Egypt. J. Chem. **Vol. 65,** No. SI13B pp. 1567 - 1588 (2022)

Egyptian Journal of Chemistry

http://ejchem.journals.ekb.eg/

Mechanisms of the anti-fibrotic impact of natural nutraceuticals in thioacetamide-induced hepatic fibrosis in obese rats

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Abstract

Hepatic fibrosis is a serious global health issue and it is the consequence of various chronic liver ailments. Hepatic fibrosis is manifested by replacement of liver tissue by fibrosis, scar tissue, and regenerative nodules. The rational of the present attempt was to evaluate the anti-fibrotic outcome of lycopene and resveratrol against hepatic fibrosis induced in obese rats. The generation of hepatic fibrosis model was carried out by thioacetamide (TAA) intraperitoneal administration (200 mg/kg b.wt) twice a week for 6 weeks. Obese model was created by feeding the rats with high fat diet (HFD) for 24 weeks. The lean control group, was administered saline intraperitoneally twice weekly for 6 weeks after feeding a standard chow diet for 24 weeks. The obese control group received intraperitoneal injection of saline twice a week for 6 weeks after feeding a high fat diet (HFD) for 24 weeks. Hepatic fibrosis lean group received intraperitoneal injection of TAA (200 mg/kg b.wt) twice a week for 6 weeks after feeding a standard chow diet for 24 weeks. Hepatic fibrosis obese group received intraperitoneal injection of TAA (200 mg/kg b.wt) twice a week for 6 consecutive weeks after feeding HFD for 24 weeks. Lycopene prophylactic group was given 20 mg/kg b.wt of lycopene by intragastric gavage tube daily for 6 weeks simultaneously with intraperitoneal injection with TAA (200mg/kg b.wt) before feeding HFD for 24 weeks. Resveratrol prophylactic group received 30 mg/kg b.wt of resveratrol intragastrically daily for 6 weeks simultaneously with intraperitoneal injection of TAA (200 mg/kg b.wt) before feeding HFD for 24 weeks. Lycopene therapeutic group was given 20 mg/kg b.wt of lycopene by gastric intubation with an oral gavage daily for 6 weeks after feeding HFD and TAA administration. Resveratrol therapeutic group received 30 mg/kg b.wt of resveratrol orally by gastric tube daily for 6 weeks after feeding HFD and TAA administration. Anthropometric parameters including body weight, body length, WC, AC and BMI were measured in lean control group and obese control group to ensure obesity manifestation. Also, serum glucose and insulin levels as well as insulin resistance (IR) value were determined in these groups to assure the development of obesity-related complications. After finalizing the treatment period, serum ALT, AST, cholesterol, TG and LDL, leptin, TGF-β and fibronectin levels were quantified. Also, the immune reaction for hepatic α-SMA, CD31 and CD34 was investigated. Moreover, histological examination of liver tissue sections was carried out in the different studied groups. The obtained findings indicated significant higher values of the anthropometric parameters in obese control group than in lean control one. Also, serum glucose, insulin and IR values showed significant elevation in obese control group versus the lean control group. These results evidenced the success of obese model. The present results also highlighted that the severity of hepatic fibrosis in obese rats is superior than that in the lean counterparts as indicated by the significant increased values of ALT, AST, cholesterol, TG, LDL, leptin, TGF-β and fibronectin as well as the hepatic immune reaction for α-SMA, CD31 and CD34. The administration of lycopene or resveratrol in obese rats with hepatic fibrosis recovered the values of serum ALT, AST, cholesterol, TG, LDL, leptin, TGF-β and fibronectin significantly as well as ameliorated the severity of immune reaction for hepatic α-SMA, CD31 and CD34 markedly. The net results were greatly supported by the histomorphological hallmark of liver tissue sections of the different studied groups. Based on these outcomes, this research work explored the pharmacological activity of lycopene and resveratrol against hepatic fibrosis from a holistic perspective. The behind mechanism of this effect was closely related to the intervention of inflammatory pathway through their anti-oxidative properties.

KEY WORDS : Hepatic fibrosis, Obesity, Thioacetamide, Lycopene, Resveratrol.

Introduction

Hepatic fibrosis is a meaningful, life-threatening disorder with high morbidity and mortality that results from various reasons [1]. Hepatic fibrosis has become one of the widely prevalent liver diseases globally, and it has also become one of the leading manifestations for liver transplantation [2]. The worldwide prevalence of liver diseases registers for approximately 2 million deaths per year all over the world, among which 1 million deaths arise due to cirrhosis complication that represents the 11th major cause of death across the globe [3].

Hepatic fibrosis is a mainstream pathological sequel of chronic liver disease, including chronic infections by hepatotropic viruses (hepatitis B and hepatitis C viruses) **[4,5]**, alcoholism **[6]**, chronic metabolic variations, (e.g., non-alcoholic fatty liver disease **[7]**, autoimmune situations, (e.g., autoimmune hepatitis) **[8]** and the

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Receive Date: 22 April 2022, Revise Date: 15 March 2023, Accept Date: 03 July 2022

DOI: 10.21608/EJCHEM.2022.135256.5952

prorated chronic motivation of the wound-healing response **[9]**. Obesity, metabolic syndrome, toxins, drugs, hereditary factors, cholestasis and parasites, are considered as, other factors that deteriorate the liver cells, disrupting the dynamic parity between collagen fiber generation, accumulation, dissolution and absorption, thus resulting in the occurance of hepatic fibrosis **[10]**.

Hepatic fibrosis is manifested by the abnormal deposition of extracellular matrix (ECM) elements involving collagen fibers that arise in most traits of chronic liver disorders. These and non-collagenous constituents of hepatic fibrosis frequently lead to hepatic dysfunction and portal hypertension **[11]**. Hepatic fibrosis is an active process of hepatic homeostasis moderated by different cellular molecules in response to an inflammatory reaction; the major cell type involved appears to be hepatic stellate cells (HSCs). HSCs play the central role in hepatic fibrosis and the other types of liver cells also have significant roles in fibrosis **[12]**. In fact, the activation of HSCs relies on the interplay with other hepatic cells, involving hepatocytes, liver sinusoidal endothelial cells, inflammatory cells and biliary cells. These cells react with each other and stimulate or inhibit HSCs activation *via* realization of cytokines and other signaling molecules. Targeting the interaction between HSCs and other hepatic cells could be a new choice for treatment of hepatic fibrosis **[13, 2**].

Chronic inflammation leads to the activation of HSCs that undergo trans-differentiation into proliferative, migratory and contractile myofibroblasts **[14]**, exhibiting pro-fibrogenic transcriptional and secretory characteristics (so-called "cell activation") and discharge ECM proteins, mostly cross-linked collagens type I and type III, that replace damaged normal tissue **[15]**. The accumulation of ECM proteins forms scar tissue in the space of Disse that results in sinusoidal capillarization, manifested by lack of endothelial fenestrations **[16]**. Over time the increased ECM proteins production leads to the formation of hepatic fibrosis **[14].** Thus, the pathogenesis of hepatic fibrosis is convoluted and it comprises various sorts of liver cells and inflammatory reactions. Therefore, the prime pathological manifestations of hepatic fibrosis are collagen deposition and destruction of liver architecture **[2].** Indeed, the progression of hepatic fibrosis to cirrhosis provokes more significant mortality and higher frequency of hepatocellular carcinoma **[17].**

Obesity is a recognized prognostic significance of NAFLD, which is accompanied with the evolution of hepatic fibrosis **[18]**. Obesity raises hepatic NF-κB level that results in hepatic inflammation owing to an enhancement of free fatty acid (FFA). FFA recognized by Kupffer cells promotes sterile inflammation and stimulates pattern recognition receptors (PRRs), such as Toll-like receptor 4 (TLR4) in the hepatocytes **[19]**. As the Kupffer cells were activated by inflammation, the HSCs become activated, TGF-β, leading to hepatic fibrosis and enhanced secretion of ECM. The hormone derived from adipocyte, leptin also participates to induce the Kupffer cells to produce collagen **[20]**. The findings of **Setyaningsih et al. [21]** research hypothesized that FFA synthesized by white adipose tissue is delivered to the liver through the hepatic artery and portal vein. Furthermore, the up-regulation of inflammatory mediators in adipose tissue during obesity leads to hepatic fibrosis **[20]** which may be interposed by ET-1 and TGF-β1 signaling **[21].**

Fibronectin is considered as a valuable marker in anticipating the advancement of fibrosis obese patients who have aberrant liver enzymes. Perivenular fibronectin deposition could be a precise and primary sign of active liver fibrogenic processes **[22]**. **Acharya et al. [23]** stated that when there is hepatic fibrosis, there is about a 6–8 fold elevation in the generation of ECM constituents. Also, when the non-fibrogenic type IV collagen is substituted by fibrogenic type I and II collagen, there is a more relase of fibronectin, hyaluronic acid and α-smooth muscle actin (α-SMA) into the ECM.

The medical management of hepatic diseases including hepatic fibrosis is actually insufficient; so far no available medicine has successfully prohibited the progression of hepatic diseases. However, number of clinical trials and pre-clinical validation studies were in progress. Despite these efforts, the current medications for hepatic fibrosis bread various adverse effects. Therefore, the increased number of hepatic fibrosis patients spot lights the imperative poverty to develop mechanistic-based therapies for cuting fibrosis. Hence, more supplementary researches should be conducted to achieve alternative drugs for the treatment of hepatic fibrosis of trustable efficacy and safety **[24]**.

Scientific research spanning over more than five decades has confirmed the direct and potential preventive as well as therapeutic benefits of natural remedy against chronic disorders, and the traditional medicine still contributes significantly in the treatment regimen, particularly in the developing nations. Lycopene, that is a tetraterpene compound. It is extensively found in tomato and tomato-based products and it is considered as an intermediate compound of carotenoid synthesis in the plants **[25].** Some colored fruits and vegetables specially with red and orange colors is have to this liposoluble pigment

[26]. Meanwhile, some of non-red or non-orange plants, such as asparagus and parsley possess this carotenoid compound **[27].** It is mainly recognized as a non-pro-vitamin A carotenoid **[28].** Lycopene has been reported to be effective as anti-cancer, anti-diabetic **[29]**, cardioprotective **[30]**, antioxidative **[31]**, anti-inflammatory **[32]**, hepatoprotective **[33]**, neuroprotective **[34]**, bone protective **[35]**, and as a protective agent against various toxins **[27].** It cannot be produced in the human body, therefore, it must be consumed in a daily diet **[28].**

A non-flavonoid phenol resveratrol (3,5,4′-trihydroxy-trans-stilbene) is synthesized by various plants in response to bacteria or fungi colonization. It is existed mostly in grape peel, blueberries, raspberries, mulberries, and peanuts **[36]**. A multitude of researches have shown that resveratrol could prohibit or delay the advancement of a wide diversity of ailments, involving malignant tumors, neurodegenerative disorders, cardiovascular diseases, ischemic lesions, and viral infections **[37]**. Resveratrol possesses anti-inflammatory, antioxidative, anti-proliferative, and chemopreventive activities **[38]**. **Hosseini et al. [39]** demonstrated that resveratrol can mitigate NAFLD *via* the demethylation of the nuclear factor erythroid 2-related factor 2(Nrf2) promoter. Resveratrol biological activity is mainly associated with various signaling pathways and immune responses owing to its significant anti-inflammatory effects and oxidation resistance **[40]** Resveratrol could improve liver functions and block oxidative stress, thus attenuating hepatic fibrosis **[41]**. It has been reported that resveratrol inhibits HSC activation to hamper hepatic fibrosis *via* suppressing the PI3K/AKT signaling pathway **[42]. Koushki et al. [43]** demonstrated that resveratrol diminishes the accumulation of hepatic fat induced by diet *via* augmenting fatty acids oxidation and lowering the lipogenesis.

Therefore, developing efficient anti-fibrosis approaches may change the inherent history of the chronic liver diseases and hence mitigate hepatic fibrosis. For this reason, the goal of the present approach was to explore possible protective / therapeutic influence of lycopene and resveratrol against hepatic fibrosis induced in obese rats and to shed more light on the precise mode of action and the related signal pathway underlying the possible effects of these remedies to combat hepatic fibrosis.

MATERIAL AND METHODS

Chemicals

- Thioacetamide (TAA)≥99.0%, product number 163678, CAS number 62-55-5, MDL number MFCD00008070, formula CH_3CSNH_2, molecular weight75.13 was purchased from Sigma –Aldrich Company (St. Louis, MO,USA).
- Lycopene ≥90 % from tomato, product numberL9879, CAS number 502-65-8, MDL numberMFCD00017350, formula $C_{40}H_{56}$ and formula weight 536.87 g/mol was procured from Sigma-Aldrich Company (St. Louis, MO, USA).
- Resveratrol ≥99% (HPLC), product number R5010 , CAS number501-36-0, MDL numberMFCD00133799, formula $C_{14}H_{12}O_3$and formula weight 228.24 g/mol was acquired from Sigma-Aldrich Co. (St. Louis, MO, USA).
- All reagents, solvents and chemical compounds used for analysis were of analytical grade and were not further purified.

Animals

Eighty adult female albino *Wistar* rats, whose weights at the time of randomization ranged from120-130 g, were obtained from Animal Care Unit of the National Research Centre (NRC) Egypt. The animals were fed with standard chow diet and water *ad libitum*, and they were allowed to acclimatize in their new environment for 14 days before commencement of the experiment. They were housed in transparent plastic cages with wood shaving(5 per cage)at freely ventilated room with controlled room temperature (25°C ± 1°C), humidity (50% ± 10%), and alternating 12 h cycles of light and dark. Animal caring and treatments throughout the experiment were carried out according to the approval and guidelines given by the Institutional Ethical Committee for Medical Research of the National Research Centre, Egypt (Code No: 12-115).

1- Research Protocol

After the habituation period, sixty rats were subjected to obesity by feeding high fat diet (HFD) for 24 weeks. After this period, the success of obesity model was confirmed by measuring the anthropometric parameters including the abdominal circumference (AC) (immediately anterior to the forefoot), thoracic circumference (TC) (immediately behind the foreleg), body length (nose-to-anus or nose-anus length) of the anaesthetized rats. The body weight and body length were used to determine the body mass index

$$\text{BMI} = \frac{\text{Body weight (g)}}{\text{Length (cm}^2\text{)}}$$

Then, these animals were fasted overnight , anaesthetized by sodium pentobarbital and1ml blood was withdrawn from each rat in the anti-coagulant coated tubes to obtain plasma by centrifugation under cooling at 1800xg for ten min. Plasma glucose level was measured colorimetrically using kit purchased from Stanbio Laboratory. Boerne, Texas, USA, according to the method of **Howantiz and Howantiz [44]**. Plasma insulin level was estimated by enzyme linked immunosorbent assay (ELISA) procedure using DRG kit (Germany) as described by **Temple et al. [45]**. The homeostasis model assessment of basal insulin resistance (HOMA-IR) was used to calculate the index from the product of the fasting concentration of plasma glucose (mmol/L) and plasma insulin (mU/ml) divided by 22.5 according to the equation mentioned in the study of **Duncan et al. [46].**After that, the rats were allocated to 8 groups (10 rats/group) as follow:

(1) Lean control group which was administered saline intraperitoneally twice a week for 6 week after feeding a standard chow diet for 24 weeks. (2)**Obese control group** which received interaperitoneal injection of saline twice a week for 6 weeks after feeding HFD for 24 weeks. (3) **Hepatic fibrosis lean group** which received interaperitoneal injection of TAA (200 mg/kg b.wt) twice a week according to **Bruck et al. [47]** for 6 consecutive weeks after feeding a standard chow diet for 24 weeks. (4) **Hepatic fibrosis obese group** which received interaperitonial injection of TAA (200 mg/kg b.wt) twice a week for 6 consecutive weeks after feeding HFD for 24 weeks. (5)**Lycopene prophylactic group** which was treated daily with an oral dose of 20 mg/kg b.wt **[48]** for six weeks simultaneously with the interaperitoneal injection of TAA (200mg/kg b.wt) before feeding HFD for 24 weeks. (6) **Resveratrol prophylactic group** which was treated with daily oral dose of 30 mg/kg b.wt of reseveratrol **[49]** for six weeks simultaneosly with the interaperitoneal injection of TAA (200mg/kg b.wt) before feeding HFD for 24. (7)**Lycopene therapeutic group** which was fed with HFD for 24 weeks and then received interaperitoneal injection of TAA (200mg/kg b.wt) for six weeks, after that it received 20 mg/kg b.wt of lycopene by oral administration daily for other 6 weeks. (8)**Resveratrol therapeutic group** which fed with HFD for 24 weeks and then received interaperitoneal injection of TAA (200mg/kg b.wt) for six weeks, after that it received 30 mg/kg b.wt of resveratrol by oral administration daily for other 6 weeks**.**
After finalizing the experiment, all rats were fasted overnight then, humanely euthanized *via* exsanguination under sodium pentobarbital anesthesia. The blood samples were drained into the anti-coagulant free tubes and centrifuged under cooling at 1800xg for ten min. to obtain sera which were retained in aliquots at -20ºC pending further analysis. After blood collection, all animals were rapidly sacrificed and the liver specimens were excised, rinsed with saline solution, blotted dry on filter paper and fixed in formalin saline solution (10%) for Immunohistochemical and histological procedures.

Biochemical assays

Serum aspartate transaminase (AST) and alanine transaminase (ALT) activities were estimated colorimetrically using kit purchased from Quimica Clinica Aplicada S.A. Co., Spain, following the method of **Reitman and Frankel [50]**. Serum cholesterol (Chol) concentration was determined by a colorimetric method using kit obtained from Stanbio Laboratory, Boerne, Texas, USA, according the method of **Allain et al. [51].** Serum LDL-cholesterol (LDL) level was assayed by using colorimetric kit procured from Quimica Clinica Aplicada S.A. Co., Spain, as described in the method of **Assman et al. [52].** Serum triglycerides (TG) concentration was quantified colorimetrically using kit acquired from Stanbio Laboratory, Boerne, Texas, USA, as described by **Fassati and Prencipe [53]** method**.** Serum TGF-β1 level was detected by enzyme linked immunosorbent assay (ELISA) using kit purchased from DRG Diagnostics Co., Germany, following the method described by **Kropf et al. [54]**. Serum leptin level was measured by ELIZA kit produced by BioSource Co., Europe according to the method of **Friedman and Halaas [55].** Serum fibronectin level was evaluated by ELISA using kit obtained from ASSAYPRO, USA, following to the method described by **Wu et al. [56].**

Immunohistochemical procedure

To perform immunohistochemical study, the fixed liver tissues were washed under running tap water and dehydrated with 50%, 70%, 90% and 100% ethanol. Then, they were cleared in xylene and finally embedded in molten paraffin bee wax. Four microns paraffin sections were used for immunohistochemical detection of α-SMA, CD31 and CD34expression. The sections were deparaffinized in xylene, rehydrated in ethanol, water, and phosphate-buffered saline. After washing in distilled water, the sections were immersed in 3% hydrogen peroxide for 15 min. Then, phosphate-buffered saline was applied, and the sections were sopped in equilibration buffer at room temperature for 20 min. The tissue sections were then incubated with anti- α-SMA (1:400; Abcam, Cambridge, MA, USA), CD31(1:2000; Abcam, Cambridge, MA, USA) and CD34(1:2500; Abcam,Cambridge, MA, USA) solution for 1 h at room temperature. After that, the prepared sections were incubated with phosphate-buffered saline containing normal goat serum, but without a primary antibody, and the sections were counterstained with Mayer's hematoxylin. Finally, the sections were examined under a light microscope (Olympus™, Japan) **[57]**.

Histological process

The paraffin blocks which are prepared for the immunohistochemical procedure were used for the histological examination of liver tissue sections. Four-micron paraffin sections were collected on glass slides, deparaffinized, and stained using the Hematoxylin and Eosin (H&E) staining procedure **[58]**.The histological sections were examined using an Olympus ™ (Japan) light microscope.

Statistics

In the present study, all results were expressed as Mean ± S.E of the mean. Data were analyzed by One Way Analysis of Variance (ANOVA) using the Statistical Package for the Social Sciences (SPSS) program, version 20 followed by least significant difference (LSD) to compare significance between groups **[59]**. In all analysis, $P < 0.05$ was taken to indicate statistical significant. The percentage of difference (% difference) between any two groups was calculated using the following formula

$$\% \text{ difference} = \frac{\text{Treated value} - \text{Control value}}{\text{Control value}} \times 100$$

RESULTS

The results depicted in **Table (1)** showed the anthropometric parameters of lean control and obese control rats. The results indicated significant elevation ($p < 0.05$) in the body weight, body length, thoracic circumference (TC), abdominal circumference (AC) and body mass index (BMI) in obese control rats versus the lean control counterparts.

Table (2) illustrated the data of plasma glucose and insulin levels as well as the homeostasis model assessment of basal insulin resistance (HOMA-IR) value in obese control and lean control rats. The tabulated results revealed significant enhancement ($p<0.05$) in plasma glucose and insulin levels as well as HOMA-IR valuein obese control rats by contrast with the lean control one.

The represented data in **Table (3)** indicated that the obese control group exhibited significant raise ($P<0.05$) in serum ALT and AST activity (45.22% and 29.75% respectively) by contrast with the lean control group. Of note, a prominent enhancement ($P<0.05$) in serum ALT and AST activity is noticed in hepatic fibrosis lean group as compared to the lean control group (110.68% and 87.85% respectively). Importantly, a pronounced augmentation ($P<0.05$) in serum ALT and AST activity is demonstrated in hepatic fibrosis obese group versus the obese control group (71.83% and 55.49%respectively). On the opposite side, the administration of lycopene or resveratrol in hepatic fibrosis obese group led to significant inhibition($P<0.05$) in serum ALT and AST activity (-32.24% and -31.76% respectively) for lycopene prophylactic group, (-35.88% and -33.68% respectively) for resveratrol prophylactic group, (-19.12% and -15.53% respectively) for lycopene therapeutic group and(-20.94% and -25.21% respectively) for resveratrol therapeutic group in contrary to the hepatic fibrosis obese group.

The obtained data in **Table (4)** demonstrated that obesity produced significant rise ($P<0.05$) in serum cholesterol, triglycerides (TG) and low density lipoprotein (LDL) levels (53.83%, 34.2% and 69.93%, respectively) when compared to the lean control group. Noteworthy, hepatic fibrosis lean group experienced significant heightening ($P< 0.05$) in serum cholesterol, triglycerides and LDL levels relative to the lean control group. (26.01%, 10.99% and 21.67%, respectively). Notably, hepatic fibrosis obese group displayed significant aggravation ($P< 0.05$) in serum cholesterol, triglycerides and LDL levels (15.7%, 19.39% and 12.75% respectively)in respect with the obese control group. On the other hand, lycopene or resveratrol administration in hepatic fibrosis obese group brought about significant decline ($P<0.05$) in serum cholesterol, triglycerides and LDL levels(-28.78%, -26.26% and -32.84% respectively) for lycopene prophylactic group, (-33.52%, -30.36% and -34.6% respectively) for resveratrol prophylactic group, (-23.25%, -19.63% and -24.08% respectively) for lycopene therapeutic group and(-27.56%, -24.44% and -32.11% respectively) for resveratrol therapeutic group and as compared to the hepatic fibrosis obese group.

The findings illustrated in **Table (5)** denoted a significant accretion ($P<0.05$) in serum levels of TGF-β, fibronectin and leptin serum levels in obese control group versus the lean control group (39.35%, 52.81% and 117.33% respectively). Remarkably, hepatic fibrosis lean group disclosed significant enhancement ($P<0.05$) in serum TGF-β, fibronectin and leptin levels. (158.89%, 82.28% and 35.33% respectively) opposed to the lean control group. Significant expansion ($P<0.05$) in serum levels of TGF-β, fibronectin and leptin levels is recorded in hepatic fibrosis obese group contra to obese control group (233.88%, 107.01% and 229.33% respectively). In contrast, the administration with lycopene or resveratrol in hepatic fibrosis obese group yielded significant drop ($P<0.05$) in serum TGF-β, fibronectin and leptin levels(-53.62%, -21.46% and -25.91% respectively) for lycopene prophylactic group(-54.32%, -23.82.% and -27.73% respectively) for resveratrol prophylactic group, (-12.15%, -10.26% and -18.01% respectively) for lycopene therapeutic group, respectively) and (-22.64%. 11.55% and 21.86%) for resveratrol therapeutic group in opposition to the hepatic fibrosis obese group.

Immunohistochemical findings

Immunohistochemical observations in the current study indicated that, the severity of immunohistochemical reactions in the hepatic tissue differ significantly among the various experimental groups. The immunohistochemical reactions forα-SMA, CD31 and CD34were severe and prominent in the hepatic fibrosis obese group (**Fig. 1B, 2B and 3B**) respectively followed by the hepatic fibrosis lean group(**Fig. 1C, 2C and 3C**) respectively, then, obese control rats (**Fig. 1D, 2D and 3D**) respectively and lastly, the prophylactic lycopene group (Fig. 1E, 2E and 3E)respectively. Whereas, the weak and faint immunohistochemical reactions for α-SMA, CD31 and CD34 were noticed in resveratrol prophylactic group (Fig. 1F, 2F and 3F) respectively followed by lycopene therapeutic group (Fig. 1G, 2G and 3G) respectively, then resveratrol therapeutic group (**Fig. 1G, 2G** and **3G**)respectively.

Histological Observations

Microscopic examination of section in liver tissue of rat in the lean control group showed no histopathological change and the normal histological feature of the central vein and surrounding hepatocytes in the hepatic lobules are noticed (**Fig.4**). Microscopic investigation of section in liver tissue of rat fed with HFD (obese control group) showed fatty changes in the hepatocytes underneath the hepatic capsule accompanied by congestion in the portal vein and few inflammatory cells infiltration in the portal area **(Fig.5**). Microscopic examination of section in liver tissue of rat in the hepatic fibrosis lean group showed fibrosis with inflammatory cells infiltration that divided the hepatic parenchyma into nodules associated with congestion in the portal veins and formation of increasing numbers of newly formed bile ductules at the portal area with karyocytomegaly as well as degeneration and necrosis in the hepatocytes (**Fig.6**). Microscopic investigation of section in liver tissue of rat in the hepatic fibrosis obese group showed that the hepatic parenchyma is divided by proliferative fibroblastic cells and inflammatory cells into nodules associated with appearance of prominent nucleoli in the hepatocytes and dilatation in the portal vein (**Fig.7**). Microscopic examination of section in liver tissue of rat in the lycopene prophylactic group showed that there is a congestion in the portal vein and few inflammatory cells infiltration surround the dilated bile ducts at the portal area (**Fig.8**). Microscopic investigation of liver tissue section of rat in the resveratrol prophylactic group showed a congestion in the portal area including portal vein with few inflammatory cells infiltration (**Fig.9**). Microscopic examination of section in liver tissue of rat in the lycopene therapeutic group showed numerous numbers of newly formed bile ductules with inflammatory cells infiltration in

the portal area (**Fig.10**). Microscopic investigation of section in liver tissue of rat in the resveratrol therapeutic group showed fibrosis with inflammatory cells infiltration divided the hepatic parenchyma into nodules (**Fig.11**)

Table (1): Anthropometric parameters of obese control rats in comparison with lean control rats.

parameters / Groups	Body weight (g)	Body length (cm)	TC (cm)	AC (cm)	BMI (g/cm2)
Lean control group	144.3±3.7	20.7±0.25	11.5±0.18	12.3±0.63	0.27 ±0.01
Obese control group	375.0±12.8a	22.6±0.34a	14.8±0.85a	15.2±0.53a	0.55 ±0.03a

Data were expressed as means± standard error (SE) for 10 animals / group. a: P< 0.05 vs lean control group.
TC: Thoracic circumference AC: Abdominal circumference BMI: Body mass index

Table (2):Plasma levels of glucose, insulin and HOMA-IR value in obese control rats in comparison with lean control rats.

Parameters / Groups	Glucose (mg/dl)	Insulin (mu/L)	HOMA-IRvalue
Lean control group	94.9±4.92	12.9±0.54	3.0±0.22
Obese control group	181.2±5.78[a]	21.5±2.78[a]	9.8±1.56[a]

Data were expressed as means± standard error (SE) for 10 animals / group. a: P< 0.05 vs lean control group.
HOMA-IR: The homeostasis model assessment of basal insulin resistance

Table (3): Influence of administration with lycopene orresveratrol on liver enzymes activity in serum in hepatic fibrosis obese rats.

Parameters / Groups	ALT (U/L)	AST (U/L)
Lean control group	44.0 ± 3.3	56.8 ± 3.3
Obese control group	63.9 ± 5.9[a] 45.22%	73.7 ± 4.9[a] 29.75%
Hepatic fibrosis lean group	92.7 ± 6.7[a] 110.68%	106.7 ± 6.2[a] 87.85%
Hepatic fibrosis obese group	109.8 ± 7.2[b] 71.83%	114.6 ± 8.7[b] 55.49%
Lycopene prophylactic group	74.4 ± 2.6[c] -32.24%	78.2 ± 5.9[c] -31.76%
Resveratrol prophylactic group	70.4 ± 2.5[c] -35.88%	76.0 ± 6.4[c] -33.68%
Lycopene therapeutic group	88.8 ± 6.9[c] -19.12%	96.8 ± 3.0[c] -15.53%
Resveratrol therapeutic group	86.8 ± 6.2[c] -20.94%	85.7 ± 4.9[c] -25.21%

Data were expressed as means± standard error (SE) for 10 animals / group. a: P< 0.05 vs lean control group.
b: P< 0.05 vs obese control group c: P< 0.05 vs hepatic fibrosis obese group.
(%): percent difference with respect to the corresponding control value.

Table (4): Influence of administration with lycopene orresveratrol on serum lipidpanel in hepatic fibrosis obese rats.

Parameters / Groups	Cholestrol (mg/dL)	TG (mg/dL)	LDL (mg/dL)
Lean control group	66.5 ± 3.2	79.1 ± 5.1	14.3 ± 0.69
Obese control group	120.3 ± 4.8[a] 53.83%	106.2 ± 4.2[a] 34.2%	24.3 ± 0.97[a] 69.93%
Hepatic fibrosis lean group	83.8 ± 5.8[a] 26.01%	87.8 ± 6.4 10.99%	17.4 ± 1.2 21.67%
Hepatic fibrosis obese group	139.3 ± 6.5[b] 15.7%	126.8 ± 9.8[b] 19.39%	27.4 ± 1.8 12.75%
Lycopene prophylactic group	99.2 ± 7.2[c] -28.78%	93.5 ± 6.1[c] -26.26%	18.4 ± 1.3[c] -32.84%
Resveratrol prophylactic group	92.6 ± 2.7[c] -33.52%	88.3 ± 5.7[c] -30.36%	17.9 ± 0.52[c] -34.67%
Lycopene therapeutic group	106.9 ± 7.8[c] -23.25%	101.9 ± 5.4[c] -19.63%	20.8 ± 1.5[c] -24.08%
Resveratrol therapeutic group	100.9 ± 4.1[c] -27.56%	95.8 ± 8.7[c] -24.44%	18.6 ± 0.76[c] -32.11%

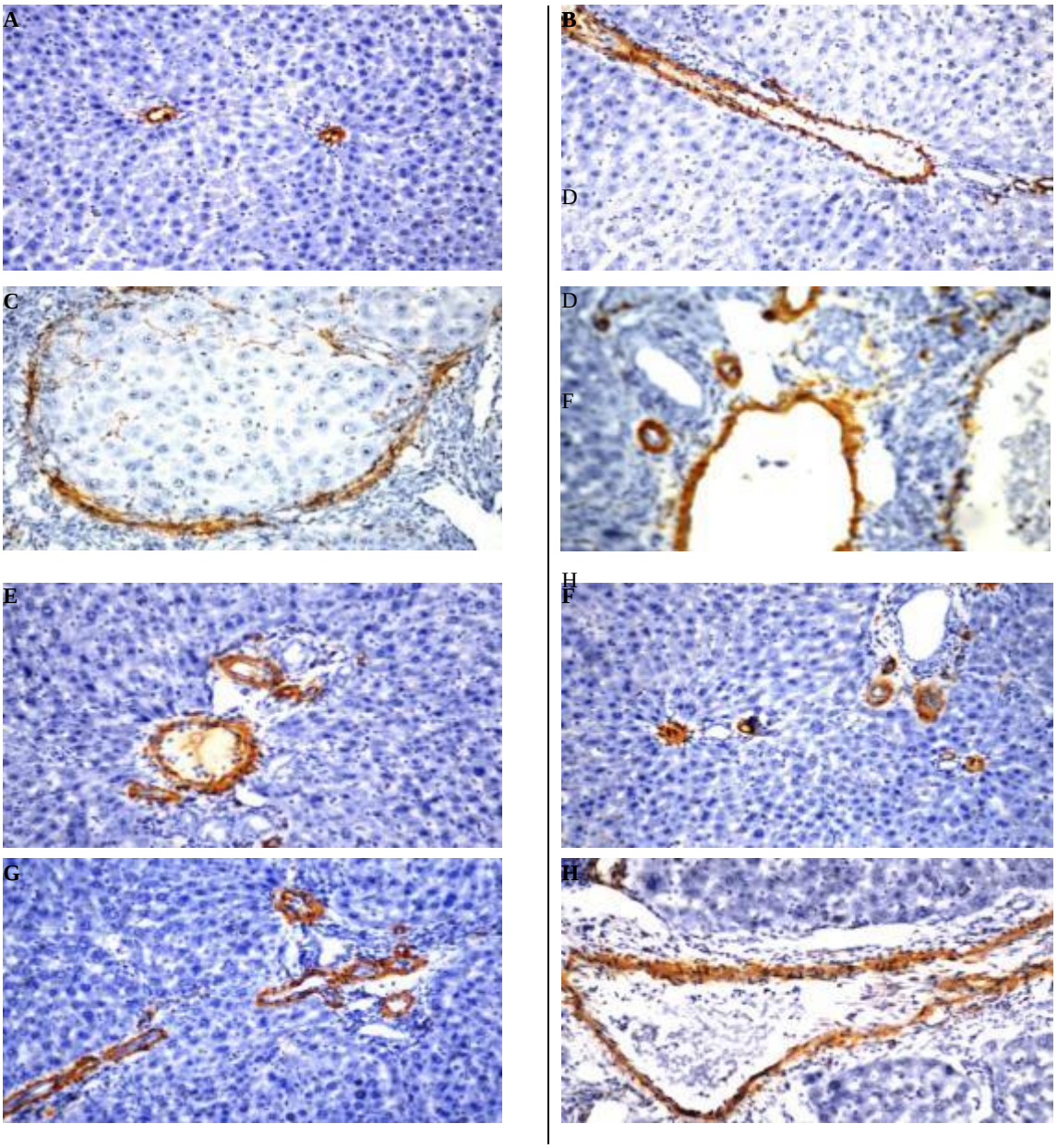
Data were expressed as means± standard error (SE) for 10 animals / group. a: P< 0.05 vs lean control group.
b: P< 0.05 vs obese control group c: P< 0.05 vs hepatic fibrosis obese group. (%): percent difference with respect to the corresponding control value TG : triglycerides LDL: low density lipoprotein

Table (5): Influence of administration with lycopene or resveratrol on serum TGF-β, fibronectin and leptin levels in hepatic fibrosis obese rats

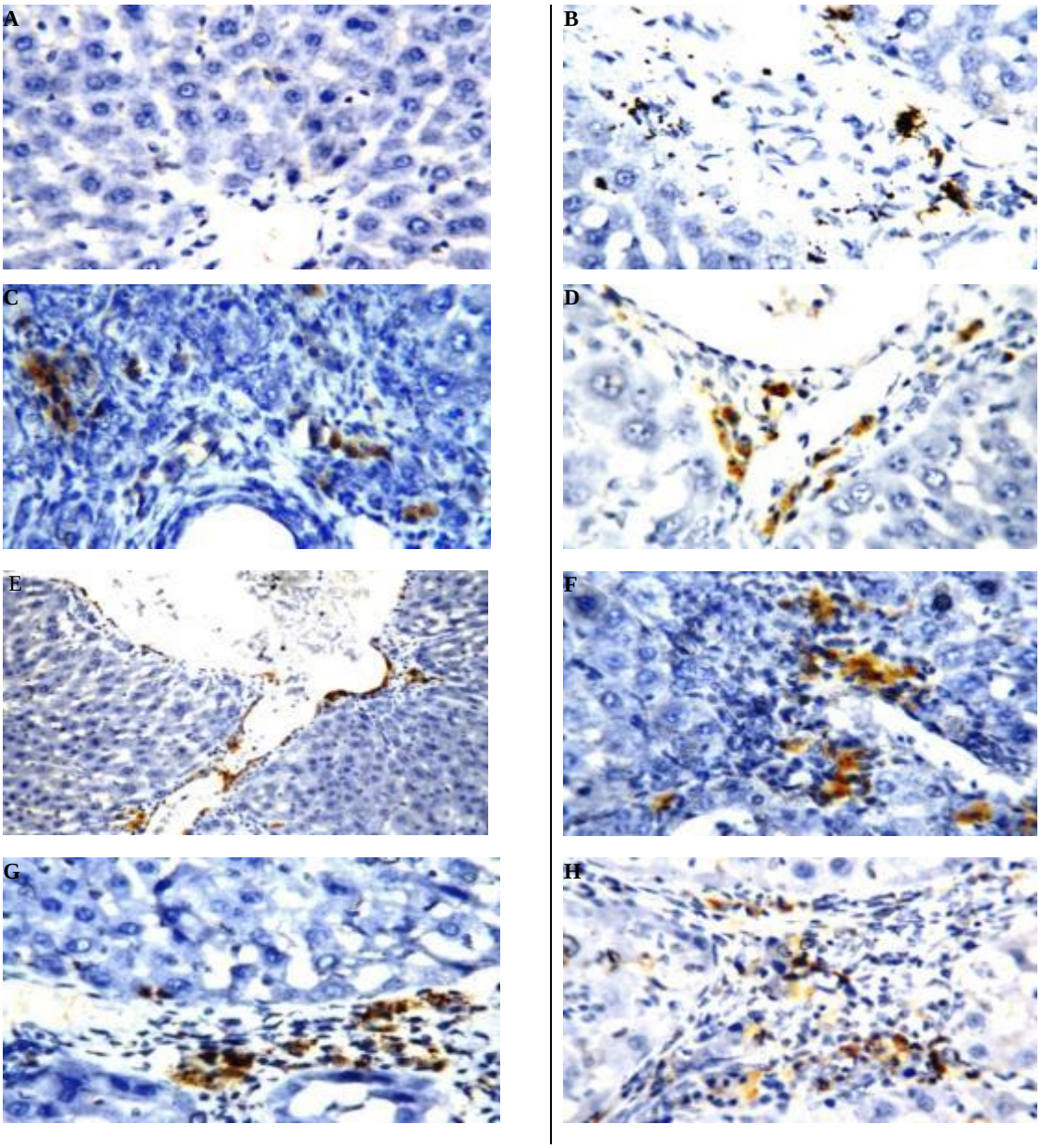
Parameters / Groups	TGF-β (pg/mL)	Fibronectin (µg/mL)	Leptin (ng/mL)
Lean control group	193.9 ± 6.5	905.2 ± 11.2	15.0 ± 0.7
Obese control group	270.2 ± 16.0[a] 39.35%	1383.3 ± 31.5[a] 52.81%	32.6 ± 2.0[a] 117.33%
Hepatic fibrosis lean group	502.5 ± 15.3[a]	1650 ± 13.7[a]	20.3 ± 1.0

	158.89%	82.28%	35.33%
Hepatic fibrosis obese group	647.4 ± 35.1[b] 233.88%	1873.9 ± 42.2[b] 107.01%	49.4 ± 1.3[b] 229.33%
Lycopene prophylactic group	300.2 ± 19.9[c] -53.62%	1471.7 ± 10.8[c] -21.46%	36.6 ± 2.4[c] -25.91%
Resveratrol prophylactic group	295.7 ± 17.9[c] -54.32%	1427.5 ± 3.1[c] -23.82%	35.7 ± 1.5[c] -27.73%
Lycopene therapeutic group	568.7 ± 26.5[c] -12.15%	1690.8 ± 30.6[c] -10.26%	40.5 ± 3.8[c] -18.01%
Resveratrol therapeutic group	500.8 ± 21.1b[c] -22.64%	1657.4 ± 52.4[c] -11.55%	38.6 ± 1.0[c] -21.86%

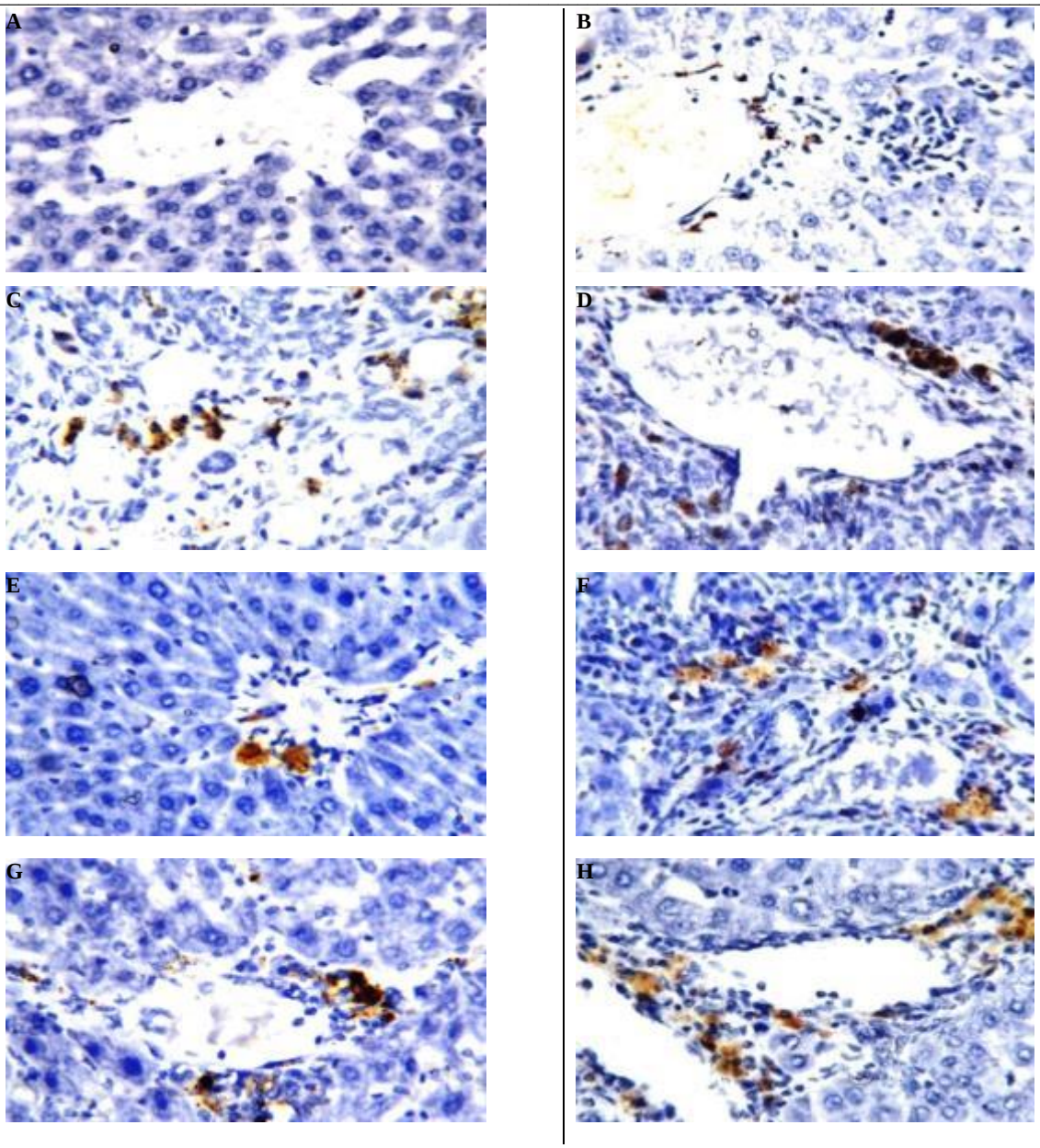
Data were expressed as means± standard error (SE) for 10 animals / group. a: P< 0.05 vs lean control group.
b: P< 0.05 vs obese control group c: P< 0.05 vs hepatic fibrosis obese group. (%): percent difference with respect to the corresponding control value TGF- β: Transforming growth factor -β



Fig(1): Immunohistochemical reaction of α-SMA in the liver tissue sections of the different studied groups.The reaction localizeson the wall of central vein, portal vein and proliferated tissue. (X40)



Fig(2): Immunohistochemical reaction of CD31 in the liver tissue sections of the different studied groups. The reaction centralizes intracellularly of the inflammatory and proliferative cells. (X80)



Fig(3): Immunohistochemical reaction of CD34 in the liver tissue sections of the different studied groups. The reaction condenses within the inflammatory and proliferative cells. (X80).

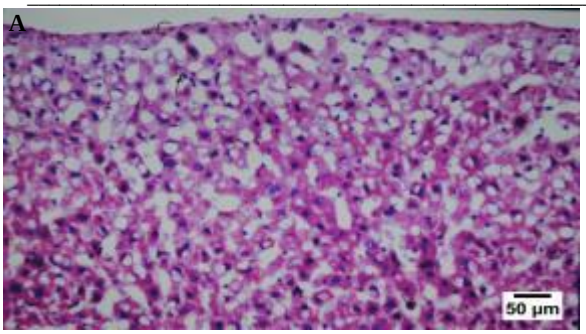


Fig.(4): Optical micrograph of liver tissue section of rat in the lean control group showing no histopathological change and the normal histological structure of the central vein and surrounding hepatocytes in the hepatic lobules are seen. **(H&E) X40**

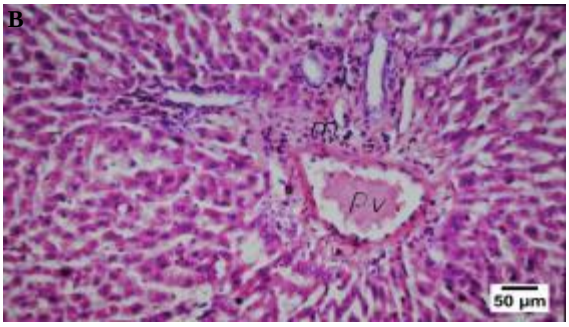


Fig.(5): Optical micrograph of liver tissue section of rat in the obese control group showing fatty changes in the hepatocytes underneath the hepatic capsule, accompanied by congestion in the portal vein and few inflammatory cells infiltration in the portal area. **(H&E) X40**

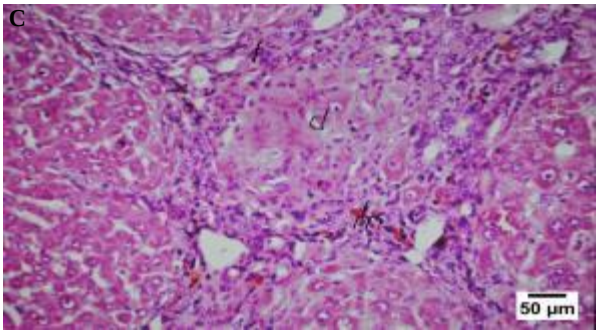


Fig.(6): Optical micrograph of liver tissue section of rat in the hepatic fibrosis lean group showing fibrosis with inflammatory cells infiltration dividing the hepatic parenchyma into nodules associated with congestion in the portal veins and formation of increasing numbers of newly formed bile ductules at the portal area with karyocytomegaly as well as degeneration and necrosis in the hepatocytes. **(H&E) X40**

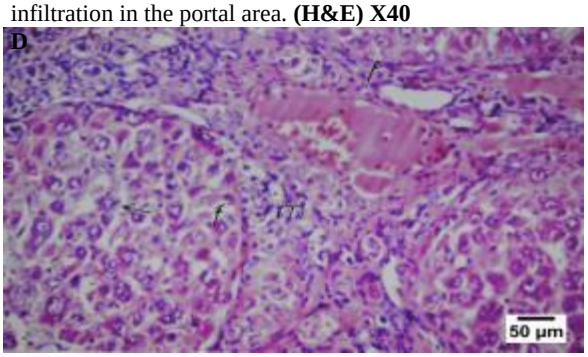


Fig.(7): Optical micrograph of liver tissue section of rat in the hepatic fibrosis obese group showing the hepatic parenchyma is divided by proliferated fibroblastic cells and inflammatory cells into nodules associated with appearance of prominent nucleoli in the hepatocytes and dilatation in the portal vein. **(H&E) X40**

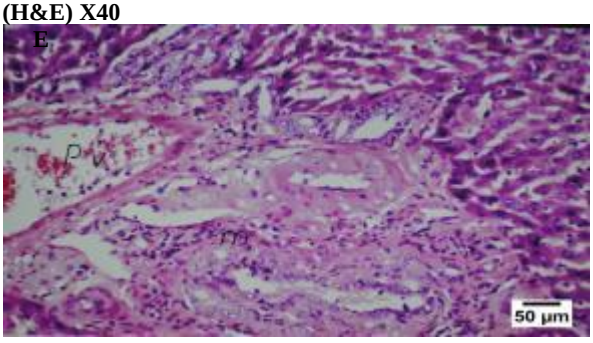


Fig.(8): Optical micrograph of liver tissue section of rat in the lycopene prophylactic group showing a congestion in the portal vein and few inflammatory cells infiltration surround the dilated bile ducts at the portal area.**(H&E) X40**

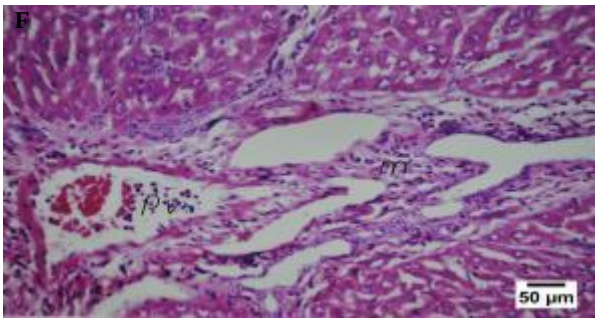


Fig.(9): Optical micrograph of liver tissue section of rat in the resveratrol prophylactic group showing a congestion in the portal area including the portal vein with few inflammatory cells infiltration. **(H&E) X40**

G

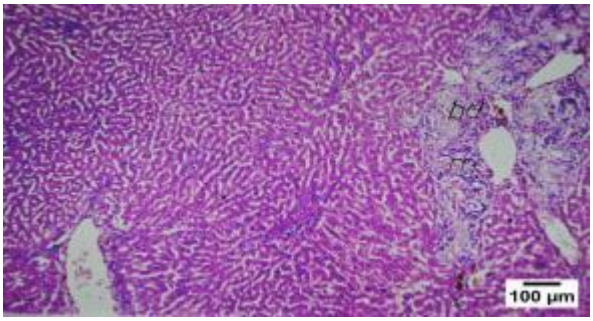
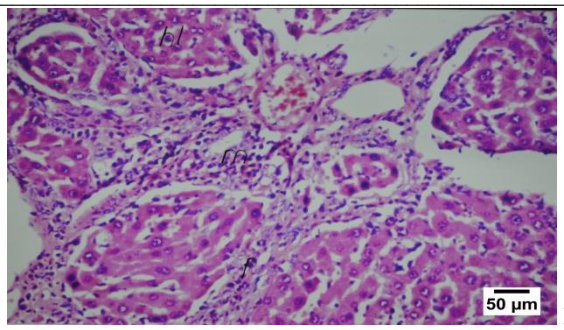


Fig.(10): Optical micrograph of liver tissue section of rat in the lycopene therapeutic group showing numerous numbers of newly formed bile ductules with inflammatory cells infiltration in the portal area. **(H&E) X10**



I

Fig.(11): Optical micrograph of liver tissue section of rat in the resveratrol therapeutic group showing fibrosis with inflammatory cells infiltration divided the hepatic parenchyma into nodules. **(H&E) X40**

DISCUSSION

The rational of the current attempt was to appraise the protective / therapeutic outcome of lycopene and resveratrol against hepatic fibrosis induced by thioacetamide (TAA) in obese rats and tracing the mechanisms by which they act.

Thioacetamide is considered as one of the most important hepatotoxin used for establishment of hepatic fibrosis. One of the key mediators of hepatic injury underlying fibrillation is the discharge of reactive oxygen species (ROS), that results in oxidative stress with consequent induction of lipid peroxidation, and in turn inflammation and fibrogenesis *via* activation of HSCs **[60]**.

To establish the obesity model, the rats were fed with a HFD for a period of 24 weeks. In view of our data, feeding with HFD for this period caused significant elevation in the anthropometric measures in rats comparative to the lean control counterparts .These results match those of **Galisteo et al. [61]**. This indicated that the total energy intake is higher in obese rats than that in the lean one. This means that diet is definitely of influence in the incidence of obesity.

The presented data showed that both TC and AC are significantly raised in obese rats. Also, BMI revealed significant elevation in obese rats. These results highlight that high fat diet induces the accumulation of fats in the thoracic and abdominal areas **[62]**, leading to increased fat deposition in tissues and organs thereby resulting in weight gain **[63]**. **Rodrigues et al. [64]** found positive correlations between the daily intake of lipids and BMI as well as fat accumulation, indicating that BMI is a simple reliable estimate of body fat and obesity in rats.

To assure the creation of obese-modeled rats, plasma glucose and insulin levels were quantified as well as the HOMA-IR was calculated. Plasma glucose and insulin levels were elevated significantly in obese rats compared with those in lean control one. These findings agonist the results of **Galisteo et al. [61]**.This could be interpreted by the existence of high lipolytic activity in the regions of fat deposition that leads to in increased mobilization of FFA to the liver. The increased flux of fatty acids to the liver augments gluconeogenesis and reduces the impact of insulin on peripheral glucose disposal **[65]**. Obesity is commonly associated with low-grade chronic systemic inflammation which potentially results in insulin resistance (IR) **[66]**. In the present study, HFD provoked significant increase in HOMA-IR, which is in alignment with the previous study of **Faheem et al. [67]**.

The results of the current investigation declared that TAA increases the liver enzymes activity in serum of both lean and obese rats. TAA has been established to heighten the activity of ALT and AST in serum **[68]**. **Tsai et al. [69]** reported that TAA induces reactive metabolites formation like thioacetamide-S-oxide radicals and ROS intermediates. Hence, the biotransformation of TAA within the cellular milieu promotes oxidative damage of the liver, which further degenerates liver tissues causing necrosis. The leakage of ALT and AST into the serum and in turn the elevation of their serum concentrations is considered as real sensible indicators in the liver diseases diagnosis **[70]**. TAA administration in obese rats aggravated the activity of liver enzymes (ALT and AST) in serum significantly. This observation is on par with that of **Mohamed et al. [71]** study which concluded a correlation of BMI with ALT and AST activity in serum. Moreover, it has been demonstrated that increased body weight enhances fat deposition in hepatocytes i.e. steatosis and it was claimed to cause hepatic fibrosis **[72]**. Another study has showed that the high prevalence of obesity leads to high incidence of chronic liver disease particularly NAFLD **[73]**. These investigators proved that TAA injection for six weeks exhibits liver dysfunction signs manifested by the up-regulated serum ALT and AST activity owing to the damage of hepatocyte membrane, which induces a leakage of transaminase enzymes into the blood circulation.

Administration of lycopene significantly inhibited the activity of serum ALT and AST in hepatic fibrosis obese rats. This result disclosed that lycopene can preserve the hepatic cellular integrity and ameliorate hepatic cellular damage so as to prevent the draining of liver enzymes (ALT and AST) into the blood circulation **[74]**. Various researches have registered that lycopene exhibited high anti-oxidative activity both *in vitro* and *in vivo* that is

exerted *via* its reaction with the free radicals. Lycopene displays unique and distinct biological activities due to its acyclic structure, hydrophobicity and large array of conjugated double bonds **[33]**.

Resveratrol supplementation in hepatic fibrosis obese rats revealed a positive impact on liver functions as evidenced by the reduction of the liver enzymes (AST and ALT) activity in serum. The obtained result is in line with **Hosseini et al. [39]** who stated that resveratrol with its anti-oxidative and anti-inflammatory properties can delay the progression of hepatic damage induced by TAA as manifested by the lowering of the activity of ALT and AST in serum of resveratrol-treated rats. The potential hepatoprotective mechanisms of resveratrol are related to its ability to inhibit inflammation, enhance apoptosis of necrotic hepatocytes and suppress oxidative stress **[75].**

TAA injection yielded significant elevation of serum cholesterol, triglycerides and LDL levels in the present approach which indicates the perturbation of lipid metabolism. During the detoxification process, TAA is metabolized by cytochrome p450 to TAA-S-oxide and TAA-S-dioxide. The metabolites of TAA are highly toxic and interfere with mitochondrial activity by changing the permeability of the cells **[76,77]**, Thus impairing the synthesis and libration of lipoproteins **[78]** leading to the accumulation of lipid. The present results are in conformity with those in the study of **El-Hadary [79]** and **Osman et al. [80]** who proved the hyperlipidemic effect of TAA. The hyperlipidemic impact of TAA could be related to the impaired fatty acid uptake and metabolism by the damaged liver cells. **Kadir et al. [81]** attributed the disturbance of lipid metabolism upon TAA administration to the reduction of functional LDL receptors which render the inability of the liver to clear LDL cholesterol from the blood stream. TAA administration in obese rats induced by HFD accelerates hepatic steatosis as HFD produces an increase in hepatic accumulation of fat droplets. Of note, the metabolism of cholesterol is paralleled by the fat content in the liver independent on body weight, suggesting that the more fat in the liver, the higher cholesterol synthesis **[71]**. Moreover, HFD enhances the collagen deposition in the rat liver over that induced by TAA, due to acute liver injury **[82]**.These scholars reported that hepatic injury leads to changes in serum lipids, lipoproteins, cholesterol, triglycerides, and some related enzymatic activity which influenced by the alterations in polyunsaturated fatty acids. Furthermore, dyslipidemia is a well-known reason of inducing oxidative stress due to elevated FFAs and triglycerides levels that render LDL less dense and more susceptible to oxidation and uptake by macrophages **[83]**.

The present data revealed that lycopene is able to reverse the elevated levels of serum cholesterol, triglycerides and LDL in hepatic fibrosis obese rats. Lycopene has been found to regulate cholesterol metabolism and prevent LDL oxidation which has been ascribed mainly to its antioxidant capacity **[84]**. The ability of lycopene to increase the faecal cholesterol excretion, along with the inhibition of hepatic 3- Hydroxy – 3 – methyl glutarine coenzyme A (HMG CoA) reductase activity have been demonstrated suggesting the reduction in the intestinal cholesterol absorption and biosynthesis **[85]**. This action of lycopene was confirmed by **Palozza et al. [86]** who concluded that lycopene can suppress the synthesis of cholesterol in human macrophages by decreasing HMG-CoA reductase activity and expression and modulation of LDL receptor cholesterol acyl transferees (ACAT) activity. **Fuhrman et al. [87]** demonstrated that the inhibitors of cholesterol biosynthesis are known to deplete the serum concentrations of cholesterol *via* augmenting the elimination of serum LDL, secondary to the stimulation of the LDL receptors. Besides that, recent report by **Albrahim and Alonazi [88]** indicated that the supplementation of diet with lycopene significantly improves the chronic consumption of HFD-mediated oxidative stress, inflammation and metabolic perturbation in rats, particularly, triglycerides, cholesterol, LDL accumulation in serum.

Resveratrol supplementation in hepatic fibrosis obese rats significantly lowered serum lipids (cholesterol, triglycerides and LDL) level. This finding is in harmony with that of **Hussein et al. [89]** who cited that resveratrol has hypolipidemic effects where it significantly decreases serum cholesterol, triglycerides and LDL concentrations in obese rats compared to non-treated obese counterparts. Also, **Ahn et al. [90]** commented that the addition of resveratrol mice diet significantly reduces lipid, triglycerides and cholesterol levels, and suppresses the HFD-induced over-expression of genes involved in lipid metabolism. Hypolipidaemic effect of resveratrol was also reported by **Cho et al. [91]** as the serum total cholesterol and triglycerides concentrations as well as the hepatic tissue contents of these lipids are significantly reduced in hamsters fed with HFD and supplemented with resveratrol. This lipid lowering effect of resveratrol was accompanied by the inhibition of HMG-CoA reductase activity and the down-regulation of its gene **[92]**. The study of **Fan et al. [93]** suggested another mechanism for the hypolipidemic effect of resveratrol as they stated that resveratrol intervention significantly decreases the total cholesterol and LDL-C levels in rabbits fed with HFD diet *via* the inhibition of LDL oxidation. Resveratrol has been shown to prevent lipids peroxidative degradation and to block the oxidized LDLs uptake in the vascular wall in a concentration-dependent manner **[94]**. Another study concluded that in high cholesterol-fed rats, resveratrol stimulates the excretion of bile acids into the feces. Thus, the inhibition of hypertriglyceridemia and hypercholesterolemia upon resveratrol administration in rats may also result from the increased excretion of fecal bile acids **[95, 96]**.

The current study declared that the serum levels of TGF-β, fibronectin and leptin displayed significant enhancement in hepatic fibrosis in lean and obese rats. **El-Baz et al. [97]** documented that administration of TAA for 6 weeks results in hepatic fibrosis as manifested by the persistence of activated HSCs, portal fibroblasts and myofibroblasts as well as progressive liver inflammation evidenced by a significant rise in TGF-β level in serum through AMP-activated protein kinase (FXR/AMPK) signal pathway.

Also,the study of **El Awdan et al. [98]** indicated that the hepatotoxin TAA, can trigger the pro-inflammatory and inflammatory cytokines and mediators by macrophages (Kupffer cells) like NF-κβ and TGF-β that play important role in hepatic inflammation. Upon activation of pro-inflammatory cytokines, NF-κB functions as the driving force of fibrosis, as it stimulates TGF-β, which is accounted as the central molecule in the fibrogenesis. TGF-β performs a significant an important role in obesity pathogenesis, affecting the liberation of inflammation molecules, and inducing remodeling and collagen accumulation in the adipose tissue (AT). HFD was accompanied by elevated TGF-β levels and plasminogen activator inhibitor-1 (PAI-1) mRNA in epididymal and retroperitoneal visceral fat depots **[99]**. Also, TGF-β encourages macrophages accumulation, collagen accumulation and remodeling in fat tissue of obese mice **[100]**, as well as reinforcing the discharge of pro-inflammatory molecules in culture models of rodent's macrophages and adipocytes **[101]**. A multitude of studies suggest the correlation between TGF-β serum levels and BMI **[102]**; the protein and/or mRNA levels of TGF-β in AT also seem to be enhanced in obesity, both in mice **[101]** and humans **[103]**.

Usually after acute liver insult, parenchymal cells regenerate and substitute the necrotic and apoptotic cells; this process is correlated with an inflammatory response and a restricted accumulation of ECM. When liver damage continues, the regenerative response depletes and hepatic cells are replaced by bountiful ECM, produced from active HSCs, commonly consisted of collagen type I-III-IV, fibronectin, elastin, laminin, and proteoglycans. The conversion of HSCs from deactivated to an activated situation is manifested by a myofibroblast-like phenotype accountable for proliferation and exaggerated ECM accumulation which is governed by inflammatory molcules, including TGF- β **[104]**. TGF-β is the most important fibrogenic factor that accumulates and forms scar tissue in the space of Disse resulting in sinusoidal capillarization. Proteins like, fibronectin are also demonstrated in the perisinusoidal space upon HSCs activation. **Sanz-García et al. [105]** stated that pro-inflammatory cytokines, mechanical alterations and other factors are sensed by myofibroblasts, that launch a transcriptional program for the expression and liberation of these proteins. It has been shown a positive correlation between plasma fibronectin and BMI, serum triglyceride level, plasma fibrinogen and serum cholinesterase activity. It is considered that metabolic perturbations associated to upper body obesity may result in an elevated hepatic secretion of very low density lipoprotein (VLDL) and of different plasma proteins including fibronectin **[106]**. Fibronectin expressions in both visceral adipose tissue (VAT) and subcutaneous adipose tissue (SAT) have an important relations with obesity indicators and leptin level in the serum. These findings suggest that adipose tissue fibronectin may have a significant role in the modulation of adipocyte-specific gene expression and may have a pathophysiological role in obesity-related ailments in humans **[107]**.

Leptin is the product of the obese (ob) gene and it is commonly expressed by the adipose tissue, whilst other sites also implicate to its expression. The discharge of both TGF-β and leptin from activated Kupffer cells (KCs) and HSCs and the deposition of ECM proteins like collagen I and collagen III are responsible for hepatic fibrosis **[108]**. In mice, leptin promotes hepatic fibrosis and stimulates of α1(I)-procollagen mRNA produced by frequent administrations of TAA for four weeks **[109]**. Besides, leptin and its receptors (*Ob-Rb*) have a pivotal role in profibrogenic process particularly by promotion of the deposition of α-SMA and type I collagen in the liver of Zucker rats **[110]**. Thus, leptin can aggravate the amplitude of TAA-induced liver fibrosis in mice and it may be a significant factor in liver fibrosis development **[111].**

A significant positive correlation has been found between serum leptin level and BMI **[112]**, leptin protein, and mRNA expression levels showed high values in the model group of obesity induced by feeding rats with HFD for 8 weeks **[38]**.

Lycopene administration in hepatic fibrosis obese rats in the current attempt elicited significant suppression in the levels of serum TGF-β, fibronectin and leptin. **Albrahim and Alonazi [88]** reported that the overproduction of ROS may participate in the development of fibrosis through activation of TGF-β1 and inturn production of ECM proteins. Lycopene protected hepatocytes *via* down-regulation of the fibrosis markers expression including TGF-β1. Lycopene has been demonstrated to inhibit the activity of HSCs by repressing lipid peroxidation *via* its special free radical scavenging character, and suppressing fibrogenesis process in hepatic fibrosis model **[113]**.

Ignotz and Massagué [114] proved that TGF-β increases the expression of the major ECM proteins; fibronectin and collagen. Thus, one can postulate that the reduction of serum fibronectin level in the current study is a consequent of the slowdown of TGF-β expression due to lycopene supplementation.

Adipokines such as leptin are cell signaling proteins liberated from adipocytes. Adipokines regulation seems to have a key role in adiposity, metabolic syndrome, and obesity. Lycopene administration has been found to decrease the circulating levels of leptin in rabbits **[115].**

Likewise, administration of resveratrol in hepatic fibrosis obese rats in the present investigation brought about significant reduction in serum TGF-β, fibronectin and leptin levels. **Abdu and Al-Bogami [116]** concluded that resveratrol possesses anti-fibrotic and hepatoprotective properties *via* promoting the anti-oxidative mechanism and ameliorating oxidative stress, thus preserving structural integrity of biomembranes and preventing liver steatosis as well as fibrosis indicating great effectiveness in the prohibtion of fibrosis. **Hussein et al. [89]** proved the anti-inflammatory effect of resveratrol *in vivo* as they cited that the administration of resveratrol in liver fibrosis rat model induced by TAA significantly decreases the concentration of serum TGF-β1 as compared with TAA alone. Also, the findings of **Lee et al. [117]** indicated that, oral administration of resveratrol in rats remarkably down-regulates TGF-β mRNA expression. The suggested

mechanism by which resveratrol could prevent serum TGF-β elevation has been reported by **Chávez et al. [118]**, providing a satisfactory anti-fibrotic action of resveratrol, that resveratrol could inhibit NF-κB activity which up-regulates TGF-β. Thus, it is possible that the effect of resveratrol on TGF-β may be mediated by NF-κB.

Mahmood et al. [119] mentioned thatfibronectin and type IV collagen levels in the renal cortex are diminished by resveratrol through lowering the increased levels of oxidative stress and inflammation *via* the scavenging of ROS. Additionally, the recent *in vivo* study has indicated that the administration of resveratrol diminishes the deposition of fibronectin and collagen type IV to ameliorate diabetic nephropathy **[120]**. Moreover, an investigation of molecular pathways demonstrated that resveratrol can alleviate TGF-β expression as well as the phosphorylation of Smad2 and Smad3 for diabetic nephropathy alleviation **[121]**. Thus, one can propose that the decline in serum fibronectin level in the current study may be mediated through suppressing the levels of TGF-β.

The synthesis and liberation of leptin are governed by multiple factors, including insulin and steroid hormones. Resveratrol has been found to markedly reduce *de novo* lipogenesis, enhance lipolysis and alter the anti-lipolytic action of insulin. Resveratrol was reported to increase insulin sensitivity and this effect is accompanied by the reduction in the circulating leptin and insulin levels **[122].**

The immunohistochemical reactions of α-SMA, CD31 and CD34 showed severe and intense reactions in hepatic fibrosis lean and obese groups. HSCs are the common type of cells implicated in hepatic fibrosis. In healthy livers HSCs are inert, stay in the space of Disse, save vitamin A in lipid droplets and they render as hepatic pericytes **[123]**. Meanwhile, in response to persistent liver insult, HSCs down-regulate the expression of some genes like glial fibrillar acidic protein and peroxisome proliferator-activated receptor gamma (PPARγ), lose lipid droplets and they are transdifferentiated into myofibroblasts. Myofibroblasts begin expressing fibrogenic genes such α-SMA and collagen type I. Then, they proliferate and relocate to the site of liver injury where they produce ECM **[124]**. Thus, it is believed that HSCs activation is accounted to be a key event in hepatic fibrosis development **[125]**. α-SMA is a sign of HSCs activation, and in the study of **Zhao et al. [126]**, the immunofluorescence staining demonstrated that the positive expression of hepatic α-SMA is significantly increased in hepatic fibrosis induced by TAA. Moreover, **Park et al. [127]** stated that feeding mice with HFD is characterized by the up-regulation of the genes expression of the markers of hepatic fibrosis like α-SMA and collagen.

Resveratrol administration in hepatic fibrosis obese rats reduced the expression ofα-SMA as shown in the immunhistochemical observation in the current research. Resveratrol administration significantly inhibited the expression of α-SMA in the liver, induced by CCL4, to almost basal levels of the normal control **[150]** indicating the anti-fibrotic activity of resveratrol. Down-regulation of α-SMA expression in the liver is widely thought to be a promising potential event in hepatic fibrosis inhibition **[151]**. It has been reported that resveratrol can suppress the TGF-β-induced increase in the ROS, fibronectin production, and expression of α-SMA in mouse model for Duchene muscular dystrophy **[152]**.

In the current attempt, administration of resveratrol in hepatic fibrosis obese rats diminished the expression of CD31 in the liver as manifested by immunohistochemical outcomes. A down expression of CD31 in the liver of rats bearing liver cancer has been detected upon treatment with resveratrol in a dose of 100 mg/kg. These results denote the inhibiting impact of resveratrol on angiogenesis which may represent a novel mechanism underlying the chemo preventive activity of this phytochemical against hepatic carcinogenesis **[153]**.

Supplementation with resveratrol in hepatic fibrosis obese rats down regulated the expression of CD34 in the liver as evidenced in the immunohistochemical analysis in the current study. The suppressive effect of resveratrol on VEGF expression and angiogenesis in hepatocellular carcinoma (HCC) has been examined. The xenograft sections were stained for CD34 to study microvessels *in vivo*; the microvessel density was decreased upon treatment with resveratrol indicating the down expression of CD34 **[154]**. Resveratrol supplementation significantly regulated dimethylnitrosamine- mediated changes hepatic fibrosis biomarkers, early cihrrosis, ducts proliferation, vascularization, lymphocytic infiltration, focal necrosis, hydropic degeneration of hepatocytes, steatosis, as well as portal hypertension. These findings indicated the potential anti-fibrotic role of resveratrol **[155]**.

Microscopic examination of liver tissue section of rat in lean control group revealed normal hepatic architecture. Microscopic examination of liver tissue section of rat in obese control group showed fatty change in the hepatocytes underneath the hepatic capsule associated with congestion in the portal vein and few inflammatory cells infiltration in the portal area. **Oliveira et al. [156]** reported that HFD induces accumulation of cytosolic triglycerides and phospholipids in association with initial mitochondrial damage leading to the development of a mixed type of liver steatosis over time.

Microscopic examination of liver tissue section of rat in hepatic fibrosis lean group showed fibrosis with inflammatory cells infiltration that divided the hepatic parenchyma into nodules associated with congestion in the portal veins and formation of multiple numbers of newly formed bile ductules at the portal area with karyocytomegaly as well as degeneration and necrosis in the hepatocytes. These findings are in agreement with those of **Gab-Allah et al. [157]** who explained these pathological changes to the metabolism of TAA.TAA is metabolized by cytochrome P450 enzymes of liver microsomes and converted into toxic metabolites which induced oxidative stress in the hepatic cells. Oxidative stress is accountable for the alterations of cell permeability, enhancement of intracellular Ca++ concentration increment in the nuclear volume, enlargement of the nucleoli and inhibition of the mitochondrial activity **[158]**.

Microscopic examination of liver tissue section of rat in hepatic fibrosis obese group showed that the hepatic

parenchyma is divided by proliferative fibroblastic cells and inflammatory cells into nodules associated with appearance of prominent nucleoli in the hepatocytes and dilatation in the portal vein. Oxidative stress is considered to play a central role in the pathogenesis of HFD-induced hepatic fibrosis because the increased generation of ROS is known to produce lipid peroxidation, followed by stimulation of the inflammatory response, and stellate cells, leading to fibrogenesis **[156]**. Moreover, the reactive radicals of TAA intermediate metabolites promote oxidative stress in hepatocytes, leading to necrosis of the lobular center and liver damage **[159]**.

Microscopic examination of liver tissue section of rat in hepatic fibrosis obese group pre-treated with lycopene showed congestion in the portal vein and few inflammatory cells infiltration surround the dilated bile ducts at the portal area. Microscopic examination of liver tissue section of rat in hepatic fibrosis obese group post-treated with lycopene revealed multiple numbers of newly formed bile ductules with inflammatory cells infiltration in the portal area. The HFD provides great quantities of fat which saturate the liver's capability to export triglycerides resulting in its deposition in the hepatocyte which activates inflammatory pathways directly by NF-κB activation and indirectly *via* changing of intestinal permeability and translocation of lipopolysaccharides to the liver. The excessive nutrients enhance the oxidation capacity of mitochondria resulting in ROS generation, documenting a mutually dependent process between inflammation and oxidative stress. Lycopene was efficient in governing the inflammatory and oxidative pathways, as well as in promoting the endogenous antioxidant defense system. Thus, it could help in the regulation of hepatic lipids accumulation and activate β-oxidation **[160]**. Furthermore, lycopene has been demonstrated to defend hepatocytes *via* down-regulation of the expression of the fibrotic markers in hepatic tissue in liver fibrosis model **[113]**. Therefore, lycopene could be considered as a promising candidate for protecting and/or treating hepatic fibrosis via its antioxidant capacity and down-regulating the fibrotic markers.

Microscopic examination of liver tissue section of rat in hepatic fibrosis obese group pre-treated with resveratrol showed congestion in the portal area including portal vein with few inflammatory cells infiltration. Microscopic examination of liver tissue section of rat in hepatic fibrosis obese group post-treated with resveratrol showed fibrosis with inflammatory cells infiltration divided the hepatic parenchyma into nodules. Resveratrol displays profitable effects on adipose tissue under obese situation by ameliorating intracellular oxidative stress, inhibiting chronic low-grade inflammation, reducing adipogenesis and lipogenesis, and repressing the differentiation of preadipocytes to mature adipocytes **[161]**. Moreover, **Franco et al. [162]** demonstrated the beneficial role of resveratrol in preventing obesity and oxidative stress as well as decreasing the risk of hypertension, dyslipidemia and steatosis in adult rats programmed by early weaning. The antioxidant effect resveratrol leads to the decreased oxidant generation and lipid peroxidation, thus restoring of oxidant and antioxidant status of the liver tissue **[163]**.

CONCLUSION

The results received in this attempt substantiated the scientific evidence in favor of the anti-fibrotic effect of lycopene and resveratrol in obese rats afflicted with hepatic fibrosis. These natural products offer hepatoprotection and are helpful in overcoming obesity accompanied by hepatic fibrosis owing to their diverse prominent biological characteristics particularly anti-inflammatory activity. The promising role of these inherent candidates against this superimposed disease may open a line of thought to consider these natural nutraceuticals for protection and/or treatment of subjects susceptible to or with chronic liver diseases. Also, one can canpostulate this naturalistic approach as an adjunct therapy aiming to improve the effectiveness of the conventional pharmacologic drugs.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Formatting of funding sources

This study was funded by National Research Centre thesis fund.

Acknowledgment

Work is partially supported by National Research Centre Egypt

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