

Amelioration of hypercholesterolemia-induced hepatic changes with red grape juice: A histopathological study

Ahlam Abdulaziz Al-Ahmadi¹, Soad Shaker Ali², Nasra Naeim Ayuob^{2,3} and Abeer Khaled Al Ansary⁴

¹Department of Biological Sciences, Faculty of Science, ²Department of Anatomy, Faculty of Medicine, King Abdulaziz University, Jeddah, Saudi Arabia, ³Department of Histology and Cytology, Mansoura University, Egypt and ⁴Department of Nutritional and Food Science, Educational Collage for Home Economic, Umm al-Qura University, Makka, Saudi Arabia

Summary. Objectives: Hypercholesterolemia was confirmed as a risk factor for hepatic fibrosis, as well as atherosclerosis and coronary heart disease. This biochemical and histopathological study was conducted to investigate the possible protective effect of red grape against hepatic injury induced by a high-cholesterol diet (HCD).

Material and methods: Thirty male Wister rats were randomly divided into three groups (n=10): the control received saline, the induction group was fed HCD, and the treated group was fed a HCD and 0.4 ml of 100% red grape juice (RGJ) for 13 weeks. After the animals were sacrificed, liver tissue samples were taken to be processed for light and electron microscopy examination.

Results: The administration of the RGJ and HCD significantly decreased the animals' blood glucose, insulin, cholesterol, triglycerides, Low Density Lipoprotein levels and increased their High Density Lipoprotein level compared to the rats fed the HCD alone. It also decreased the periportal (macro- and microvesicular) steatosis, fibrosis, lymphocytic infiltration and blood sinusoidal congestion that were observed in HCD-fed rats alone. The RGJ reduced the number of activated myofibroblasts. This was confirmed by a reduction in the expression of alpha smooth muscle actin and desmin. The RGJ increased, although not significantly, the expression of endothelial Nitric Oxide Synthetase.

Conclusion: The administration of RGJ succeeded in alleviating the biochemical and, to some extent, the histopathological changes induced by the high cholesterol diet. Consumption of fresh RGJ or its pharmaceutical preparations is advised especially for those who are used to eat a high fat diet.

Key words: Hypercholesterolemia, Liver, Grape, Immunohistochemistry, Rat

Introduction

With today's hectic lifestyles, people pay no attention to the consumption of fresh fruits and green vegetables that boost their antioxidant status (Bagchi et al., 2002). Adding to that the consumption of a high-cholesterol diet (HCD) and a lack of sufficient physical activity, all these factors contribute to the development of hypercholesterolemia (Wu et al., 2012) which is a known risk factor for the development and progression of atherosclerosis and is closely related to many diseases (Deepa and Varalakshmi, 2005). Some previous studies with rabbit and rat models reported that a HCD induced different forms of liver injury (Buysens et al., 1996; Nanji et al., 1997; Jeong et al., 2002).

Many drugs are known to lower circulating cholesterol levels, but they are frequently associated with severe side effects (Bagchi et al., 2002). The consumption of functional foods or dietary supplements can be used to lower serum cholesterol (Kwok et al., 2010).

Grape (*Vitis vinifera*), one of the most widely

consumed fruit worldwide, contains many bioactive constituents, including flavonoids, polyphenols, anthocyanins, resveratrol and other stilbene derivatives (Çetin and Sağdıç, 2009). Grape polyphenols have important antioxidant properties (Preuss et al., 2000). Proanthocyanidin, found in large amounts in red wine and grape seed, is a bioflavonoid known to have a protective effect against oxidative injury (Guler et al., 2011).

Quiescent FSCs are inconspicuous in the normal liver. However, when activated, they are transformed into myofibroblast-like cells that produce α -smooth muscle actin (ASMA) and showed up-regulated desmin expression (Gressner et al., 1992; Washington et al., 2000). Hence, these two markers, ASMA and desmin were used in this study. Nitric oxide (NO), a gas synthesised by the enzyme nitric oxide synthase (NOS), is widely considered as an endothelium-dependent regulator of vascular tone (Kausar et al., 2000; Ogita and Liao, 2004). Endothelial cells lining the blood sinusoids and the central vein of normal liver shows eNOS expression (Michael et al., 2003; Leifeld et al., 2002). Thus, it was used in this study as an indicator of the structure of the microvasculature in hypercholesterolemic conditions. In this study, the effect of a HCD on the liver and the possible protective effect of red grape juice (RGJ) were investigated both biochemically and histopathologically using light and electron microscopy.

Materials and methods

Animals and diets

This experimental study was approved by the biomedical research ethics committee, Faculty of Medicine, King Abdulaziz University, Jeddah, Saudi Arabia. It was performed on 30 male albino Wister rats weighing between 250 and 350 g that were purchased from the animal house in King Fahd Medical Research Center. The animals were housed in stainless steel cages and maintained in a 12-hour light-dark cycle, with a room temperature of $27 \pm 1^\circ\text{C}$ under hygienic conditions. Water was offered ad libitum. The rats were randomly divided into three groups. Group I served as the control (n=10) and received a standard diet, group II served as the induction group (n=10) and was fed a HCD (rat

chow supplemented with 4% cholesterol and 1% cholic acid according to Thiruchenduran et al. (2011) for 13 weeks, and group III served as the treated group and was fed a HCD and 0.4 ml/day of 100% RGJ through a nasogastric tube for 13 weeks as per the method advised by Castilla et al. (2006). The RGJ was purchased from fruit stores in Jeddah (imported from Sheli). The RGJ was prepared in the nutrition laboratory using a sterilized blender. It was then placed in 10 ml bottles and stored in a cold room at 4°C until it was used. The component concentrations of the 100 g of red grape used in the study was assessed in the Analytical Chemistry Unit at Assuit University, Egypt, using Mass Spectrometry (MS) according to the Folin-Ciocalteu procedure (Zoecklein et al., 1990). The RGJ component concentrations are shown in Table 2.

Metabolic and biochemical assessment

The animals' food, water consumption and body weights were measured at the start of the experiment, during the experiment and at the end of the experiment. Weight gain and food efficacy were calculated. Blood samples were collected at the start of the experiment, during the experiment and at the end of the experiment for assessment of blood glucose, insulin levels, lipid profiles and liver function.

Histology

At the end of the experiment, the animals were sacrificed, and the liver was dissected, weighed, processed and embedded in paraffin blocks. Paraffin sections (5-8 μm thick) were stained with haematoxylin and eosin for routine histological examination and Masson trichrome for detection of connective tissue according to Bancroft and Gamble (2002). An Olympus microscope BX-51 with a digital camera connected to a computer with Pro Plus image analysis software version 6.0 (Media Cybernetics, Inc., USA) was used for photographing the samples. The images of the tissue sections stained with haematoxylin and eosin were analysed, and the area (μm^2) and the circumference (μm) of the hepatocytes were determined. At least ten cells in five random fields were analyzed (Zaitoun et al., 2005). For the immunostaining, the labelling intensity (area percentage) of ASMA, desmin and eNOS positive tissue

Table 1. Primary antibodies used in the study and their characteristics.

Antibodies	Functional significance	Clone	Dilution	Manufacturers
α -SMA alpha smooth muscle actin (ASMA)	Marker of smooth muscle cells and myofibroblast cytoskeleton Seki, et al., 2002	1A4 (a murine monoclonal antibody to a NH2-terminal synthetic decapeptide of ASMA)	1/1000	DakoCytomation, Heverlee, Belgium
Desmin	Marker of Fat storing (stellate) cell Knittel et al., 1999	D33	1/100	Dako, Trappes, Frances
Endothelial nitric oxide synthase (eNOS)	It has an important role in maintaining normal hemodynamics and vascular tone. Lin et al., 2004	(Polyclonal antibody)	1/100	Abcam, Cambridge, MA

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were measured in 30 fields (X40 objective lens and X10 ocular lens) in each rat liver.

Small pieces of the liver (1 mm) were fixed in 4% glutaraldehyde, postfixed in osmium tetroxide, processed and embedded in Epon. Semithin sections (0.5-1 mm) were stained with toluidine blue and

examined by a light microscope. Ultrathin sections (500-800 Å) of the glutaraldehyde-fixed specimens were counterstained with uranyl acetate and lead citrate (Reynolds, 1963) and examined with the transmission electron microscope (TEM) (JEM-100 Cx11, JEOL, Egypt) belonging to the TEM Unit of Assiut University,

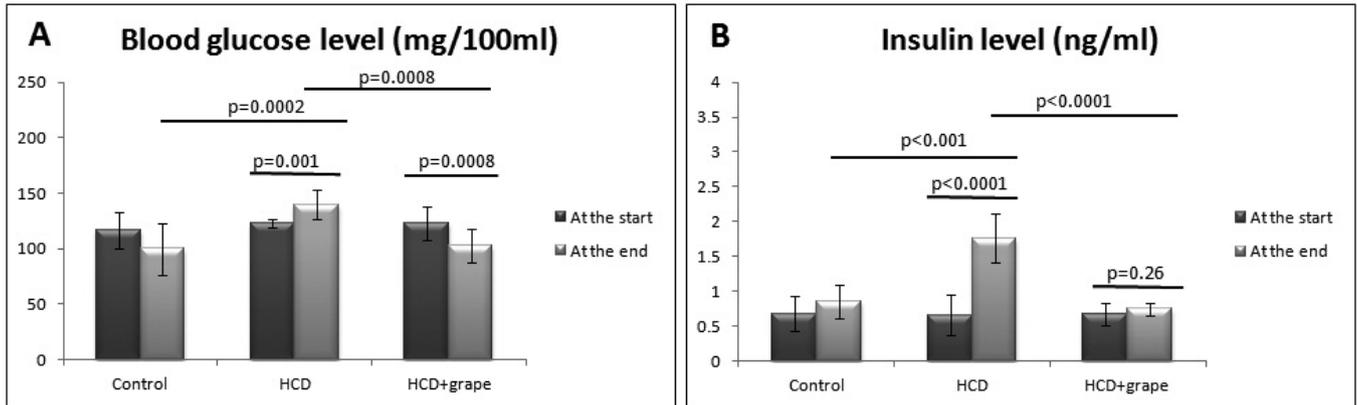


Fig. 1. Effect of administration of RGJ simultaneously with the HCD on blood glucose and insulin level. Values are means \pm SD, $P < 0.05$.

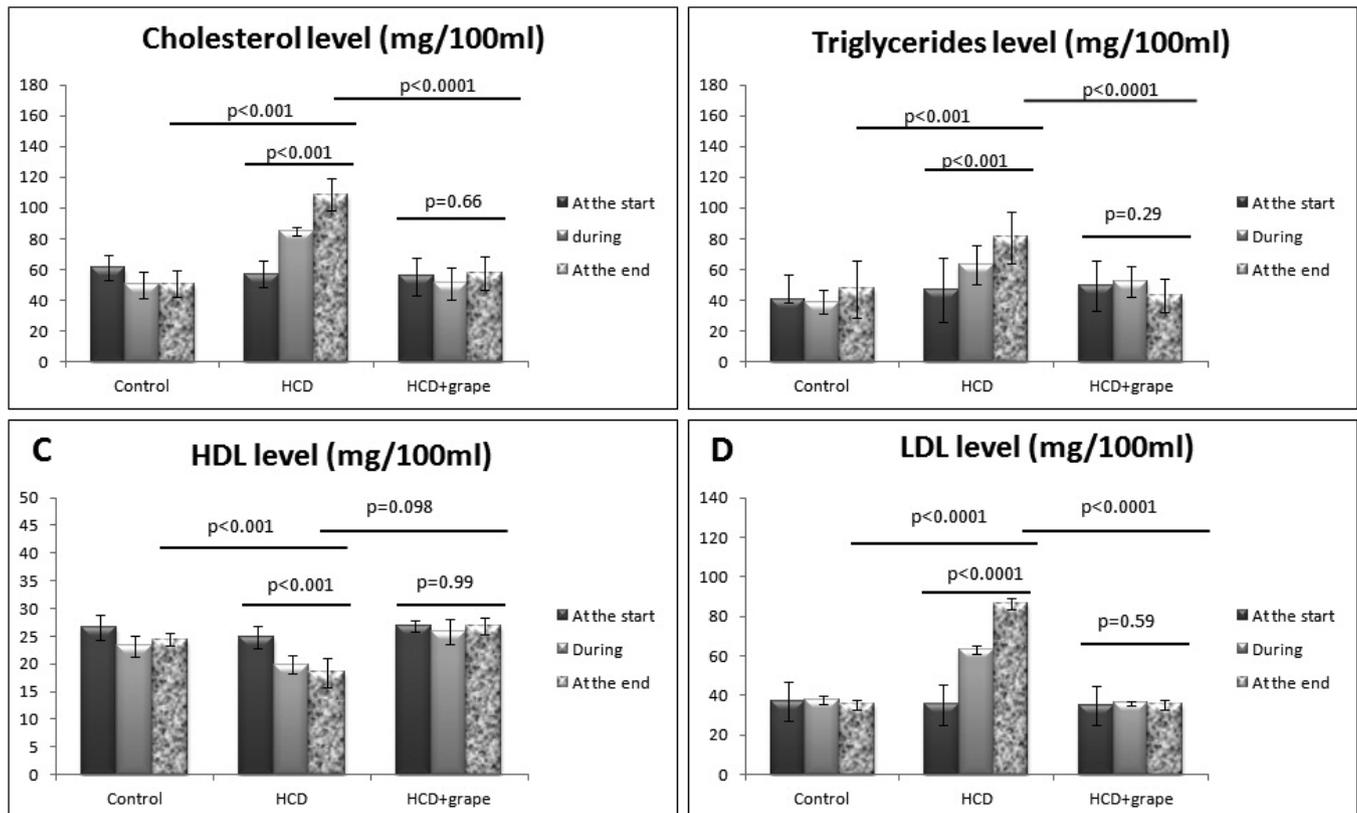


Fig. 2. Effect of administration of RGJ simultaneously with the HCD on blood cholesterol, triglycerides and Low Density Lipoprotein (LDL) and (High Density Lipoprotein) HDL levels. Values are means \pm SD, $P < 0.05$.

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Immunohistochemistry

Immunohistochemical stains were performed on neutral-buffered, formalin-fixed, paraffin-embedded tissue sections (4 μ m). The standard immunohistochemistry staining procedure was performed as described previously (Lin et al., 2004; Knittel et al., 1999; Seki et al., 2002). Briefly, deparaffinisation was performed using xylene and ethanol. The antigens were retrieved by boiling the tissue slides with 0.01 M citric buffer in a microwave for 5 min. Hydrogen peroxide was used to quench the endogenous peroxidase activity. After blocking with 10% serum-Tris buffer for 20 min at room temperature, the sections were incubated with the primary antibody at room temperature for 120 min (Table 1). Corresponding biotinylated conjugated secondary antibody from the Dako staining system was used. Slides stained with secondary antibody only were used as negative controls. The nuclei were counterstained with haematoxylin.

Statistics

Data were analysed using the statistical package for the social sciences (SPSS) version 16. For nonparametric data, analysis of variance (ANOVA) and the Kruskal-Wallis test, followed by a posthoc test (based on Dunn's procedure), were used to analyse each pair of groups and thereby avoid the multiple-comparison effect. For the parametric data, the different groups were compared using ANOVA (*f* test), followed by Bonferroni's posthoc test. A P value less than 0.05 was considered to be significant.

Results

Biochemical results

The chemical composition of red grape used in the study was analysed. It was found that 100 g of RGJ contained a large amount of water, carbohydrates and few proteins or fatty acids. The RGJ also contained significant amounts of potassium, vitamin E, vitamin B3

Table 2. The component concentrations of Red grape used in the study:

Element	amount	Element	amount
Water (g/100g)	80.52	Magnesium (g/100g)	0.002
Protein (g/100g)	0.46	Phosphorus (g/100g)	0.065
Carbohydrates (g/100g)	28.37	Vitamin E (g/100g)	0.17
Fibers (g/100g)	0.96	Vitamin B1 (mg/100g)	0.09
Mono-unsaturated fatty acids (g/100g)	0.055	Vitamin B2 (mg/100g)	0.053
Poly-unsaturated fatty acids (g/100g)	0.034	Vitamin B3 (mg/100g)	0.28
Cholesterol (g/100g)	-	Vitamin C (g/100g)	0.006
Iron (g/100g)	0.0002	Phenol compounds (g/100g)	9.37
Potassium (g/100g)	0.15	Flavones (g/100g)	0.042
Calcium (g/100g)	0.01	Anthocyanin (mg/100g)	0.345

Table 3. comparison between control, HCD and HCD plus RGJ groups regarding body weight, food and water intake, plasma/serum laboratory parameters, and hepatocyte size.

	Control	HCD	HCD+RGJ
Weight at Day 0 (g)	240.6±14.4	231±18.9	245±18.1
Weight at the end of 13th week (g)	376±14.9	418.6±32.5 P<0.0001 P#<0.0001	405.8±8.6 P1=0.015 P#<0.0001
Water and Juice intake (ml/d)	286.5±13.5	247±21.3	354.8 ±337
Food intake (g/d)	2046±106	1831±142 P<0.0001	1750±102 P1=0.005
*Weight gain (g)	56.9±11.4	81.8±15.8 P<0.0001	66.2±10.5 P1<0.0001
Food efficacy	0.028	0.045	0.038
**Relative weight of liver	2.85±0.37	4.01±0.95 P<0.0001	3.65±0.36 P1=0.03
Albumin in blood (g/L)	9.5±0.57	10.7±0.58 P<0.0001	8.5±0.7 P1<0.0001
AST (U/L)	148.8±42.05	137±60.9 P=0.34	150.5±21.9 P1=0.19
ALT (U/L)	76.5±7.9	70.6±4.2 P=0.06	71.3±3.4 P1=0.07
Alkaline phosphate in blood (U/L)	70±18	94.6±16.8 P<0.0001	57±1.4 P1<0.0001
Area of hepatocytes (μ m ²)	132.2±16.3	420.8±49.4 P<0.0001	382.9±27.03 P1<0.0001
Circumference of hepatocytes (μ m)	41.6±3.11	82.7±1.7 P<0.0001	75.6±1.2 P1<0.0001

*Food efficacy: weight gain/food intake. ** Relative weight of liver: weight of the kidney/body weight. AST, aspartate aminotransferase; ALT, alanine aminotransferase. P HCD versus control. P1 HCD versus HCD/grape juice. P# weight at the start versus weight at the end of the experiment. Significance was considered at p<0.05

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and a small amount of calcium and phosphorus (Table 2).

The weight gain and food intake of the rats that received the HCD was significantly higher compared to the control, and these were significantly reduced in the treated group fed the HCD and the RGJ. The relative weight of the liver of HCD-fed rats was significantly

higher compared to that of the control ($p < 0.0001$) and the rats fed the HCD plus the RGJ ($p = 0.03$) (Table 3). The HCD significantly increased both levels of albumin and alkaline phosphatase compared to the control, and these were significantly reduced by the administration of the RGJ simultaneously with the HCD. Levels of Aspartate Aminotransferase (AST) and Alanine

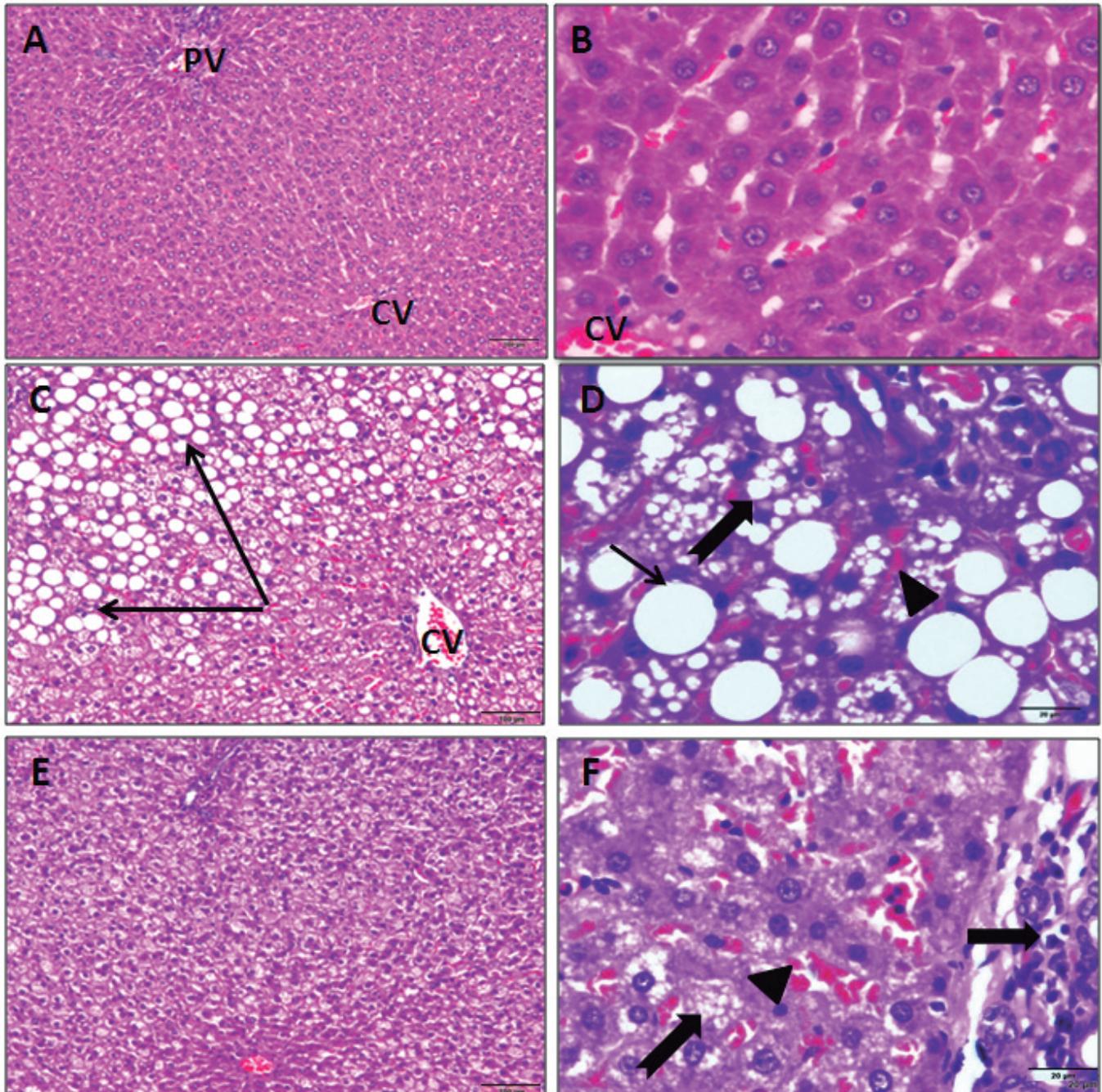


Fig. 3. Liver of control rat (A, B) showing normal structure of the hepatic lobule. Liver of hypercholesterolemic rat (C-F) showing macrovesicular (thin arrow) and microvesicular (bifid arrow) steatosis, congested blood sinusoids (arrow head) and inflammatory cell infiltrate in the portal area (thick arrow). portal vein (PV), central vein (CV). H&E. A, C, E, x 200; B, D, F, x 1000

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Aminotransferase (ALT) showed no significant changes in response to the HCD (Table 3). The blood glucose and insulin levels of the rats fed the HCD were significantly increased at the end of the experiment compared to their starting level and to the control. At the end of the experiments, the blood glucose and insulin levels of the rats fed the HCD and the RGJ had significantly decreased compared to those fed the HCD alone (Fig. 1).

The rats fed the HCD showed a significant increase in their blood cholesterol, triglycerides and Low Density Lipoprotein (LDL) levels at the end of the experiment compared to their starting levels and to the levels of the control. The levels of these lipids were significantly decreased at the end of the experiment in the rats fed the HCD plus the RGJ compared to the group fed the HCD alone (Fig. 2). In addition, the blood High Density

Lipoprotein (HDL) level of HCD-fed rats showed a significant decrease compared to its starting level and the level of the control group and it was significantly increased in the rats fed the HCD plus the RGJ compared to those that received the HCD alone (Fig. 2).

Histological findings

The liver of the hypercholesterolemic rats showed some hepatocytes with a signet-ring appearance due to the coalescence of large fat droplets (macrovesicular steatosis), and others showed multiple small lipid droplets (microvesicular steatosis). The former type was prominent in the periportal area. There was inflammatory infiltrate near the portal area, and the blood sinusoids appeared congested. Variation among

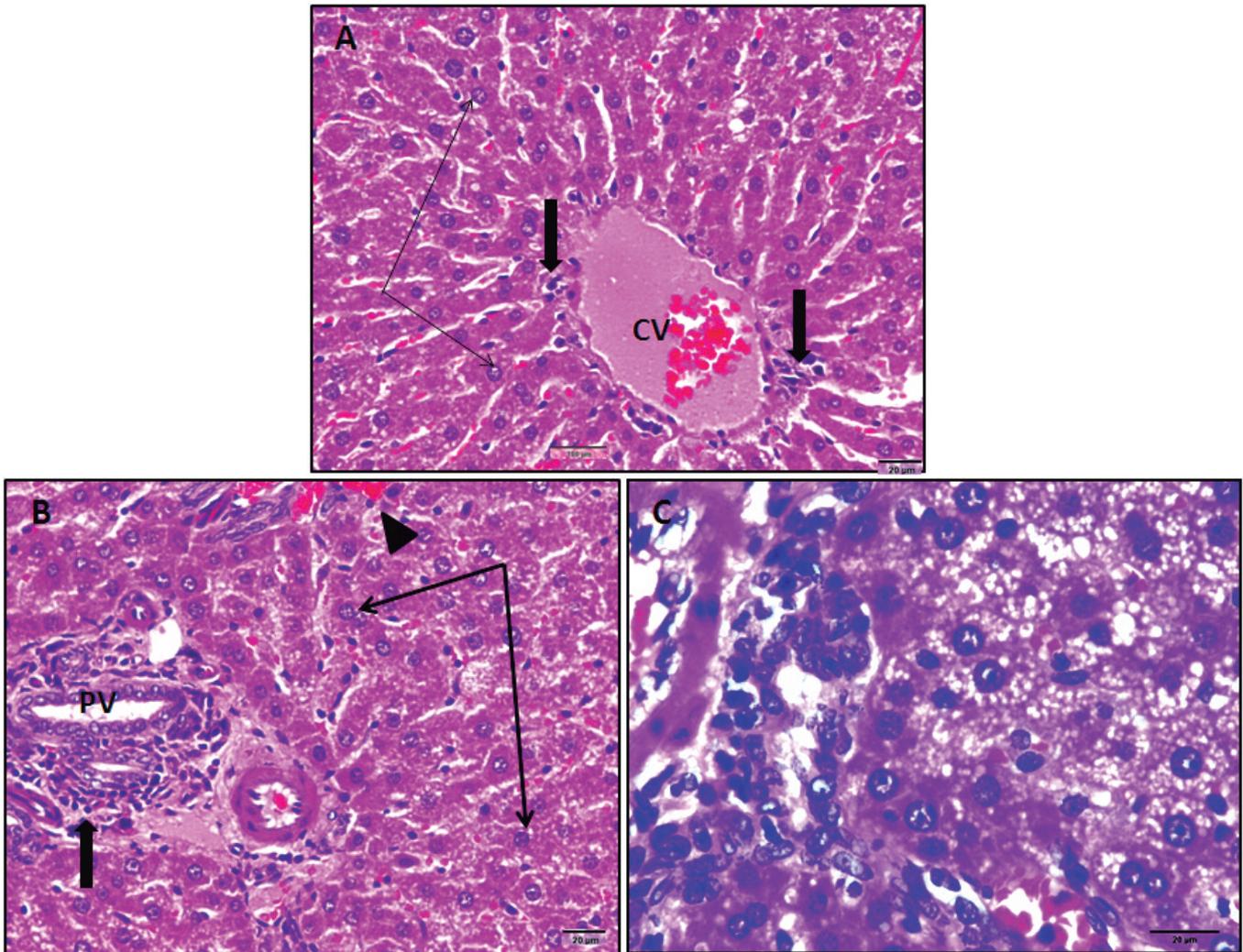


Fig. 4. Liver of hypercholesterolemic rat treated with RGJ showing the central (A) and peripheral (B) regions of the hepatic lobule. Hepatocytes with vesicular nuclei (thin arrow), some congested blood sinusoids (arrow head), lymphocyte infiltrate around PV and CV (thick arrows) and few lipid droplets (bifid arrow) are observed. portal vein (PV), central vein (CV). H&E. A, B, x 400; C, x 1000

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the individual animals existed, but within each liver, the variation was minor (Fig. 3). Most of the hepatocytes were enlarged, and some were ballooned, as evidenced by a significant increase in their area and circumference (Table 3). The livers of the hypercholesterolemic rats

treated with RGJ showed microvesicular steatosis, congested blood sinusoids and lymphocytic infiltration but to a lesser extent (Fig. 4).

The livers of the hypercholesterolemic rats showed a marked increase in collagen fibres around the portal area

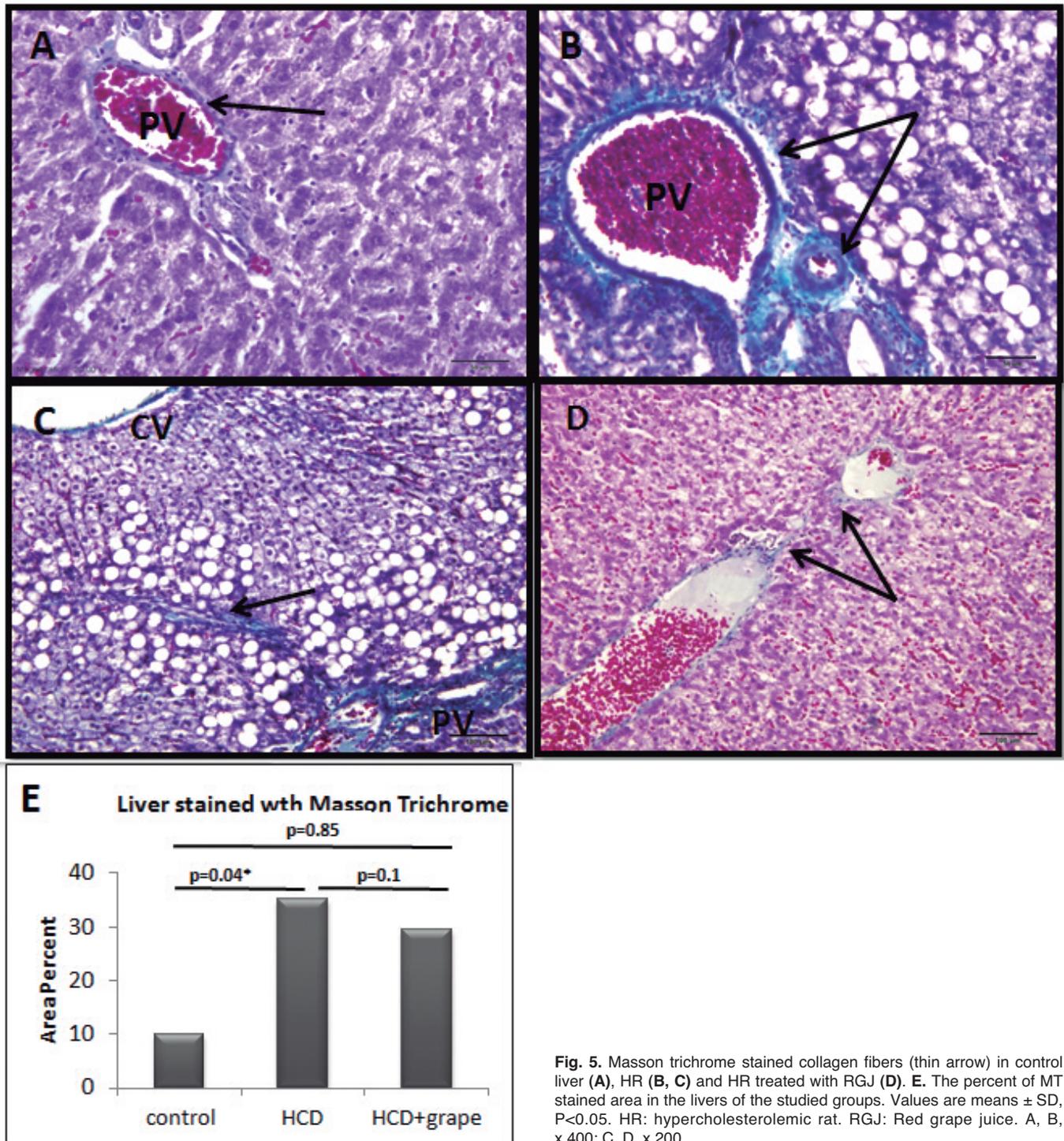


Fig. 5. Masson trichrome stained collagen fibers (thin arrow) in control liver (A), HR (B, C) and HR treated with RGJ (D). **E.** The percent of MT stained area in the livers of the studied groups. Values are means \pm SD, $P < 0.05$. HR: hypercholesterolemic rat. RGJ: Red grape juice. A, B, x 400; C, D, x 200

compared to the control. The HCD-fed rats treated with the RGJ showed a moderate amount of collagen fibres around the central vein and the portal area. Semi-quantitative assessment of the area percentage of MT-stained collagen fibres showed a significant increase in HCD-fed rats compared to the control. The treatment with the RGJ did not result in a significant reduction ($P=0.1$) in the amount of collagen fibres (Fig. 5).

The examination of the toluidine blue-stained sections of the hypercholesterolemic livers showed many elongated cells near the blood sinusoids, with dark cytoplasm, oval nuclei and few lipid droplets. Fewer numbers of these cells were seen in the livers of the RJG-treated group. Many mast cells with violet granules were observed in the periportal area of the hypercholesterolemic livers (Fig. 6).

Electron microscopic findings

The livers of the control rats showed normal hepatocytes and occasionally perisinusoidal fat-storing cells (FSCs) with a small rounded nucleus, multiple lipid droplets and few organelles (Fig. 7). The hepatocytes of the HCD-fed rats contained many different sized lipid droplets. Some of the hepatocytes had small nuclei, with dense peripheral chromatin, cytoplasmic vacuoles and swollen mitochondria. Some FSCs, which were recognised by their character (having many small lipid droplets) and their location near the blood sinusoids, had an oval nucleus, with an irregular, dilated nuclear membrane and few lipid droplets, mitochondria and microfilaments. Another type of peri-sinusoidal cell, Von Kupffer cells, were also observed, with many lipid

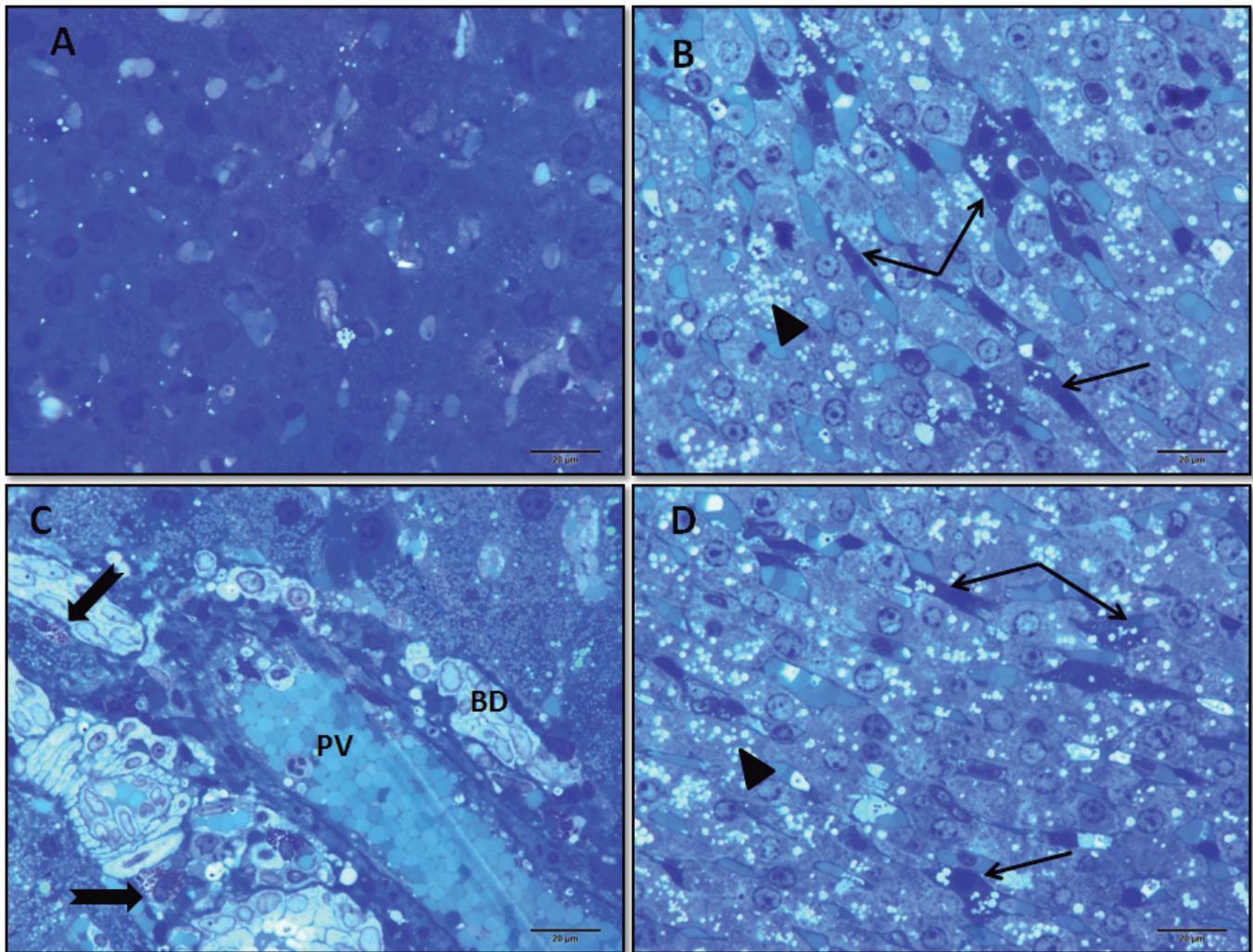


Fig. 6. livers of control rat (A), HR (B, C) and HR treated with RGJ (D) showing hepatocytes with lipid droplets (arrow head), elongated cells (thin arrow) with dark cytoplasm, the mast cells (thin arrow) in periportal area. HR: hypercholesterolemic rat. portal vein (PV), BD: bile ducts. Semithin stained with toluidine blue. x 1000

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droplets and many phagolysosomes, some of which contained myelin figures (Fig. 8).

The hepatocytes of the RGJ-treated group showed fewer lipid droplets compared to the hypercholesterolemic livers, and most of them possessed vesicular nuclei with euchromatin, intact rER and mitochondria. The elongated FSCs with oval nuclei and few lipid droplets were also less frequently observed in the livers of the HCD plus RGJ group (Fig. 9).

Immunohistochemical findings

The livers of the control rats showed negative

ASMA expression in the parenchyma and positive expression in the wall of the portal and central vein. The hypercholesterolemic liver showed strong ASMA expression along the walls of the sinusoids, as well some positive parasinusoidal cells. Semiquantitative assessment showed a significant increase ($P=0.04$) in the area percentage of ASMA expression in the hypercholesterolemic livers compared to the control. The treatment with RGJ induced a non significant ($P=0.3$) decrease in the expression of ASMA compared to that of the hypercholesterolemic rats (Fig.10A-D).

Moderate expression of desmin was observed in some perisinusoidal cells in the control livers. The

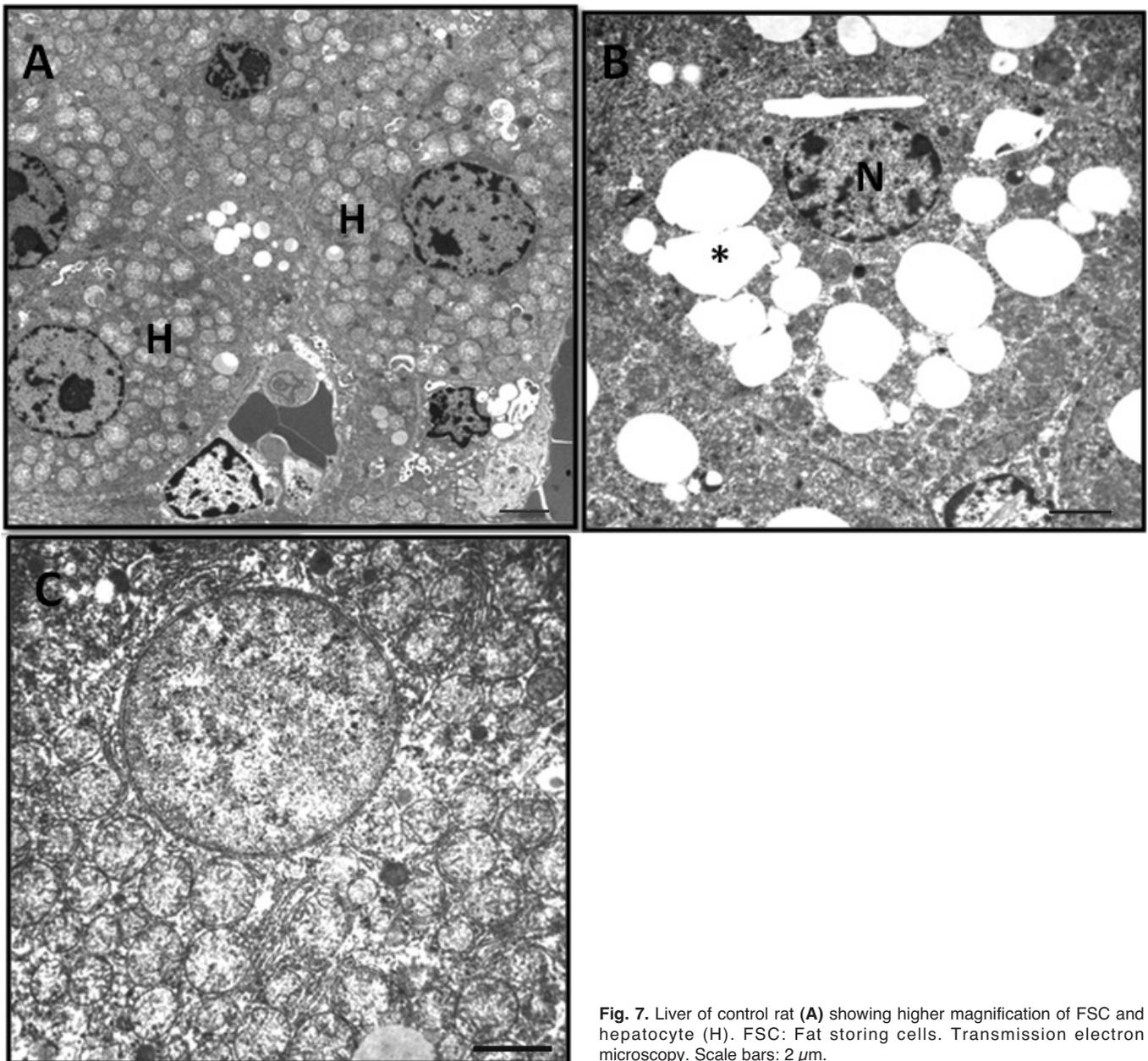


Fig. 7. Liver of control rat (A) showing higher magnification of FSC and hepatocyte (H). FSC: Fat storing cells. Transmission electron microscopy. Scale bars: 2 μ m.

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hypercholesterolemic livers showed an increase in desmin expression, although the semiquantitative analysis showed that this increase was not significant ($P=0.04$) (Fig. 10E-H).

The livers in the control group showed moderate eNOS expression in the endothelial cells lining the blood sinusoids and central vein, whereas the hypercholesterolemic livers showed weak eNOS expression in

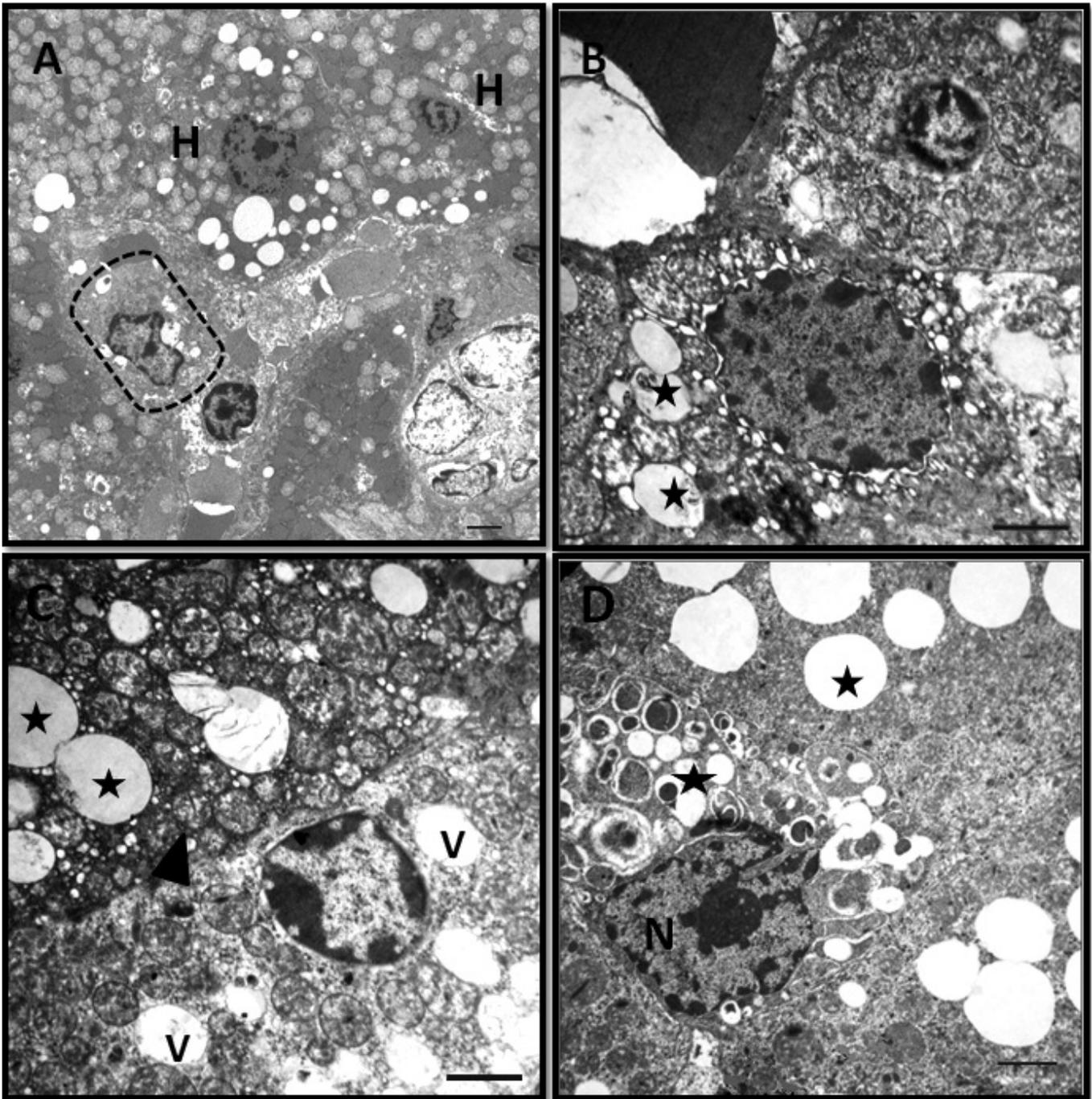


Fig. 8. Liver of HR (A) showing hepatocytes (H) with many lipid droplets. The interrupted line shape indicates FSC. A higher magnification (B) of FSC with few lipid droplets (star). C. Two adjacent hepatocytes with lipid droplets (black star) and cytoplasmic vacuoles (V). D. Perisinusoidal cell (mostly Von Kupffer) with many lipid droplets. HR: hypercholesterolemic rat. Transmission electron microscopy. Scale bars: 2 μ m.

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the same areas, while those of the hypercholesterolemic rats fed the RGJ showed moderate to weak expression. There was a significant decrease in the area percentage of eNOS expression in the livers of the hypercholesterolemic rats compared to the control. It was increased in the livers of the rats fed the RGJ, but the increase was not significant increase (Fig. 10I-L).

Discussion

Although some studies had reported a beneficial effect of flavonoid- or phenolics containing food on lipid

abnormalities and related hepatic injury in cholesterol-fed animals, the present study included, in addition to the biochemical investigation, an in-depth evaluation of the hepatic histopathological changes induced by HCD and the ameliorative effect of RGJ.

Red grape juice significantly decreased HCD-induced weight gain. In a previous study red grape extract, soy isoflavone and L-carnitine (RISC) substantially inhibited high-fat diet (HFD)-induced increase in body weight and down-regulated plasma leptin levels (Kang et al., 2011). As expected, plasma cholesterol levels were significantly increased after HCD

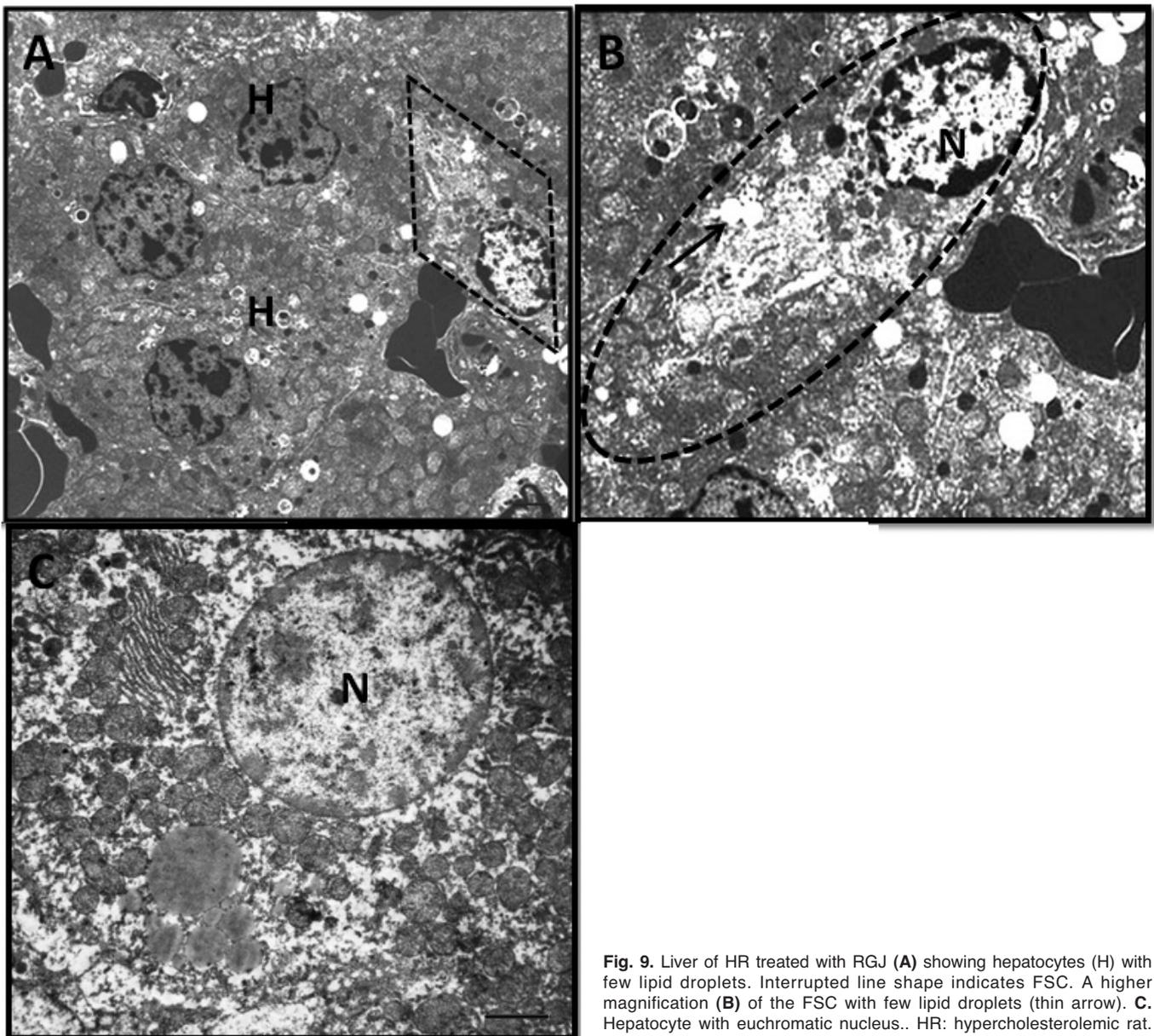


Fig. 9. Liver of HR treated with RGJ (A) showing hepatocytes (H) with few lipid droplets. Interrupted line shape indicates FSC. A higher magnification (B) of the FSC with few lipid droplets (thin arrow). C. Hepatocyte with euchromatic nucleus. HR: hypercholesterolemic rat. Electron microscopy. Scale bars: 2 μ m.

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ingestion. It was observed that RGJ significantly reduce cholesterol levels. In a previous study, RGJ hypolipidemic effect was reported in haemodialysis-receiving humans (Castilla et al., 2006). This hypolipidaemic effect of grape was attributed to its polyphenols that interfere with cholesterol absorption, decrease hepatic cholesterol concentrations (Zern and Fernandez, 2005), reduce circulating levels of LDL

cholesterol and increase the LDL receptor activity (Dávalos et al., 2006). In this study, RGJ significantly decreased the HCD-induced elevated blood glucose and insulin levels and this could be attributed to the resveratrol component of the RGJ (Baur et al., 2006; Bujanda et al., 2008; Shang et al., 2008) or the grape seed extract procyanidin (Decorde et al. 2009) as proposed in previous studies. In addition, diabetics given

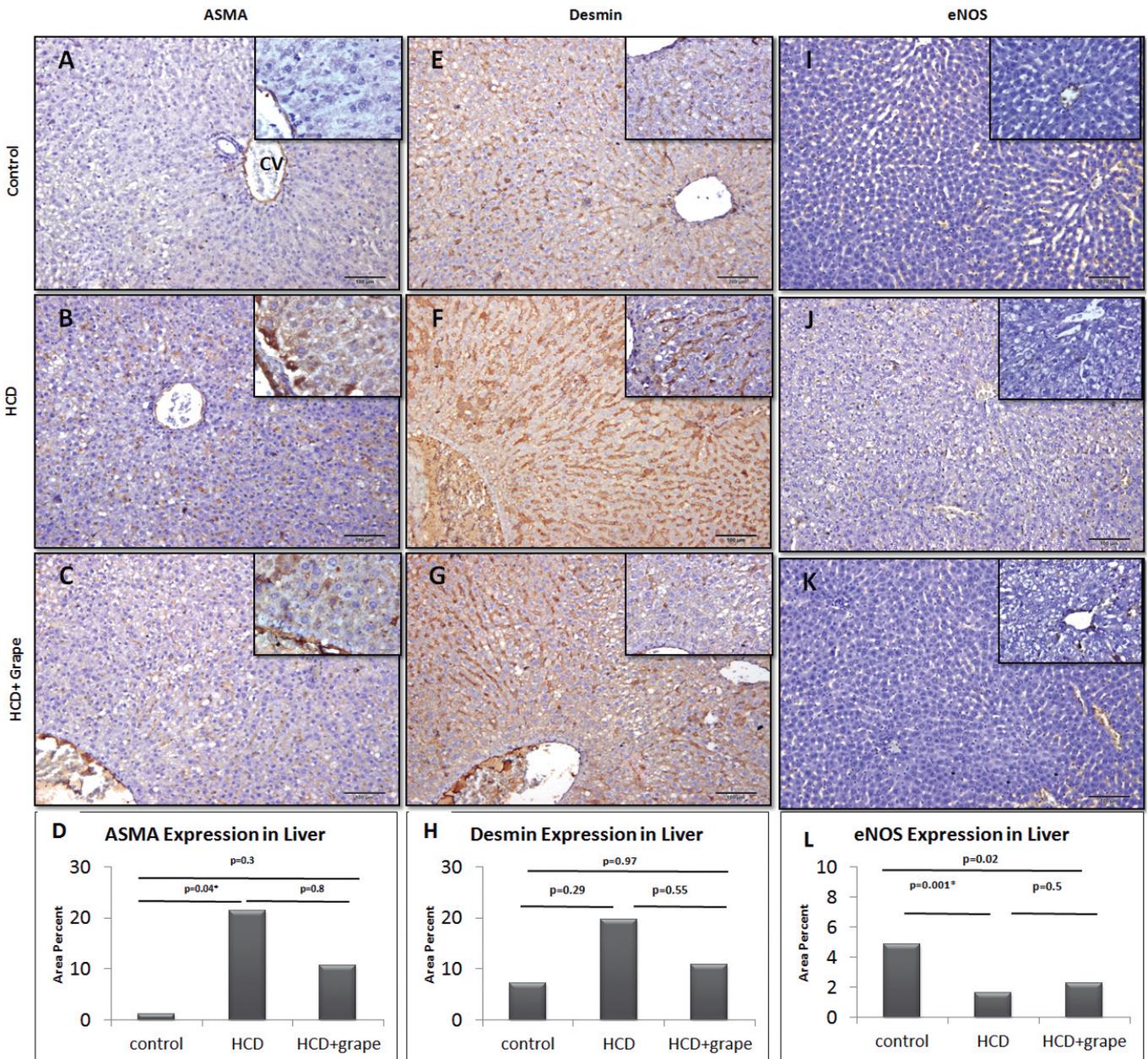


Fig. 10. immunoeexpression of Alpha Smooth muscle actin (ASMA), desmin and endothelial nitric acid synthetase (eNOS) in livers of control rats (A, E, I) hypercholesterolemic rats (B, F, J) and hypercholesterolemic rats treated with grape juice (C, G, K). Area percent of ASMA (D), desmin (H) and eNOS (L) immunoeexpression in the studied groups. Values are means ±SD, P <0.05. Immunohistochemistry. x 200; inserts, x 1000

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muscadine grape wine, and dealcoholized muscadine grape wine showed lower levels of blood glucose, insulin, and glycated hemoglobin, indicating better glycemic control (Banini et al., 2006).

Regarding the liver function, the HCD did not affect the levels of AST and ALT and this finding is consistent with that of Kainuma et al. 2006 in their study of cholesterol-fed rabbits. Albumin level was elevated in HCD group and this was reported previously by (Baur et al., 2006; Bujanda et al., 2008). Improved hepatic conditions were noted by Banini et al. among subjects with type-2 diabetes given muscadine grape wine, indicating better insulin sensitivity and decreased tendency toward impaired liver function (Banini et al., 2006). RGJ administration, in our study, was associated with decreased albumin level and this was observed by Baur et al. (2006) and Milne et al. (2007) after resveratrol administration. They added that both number and power of mitochondria had increased after resveratrol administration. This result might be a consequence of engagement of hepatocytes in restoring the number and capacity of the mitochondria rather than excreting albumin.

In the present study, both micro/macrovacular steatosis were observed in HCD-fed rats with the microvesicular variety more prominent. Macrovesicular steatosis was observed in the periportal areas together with moderate to marked periportal fibrosis. This was in line with Jeong et al. (2005) observations during their study on rats fed a sodium cholate diet. Kainuma et al. 2006 had studied an experimental cholesterol-fed rabbit model of nonalcoholic fatty liver disease (NAFLD)/nonalcoholic steatohepatitis (NASH). In this model Kainuma et al. observed the microvesicular fat deposition more predominantly than the macrovesicular variety in the periportal area. In addition, HSC activation, slender fibrosis that extended to form bridging in some places together with a slight infiltration of neutrophils was also observed. In another animal model of NAFLD, an obvious ballooning degeneration without fibrosis was observed by Shang et al., 2008. In Bujanda et al. a rat model of NAFLD induced by alternative cycles of fasting/feeding with a high carbohydrate-fat free diet, fat deposit was classified as macrovesicular (grade 3). They did not report or exclude occurrence of fibrosis in their model (Bujanda et al., 2008). Although it was statistically insignificant, RGJ induced reduction in the HCD-associated fibrosis. In a previous study, grape skin and seeds reduced Dimethylnitrosamine-induced collagen accumulation in the liver (Shin and Moon, 2010). Jie et al. (2012) also reported amelioration of Thioacetamide-induced mouse hepatic fibrosis after Grape Seed Proanthocyanidin Extract (GSPE) administration.

An impaired dilator reactivity of the blood vessels in hypercholesterolemic mice as compared to responses in control animals was recorded by Wolfle and de Wit (2005). They attributed this to either deficits in NO

production or increased oxidant scavenging. The first explanation was confirmed by our study, as expression of eNOS in livers of HCD-fed group was significantly reduced. Grape polyphenols was reported to have vasorelaxant effects (Zenebe et al., 2003) and that they improve endothelial function (Stein et al., 1999). This could explain the increased eNOS expression in the endothelial cells lining the hepatic blood sinusoids of RGJ-treated group.

α -SMA expression, as well as up-regulated desmin expression, indicated FSCs activation into myofibroblast-like (Gressner et al., 1992; Washington et al., 2000). According to that, hypercholesterolemic livers showed evidence of FSCs activation. The activated FSCs corresponded to the elongated cells observed along the blood sinusoids in the semithin and the ultrathin sections and this was consistent with that described by Buysens et al. (1996), who added that cholesterol overload can activate FSCs into fibrogenic effector cells. Grape skin and seeds were found to reduce hepatic stellate cell activation induced by dimethylnitrosamine in rats, as was assessed by α -smooth muscle actin staining (Shin and Moon, 2010) and this effect was observed in our study. The hepatocytes of HCD-fed rats showed cytoplasmic vacuoles and swollen mitochondria, and the Von Kupffer cells exhibited many myelin figures. This damage of cell organelles could be attributed to the excessive production of reactive oxygen species caused by the hypercholesterolemic diet, with subsequent impaired protein stability and membrane destruction via lipid peroxidation (Browning and Horton, 2004; Kainuma et al., 2006) or to the diminished effectiveness of the antioxidant defence system and the decrease in the activities of Catalase and Superoxide Dismutase observed in hypercholesterolemic rats (Fkia et al., 2005). Restoration of such an antioxidant defence mechanism might be responsible for the improvement observed in the hepatocytes organelles status, as well as the reduction of the activated FSCs number observed in RGJ-treated group. It is supported by the finding of Rho and Kim (2006) who reported that grape intake, especially grape pomace, had a prominent antioxidative capacity promoting liver and red blood cell antioxidant enzyme activities.

In conclusion, the administration of RGJ succeeded in alleviating the biochemical and, to some extent, the histopathological changes induced by the high cholesterol diet. It decreased the activation of fat storing cells into myofibroblasts and partially restored the eNOS activity in the liver microvasculature. Consumption of fresh RGJ or its pharmaceutical preparations is advised especially for those who are used to eat high fat diet.

Acknowledgements. This paper was funded by the Deanship of Scientific Research (DSR), King Abdulaziz University, Jeddah under grant No. 116-130-D1432. The authors, therefore, acknowledge with thanks the DSR for technical and financial support.

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Accepted March 18, 2014