.55 ± 0.19[dg]	4.46 ± 0.12[agj]	5.58 ± 0.08[dj]	5.05 ± 0.17[be]

Values are expressed as mean ± SEM; n = 12.

- a, d, g, j, m (P<0.001) - b, e, h, k, n (P<0.01) - c, f, i, l, o (P<0.05)

{ALT, AST and GGT enzymes activity (U/L) and total protein (g/dl)} in serum of female rats treated with vehicle G_1 (Control), G_2 (Ethanol), G_3 (Chinese silymarin only), G_4 (Ethanol + Chinese silymarin), G_5 (Egyptian silymarin extract only) and G_6 (Ethanol + Egyptian silymarin extract).

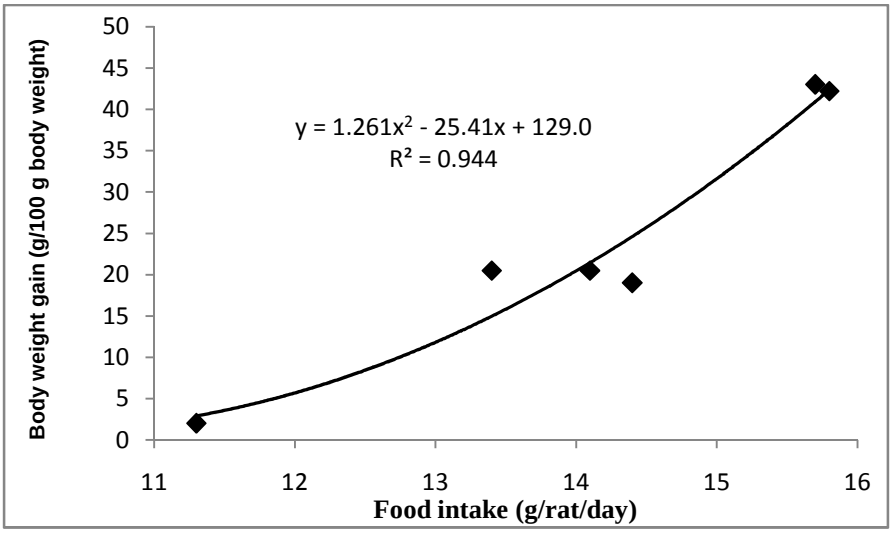

Figure 1
The relationship between rat's body weight and the food intake under the different treatments of liver fibrosis

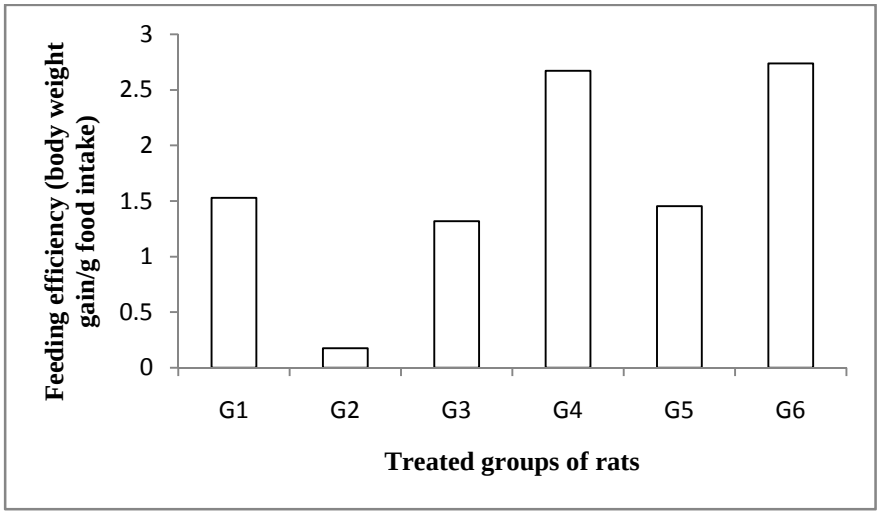

Figure 2

The feeding efficiency for rats as unit body weight gain under the different treatments of liver fibrosis

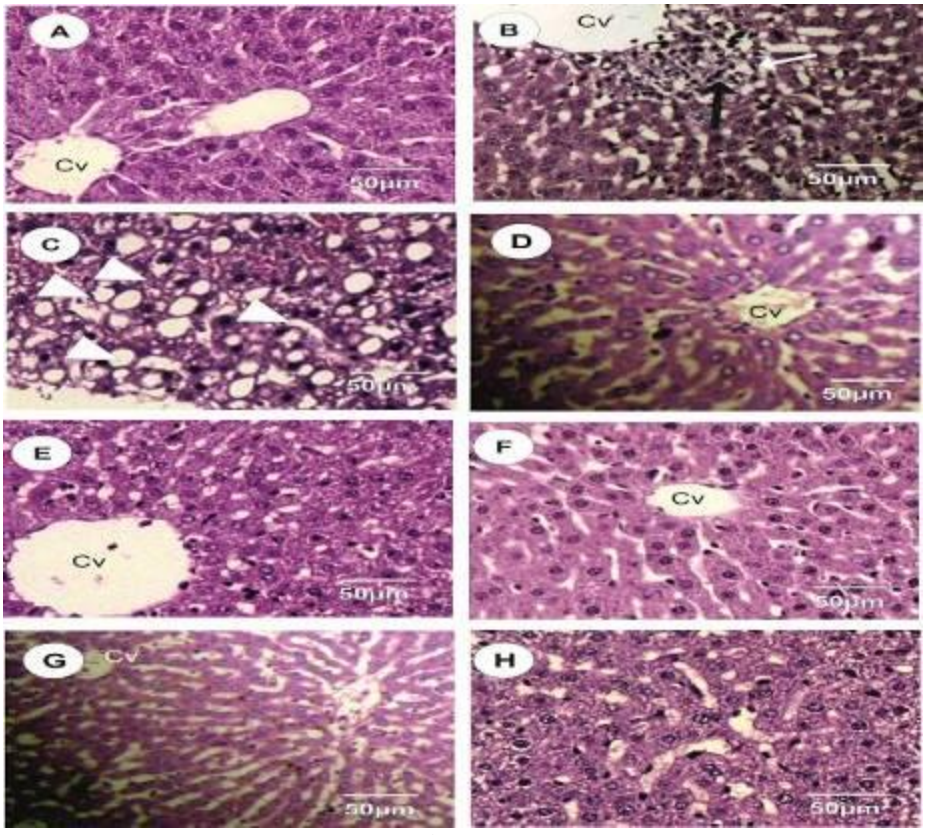

Figure 3

Photomicrographs of rat liver sections stained by haematoxylin and eosin.

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