

Histological Effect of Ginkgo Biloba on Liver and Heart of Adult Male Albino Rat

Original
Article

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ABSTRACT

Background: Ginkgo biloba (EGb) is a dietary supplement used for various purported therapeutic benefits. It contains powerful antioxidants, can help to fight inflammation, induces psychic disorders, and improves brain function. Some researchers recommended using Ginkgo Biloba as a protective agent.

The Study Aims: Are to evaluate the histological effect of chronic administration with different doses of Ginkgo Biloba on the liver and heart of adult male albino rats.

Material and Methods: The study was performed on a total number of 30 adult male albino rats; weighing 170–200 g. Rats were divided into three equal groups (10 rats each) and treated once a day. Group I (control group; n=10), each one received distilled water (1ml/day). Group II (low dose group; n=10): received EGb at a dose of 100 mg/kg BW. Group III (high dose; n=10): received a dose of EGb 200 mg/kg BW; orally by daily gavage for 4 weeks. Rats were sacrificed and prepared for histological study.

Result: In specimens of liver tissue, there were mild degenerative changes. In a low dose, the changes were in the form of cytoplasmic vacuolation and nuclear condensation. In the high dose of Ginkgo biloba, there were degenerative changes of the hepatic parenchyma varied from mild (m) degree to extensive (e) degrees. In the specimens of cardiac tissue, there were cytoplasmic vacuolations and atrophy of cardiomyocytes especially in group III, degenerative changes of the coronary artery occurred, and degeneration of the Purkinje cell fibers of the entire ventricular conducting system.

Conclusion: administration of Ginkgo biloba has degenerative effects on the liver and heart particularly in high doses

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INTRODUCTION

Ginkgo Biloba leave extract is the most widely herbal treatment. Its helps in scavenging free radical; lowering oxidative stress; and in reducing neural damages. Also, it has an antitumor effect and helps in the improvement of brain functions^[1]. GBE has also been used for the treatment of several cardiovascular diseases^[2]. Most of the studies have attributed the cardioprotection of GBE to enhanced antioxidant activity^[3]. The active constituents of Ginkgo Biloba extract (EGb761) exert their effects through interaction with multiple molecular mechanisms and signaling pathways. An The extracellular signal-regulated kinase (ERK1/2) signaling and cell cycle control gene-dependent regulation has been proposed in gastric cancer^[4]. Due to its anti-oxidant and cytoprotective properties, it is currently one of the most widely used botanical compounds worldwide. It is administrated for the prevention and treatment of a variety of diseases such as cognitive function disorders, peripheral blood flow insufficiency, tinnitus, and vertigo^[5]. Ginkgo biloba partly promotes anti-oxidative effects by inducing Keap1-Nrf2-signaling (which is the major regulator of the cytoprotective

responses to oxidative stress)^[6]. Several reports indicate that Ginkgo Biloba extract (EGb761) has anti-tumorigenic properties in a variety of tumors, including hepatocellular carcinoma (HCC)^[7]. The aim of this study is to determine the effect of chronic administration of Ginkgo Biloba on the liver and heart.

MATERIAL AND METHODS

Chemicals

In the present study ginkgo Biloba leaf powder extract (EGb) was obtained in the form of gelatin capsules manufactured by EIMC United Pharmaceuticals (for EMA Pharm Pharmaceuticals, Egypt) (24% ginkgo flavones glycosides - 6% total ginkgolides; lactones).

Animals

The study was performed on a total number of 30 adult male albino rats; weighing 170–200 g each. The animals were housed in separate cages (five rats per cage) under standard laboratory and environmental conditions with free access to food (commercial rat food) and tap water. They were obtained from the Animal House of the Faculty

of Medicine, South Valley University. Animal handling and treatment were carried out following The Laboratory Animal Protection Law proposed guidelines and protocols.

Experimental design

The rats were divided into three equal groups (10 rats each) and treated once a day, as follows:

1. Group 1 (control group; n=10): received distilled water (1ml/rat) orally by daily gavage for 4 weeks.
2. Group 2 (low dose; n=10): received EGb at a dose of 100 mg/kg; orally by daily gavage for 4 weeks. This dose is equivalent to the human therapeutic dose.
3. Group 3 (high dose; n=10): received a dose of EGb 200 mg/kg; orally by daily gavage for 4 weeks.

Twenty-four hours after the last dose, rats were anesthetized by intraperitoneal injection of 50 mg/kg sodium pentobarbital (Liu *et al.*, 2007)^[6] then they were sacrificed by decapitation.

Histological study

Animals were processed for electron microscopy. Using a sharp knife, each tissue was cut in a sagittal plane into two halves and small cubes of the left ventricle and liver were taken and kept in 5 % glutaraldehyde in sodium cacodylate buffer at 4 C for 2-48 hours. Samples were washed in sodium cacodylate buffer at ph 1.5 for three changes, 20 minutes each. Then samples were postfixed in 1% osmium tetroxide for two hours and were washed in four changes of sodium cacodylate buffer, 20 minutes each. Samples were dehydrated in ascending grades of alcohol as follows:30% for 30 minutes, 50% for 30 minutes, 70% overnight in a refrigerator, 90% for 30-60 minutes, and then in 100% twice 30 minutes each. Samples then were put in the following solutions: Propylene oxide for 30-60 minutes,propylene oxide placed in epons – Araldite formulation (1:1) overnight, pure epon – Araldite formulation for 30-60 minutes. The tissues were embedded in a pure epon Araldite mixture for 24 hours and then, tissue blocks were polymerized in an oven at 35c for 24 hours, at 45c for 24 hours and at 60c for 24 hours. Sections were cut at 1.0 um and specimens were trimmed with a razor blade, and stained with a 2 % aqueous toluidine blue then dried on a hot plate at 40 c centigrade (Ayache *et al.*, 2010)^[15]

Presentation of data

In liver specimens data was presented in the form photomicrographs comparing the treated specimens with the control group. The transverse diameter of each central vein of hepatocytes (the minimal diameter is defined as the closest possible distance between the two parallel tangents of an object) was measured. In muscle specimens data was presented in the form photomicrographs comparing the treated specimens with the control group. The transverse diameter of each of muscle fiber was measured in at least

5 muscle fibers in 5 bundles in 5 slides in 5 different animals or rats. Mean, standard deviation, and p-value were measured by SPSS version 23. ANOVA (Analysis Of Variance) test: It was used when comparing the means of these variables between different groups. The significance was measured according to the level of significance.

(*P value*) as follows:

- $P > 0.05$ (NS) No significant difference
- $P < 0.05$ (*) Significant.
- $P < 0.01$ (**) moderately significant.
- $P < 0.001$ (***) highly significant.

RESULTS

Histopathological results:

A1: Examination of sections of liver

Group I

Examination of liver sections of the group I (control group) revealed normal liver architecture with normal central veins and radiating hepatic cords (Figure 1).

Group II

Examination of liver sections of the group II ((low dose group) revealed mild degenerative changes of the hepatic parenchyma by pale cytoplasm, nuclear condensation, and cytoplasmic vacuolation, increase the Kupffer cells in the blood sinusoids, activated Kupffer cells undergo hypertrophy, and acquired vascular cytoplasm and phagocytic inclusions (Figure 2)

Group III

Examination of liver sections of the group III ((high dose group) revealed degenerative (Figure 3), vascular changes (Figure 4) and fibrotic changes (Figure 5).

Degenerative changes varied from mild degree to extensive degrees as the presence of fatty droplets, vacuolation of the cytoplasm of hepatocytes, Activation, and hypertrophy of the Kupffer cells which contained phagocytic inclusions (Figure 3).

Vascular changes were in the form of cellular debris (fragments of the cytoplasm) in the perivascular space, activated Kupffer cells with phagocytic inclusions, vasculitis of the central vein, lymphoid cells in the blood sinusoids, and aggregation of activated Kupffer cell and lymphoid cells (arrowheads) in the space of Disse (Figure 4).

There was a fibrous tissue in the hepatic parenchyma which indicates fibrosis. Also, there was some lymphoid and necrotic cells which indicate the presence of an allergic reaction (Figure 5).

A2: Examination of sections of the heart

Group I

Examination of sections of the left ventricle of the group I (control group) revealed that the cardiac muscle fibers are branching and anastomosing with central, oval one or two nuclei, obvious transverse striations and dark intercalated disc (Figure 6).

Group II

During the administration of the low dose of the Ginkgo Biloba, cardiomyocytes mostly underwent mild degeneration that exhibited slight vacuolation (Figure 7A). Notable vacuolation was observed in some cardiomyocytes (Figure 7B) as well as extensive degeneration of the cardiomyocytes occurred that had lost most of the myofibrils (Figure 7F). Cardiomyocytes atrophied and had a pyknotic nucleus (Figure 7C). Mast cells and their secretion were detected in the subendocardium. Purkinje cell fibers underwent atrophy (Figures 7D, E).

Group III

During the administration of the high dose of the Ginkgo Biloba, mild (Figures 9F, 8A,D) to sever

(Figures 8B,E) degeneration of the cardiomyocytes. Cytoplasmic condensation and atrophy of the cardiomyocytes (Figure 8C). Degeneration changes of the coronary artery occurred (Figure 8F). Degeneration of the Purkinje cell fibers of the entire ventricular conducting system including Purkinje cell fibers of the subendocardial (Figure 9D), intramuscular (Figure 9C), and subepicardial regions (Figures 9A,B). Mast cells were identified in the subendocardial (Figures 9D,E) and intramuscular regions (Figure 9F).

Morphometric analysis

Diameters of the studied central vein in different groups show great variations. Compared to the normal groups, the variations ranged from narrowing of the central vein in group II and marked dilatation in the central vein in group III (Table 1, Figure10).

Diameters of the studied muscle cells in different groups show great variations. Compared to the normal groups, the variations were in the form of a mild increase in the transverse diameter of the muscle fibers and markedly increase in it in group III (Table 2, Figure11).

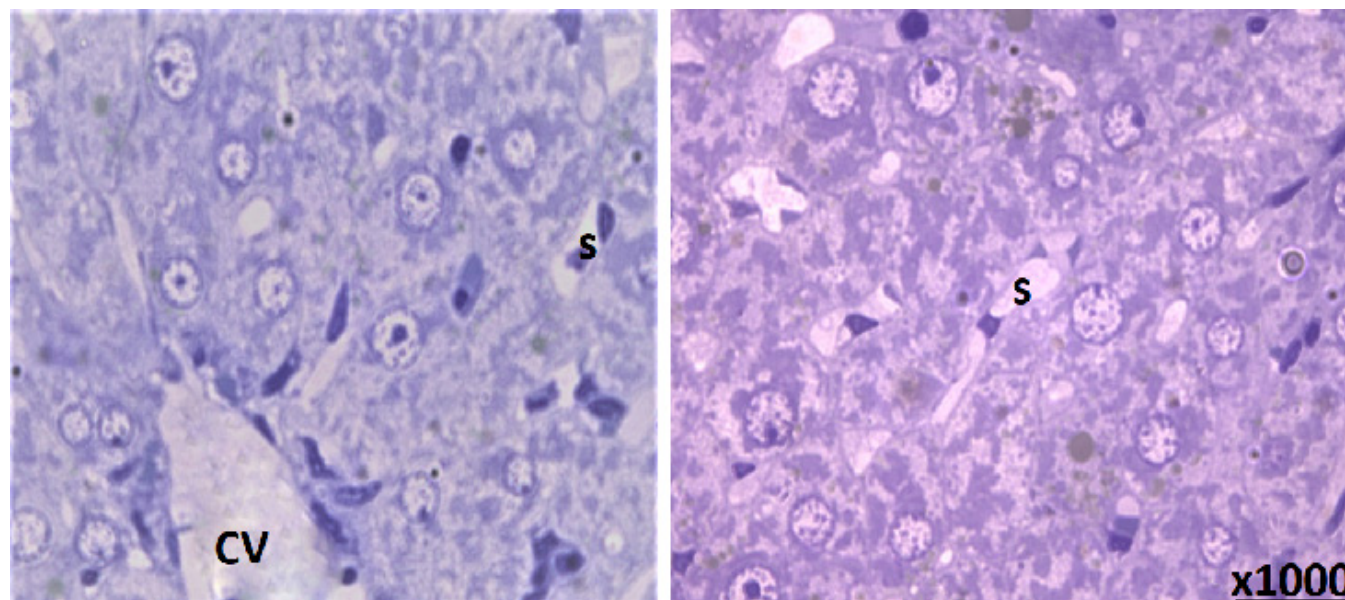


Fig. 1: Photomicrograph of sections of the liver of the group I stained with toluidine blue showing normal histological architecture. The cells appeared as cords radiating from central veins. Toluidine blue X 1000.

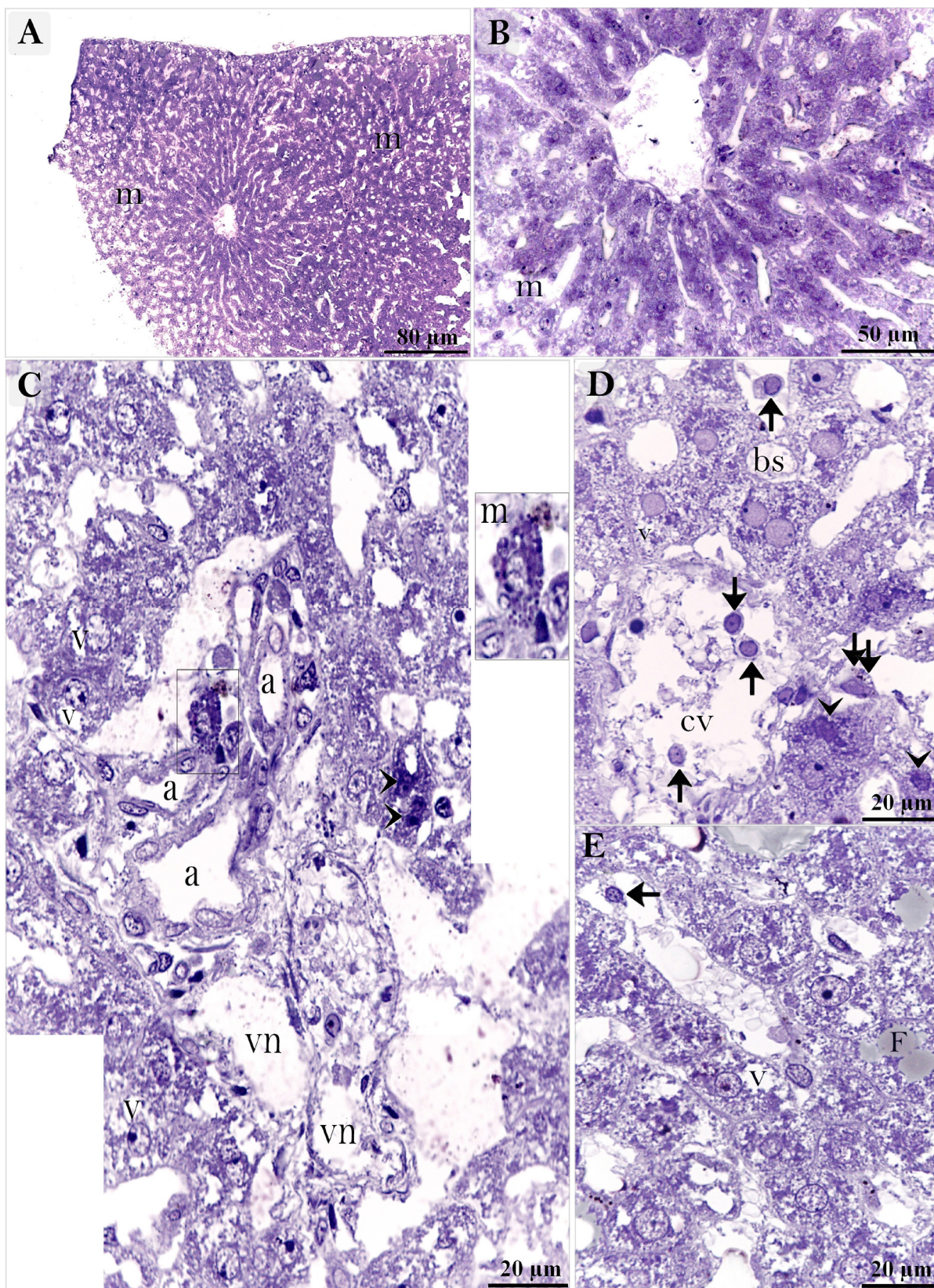


Fig. 2 (low dose): Mild degenerative changes of the hepatic parenchyma treated by a low dose of Ginkgo biloba Semithin sections stained by toluidine blue. A, B: Mild (m) Degenerative changes of the hepatic parenchyma was identified by the pale cytoplasm where the vacuolation occurred. C: the portal area contained branches of the portal vein (vn) and hepatic artery (a). Note Mast cells (m) rich in mast cell granules. Hepatocytes showed mild degenerative changes such as nuclear condensation (arrowheads) and cytoplasmic vacuolation (v). D: increase the Kupffer cells (arrows) in the blood sinusoids (bs) and the central vein (cv). Note activated Kupffer cells undergo hypertrophy and acquired vascular cytoplasm and phagocytic inclusions. note nuclear condensation (arrowheads). E: fatty degeneration of the hepatocytes. note fat (F), cytoplasmic vacuolation (v), Kupffer cells (arrows).

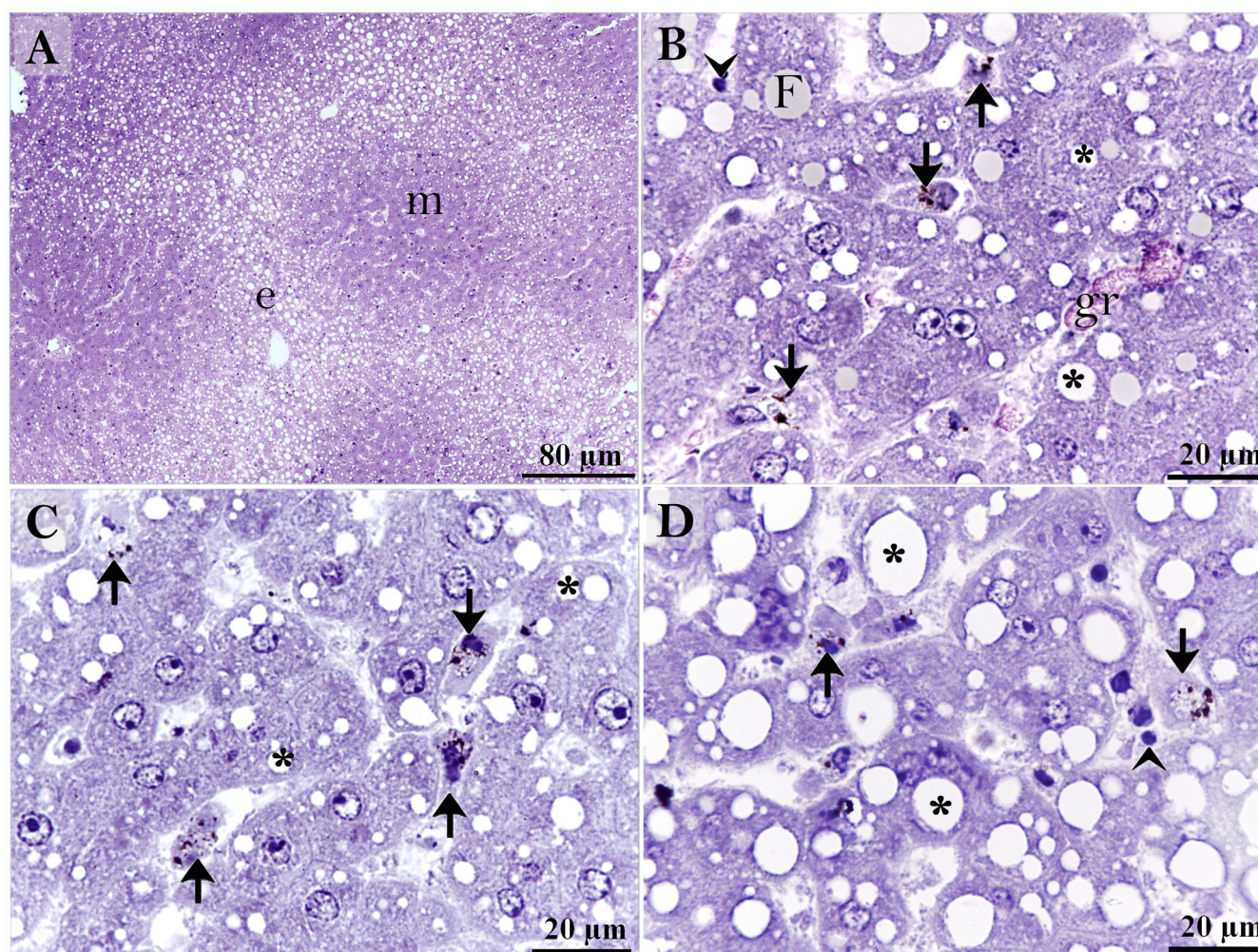


Fig. 3 (High dose): Degenerative changes of the hepatic parenchyma treated by a high dose of Ginkgo biloba. Semithin sections stained by toluidine blue. A: Degenerative changes of the hepatic lobules varied from mild (m) degree to extensive (e) degrees. B: Mild Degenerative changes of hepatic parenchyma. note fatty droplets (F), vacuolation (asterisks) of the cytoplasm of hepatocytes. Activation and hypertrophy of the Kupffer cells which contained phagocytic inclusions (arrows).. note metachromatic granules (gr) of the mast cells in the blood sinusoids. C: Mild Degenerative changes of hepatic parenchyma. vacuolations (asterisks) of the cytoplasm of hepatocytes. Activated Kupffer cells with phagocytic inclusions (arrows). D: Extensive degenerative changes of the hepatic parenchyma. Large vacuolation (asterisks) occupied most of the cytoplasm of hepatocytes. lymphoid cells (arrowhead) in the hepatic sinusoids, and activated von-kupffer cells (arrows).

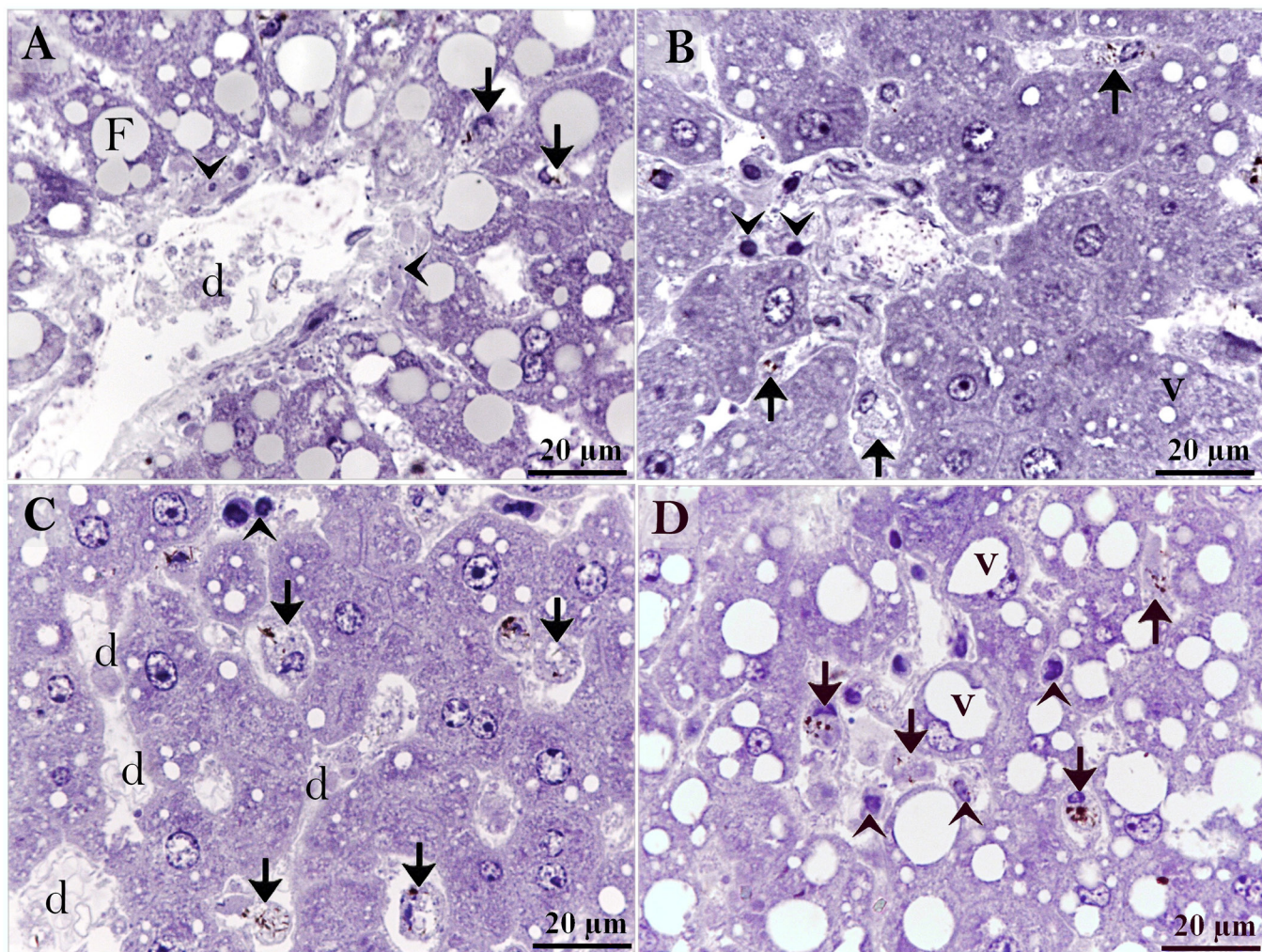


Fig. 4 (High dose): Vascular changes of hepatic veins and sinusoids responding to treatment by a high dose of Ginkgo biloba. Semithin sections stained by toluidine blue. A: vasculitis of the central vein. note cellular debris (fragments of the cytoplasm) (arrowheads) in the perivascular space. note cell debris (d) inside the lumen of the central vein. fatty degeneration (F) of hepatocytes. Activated Kupffer cells with phagocytic inclusions (arrows). B: vasculitis of the central vein. note perivascular lymphoid cells (arrowheads). vacuolation (v) of degenerated hepatocytes. Activated Kupffer cells with phagocytic inclusions (arrows). C: lymphoid cells (arrowheads) in the blood sinusoids. cell debris (d) obliterated the blood sinusoids. Activated Kupffer cells with phagocytic inclusions (arrows). D: Aggregation of activated Kupffer cell and lymphoid cells (arrowheads) in the space of Disse. vacuolation (v) occupied most of the cytoplasm of the hepatocytes which exhibited eccentric nucleus. Activated Kupffer cells with phagocytic inclusions (arrows).

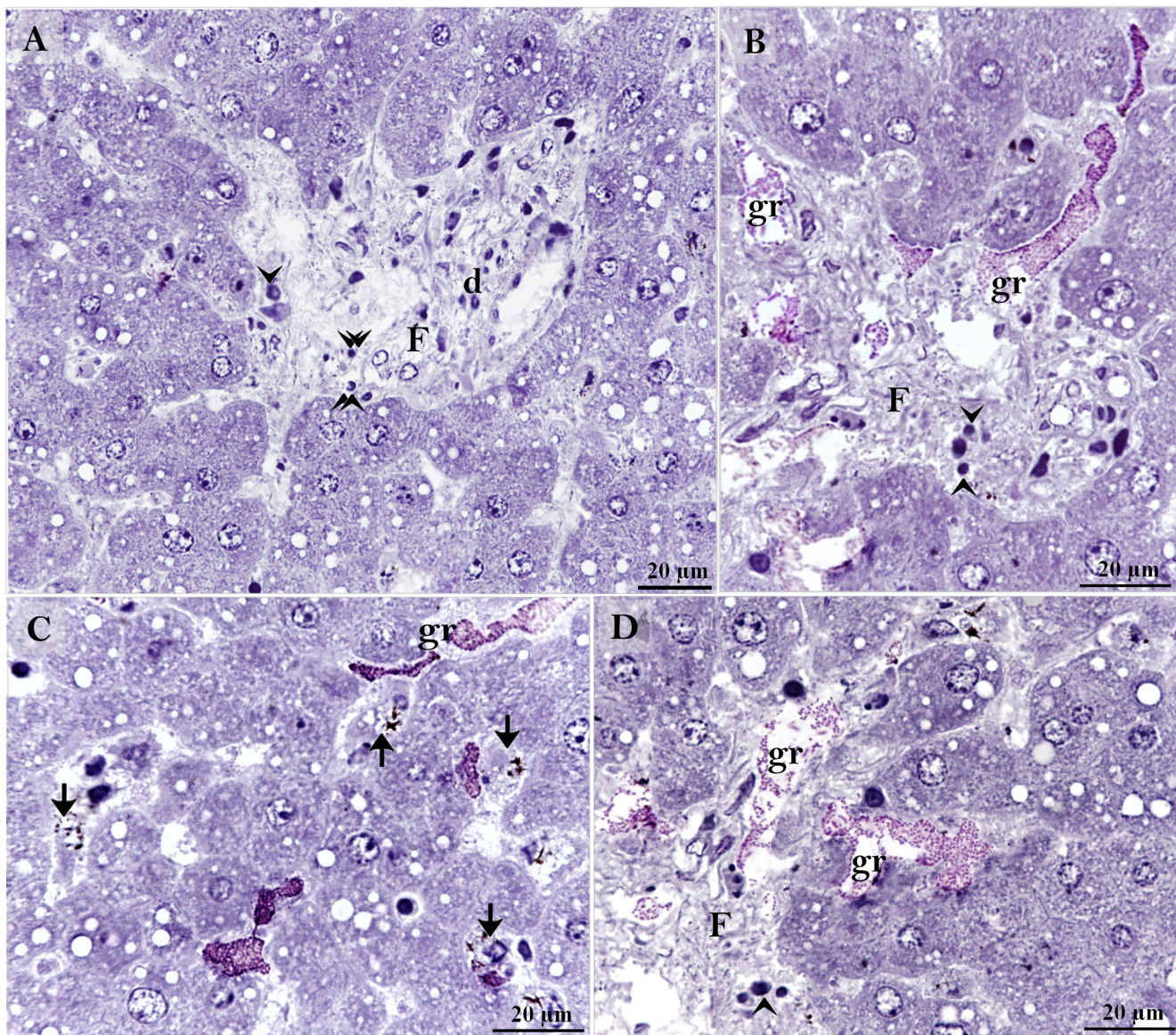


Fig. 5 (High dose): Fibrotic changes of the hepatic parenchyma. Semithin sections stained by toluidine blue A: Fibrous tissue (F), lymphoid cells (arrowheads), necrotic cells (double arrowheads), cell debris (d). B: Fibrous tissue (F), lymphoid cells (arrowheads), metachromatic granules (gr). C: metachromatic granules (gr) in the blood sinusoids. D: lymphoid cells (arrowheads), metachromatic granules (gr) associated with the fibrous tissue.

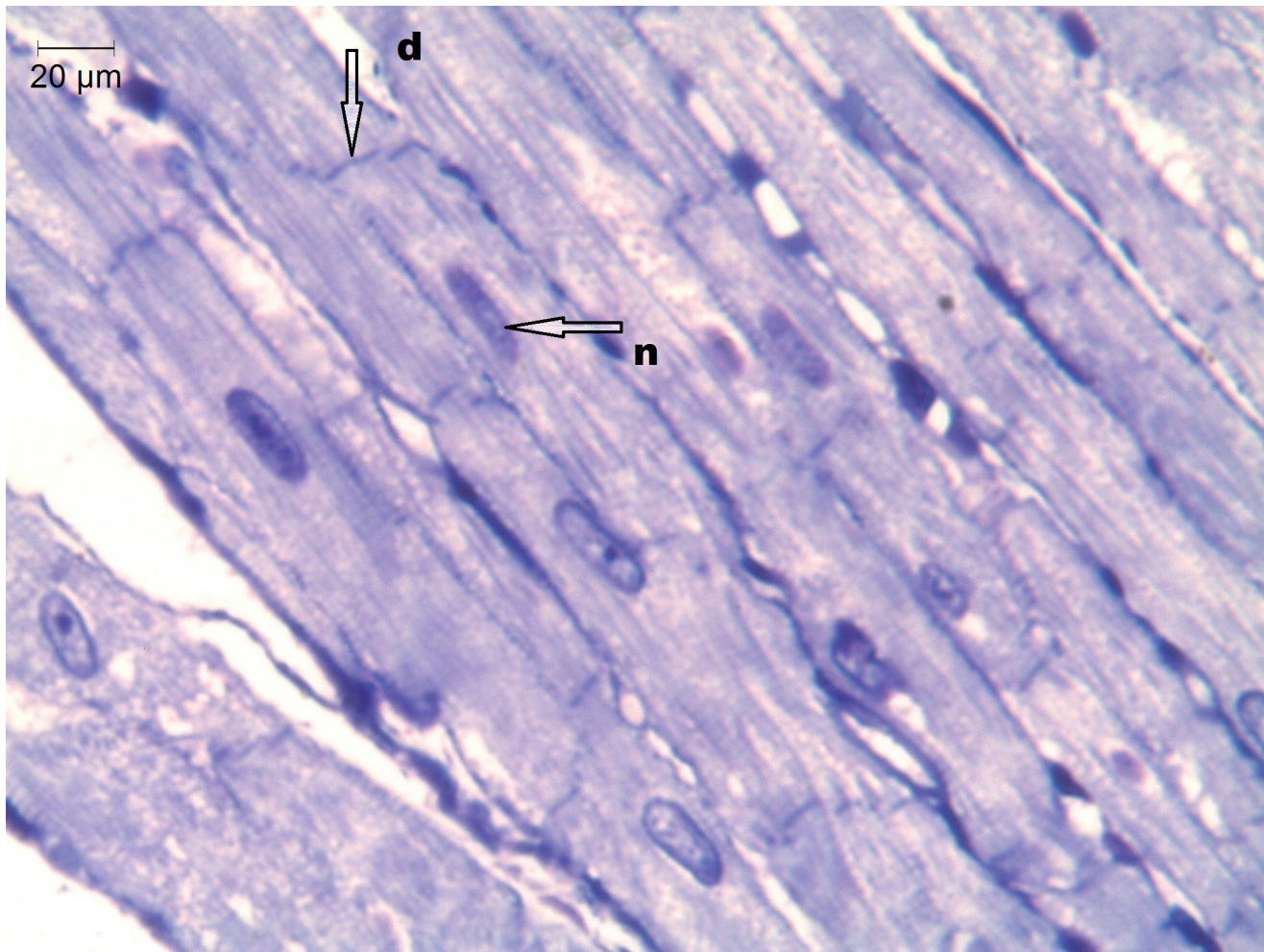


Fig. 6 (heart specimen): Photomicrograph of a section in the left ventricle of group I showing longitudinal cardiac muscle fibers. Notice their central large oval nuclei (arrow with n), transverse striations, and intercalated disc (arrow with d). Toluidine blue x1000.

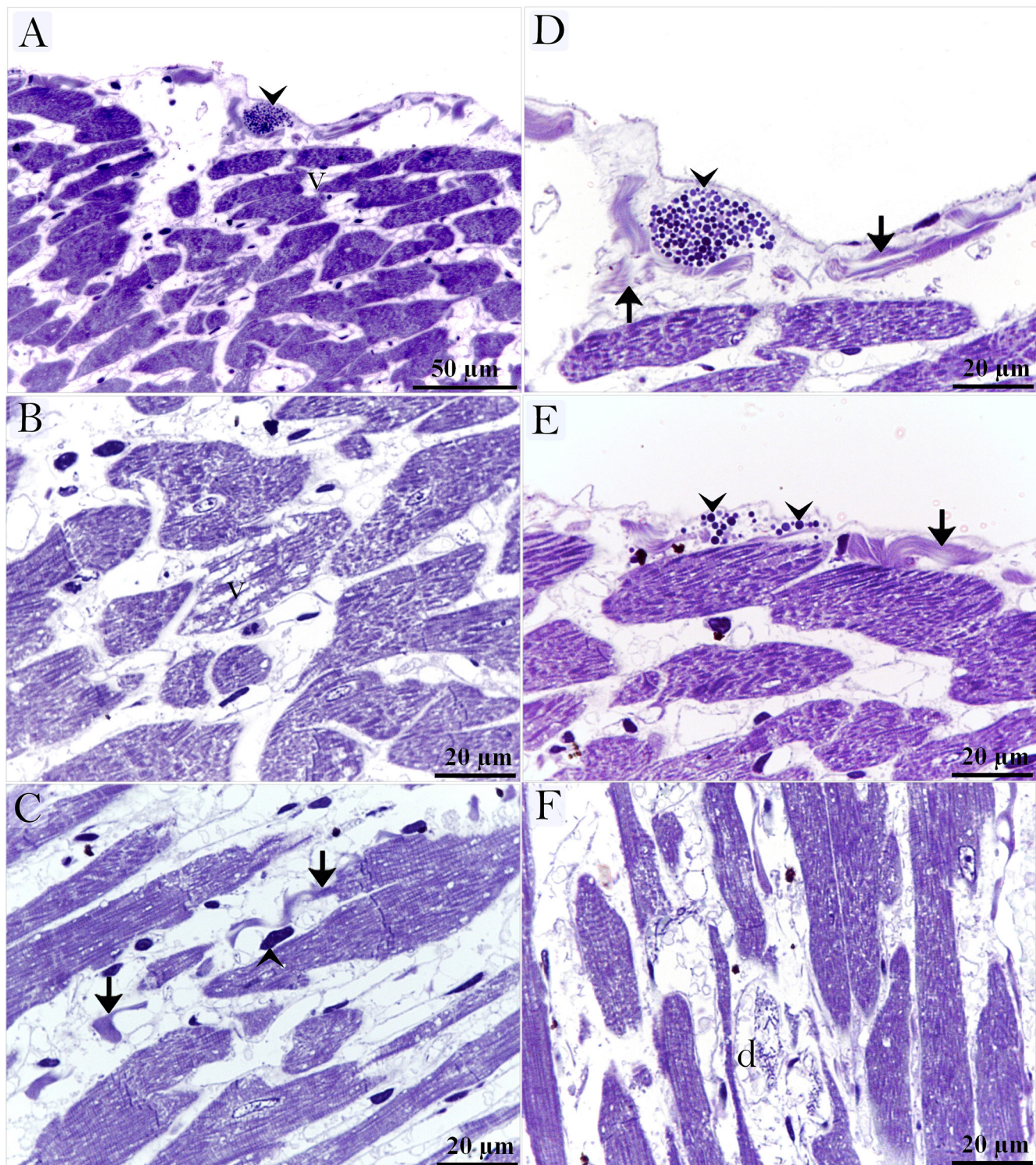


Fig. 7 (heart specimen): Effect of the low dose of the Ginkgo Biloba on the longitudinal cardiac muscle of the rats. A: slight vacuolation (v) of the cardiomyocytes and inflammatory cells (arrow head). B: notable vacuolation (v) of the cardiomyocytes. C: atrophy of cardiomyocytes (arrows). note pyknotic nucleus (arrowhead). D: mast cell in the subendocardium. atrophy of the Purkinje cell fibers (arrows). E: mast cell granules (arrowheads) in the subendocardium. atrophy of the Purkinje cell fibers (arrows). F: degenerated cardiomyocytes (d) that had lost most of the myofibrils.

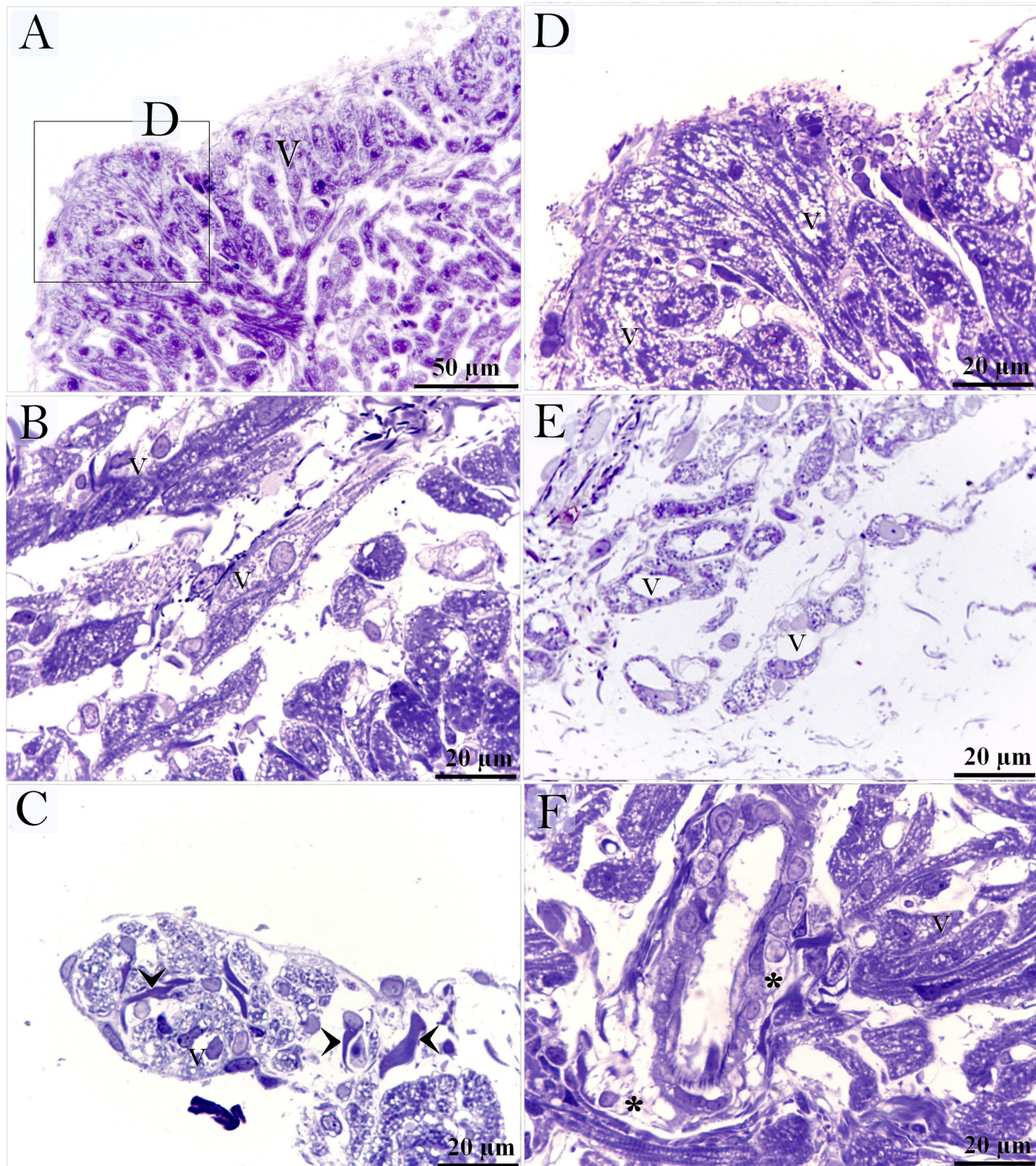


Fig. 8 (heart specimen): Effect of the high dose of the Ginkgo Biloba on the longitudinal cardiac muscle of the rats. A, D: Vacuolation (v) of the cardiomyocytes. B, E: extensive vacuolation (v) which occupied almost cytoplasm of the cardiomyocytes. Vacuolation (v) of the cardiomyocytes. C: cytoplasmic condensation and atrophy (arrowheads) of the cardiomyocytes. Vacuolation (v) of the cardiomyocytes. F: degenerative changes of the coronary eatery branches. Note vacuolation (asterisks) of the muscular cells of the tunica media.

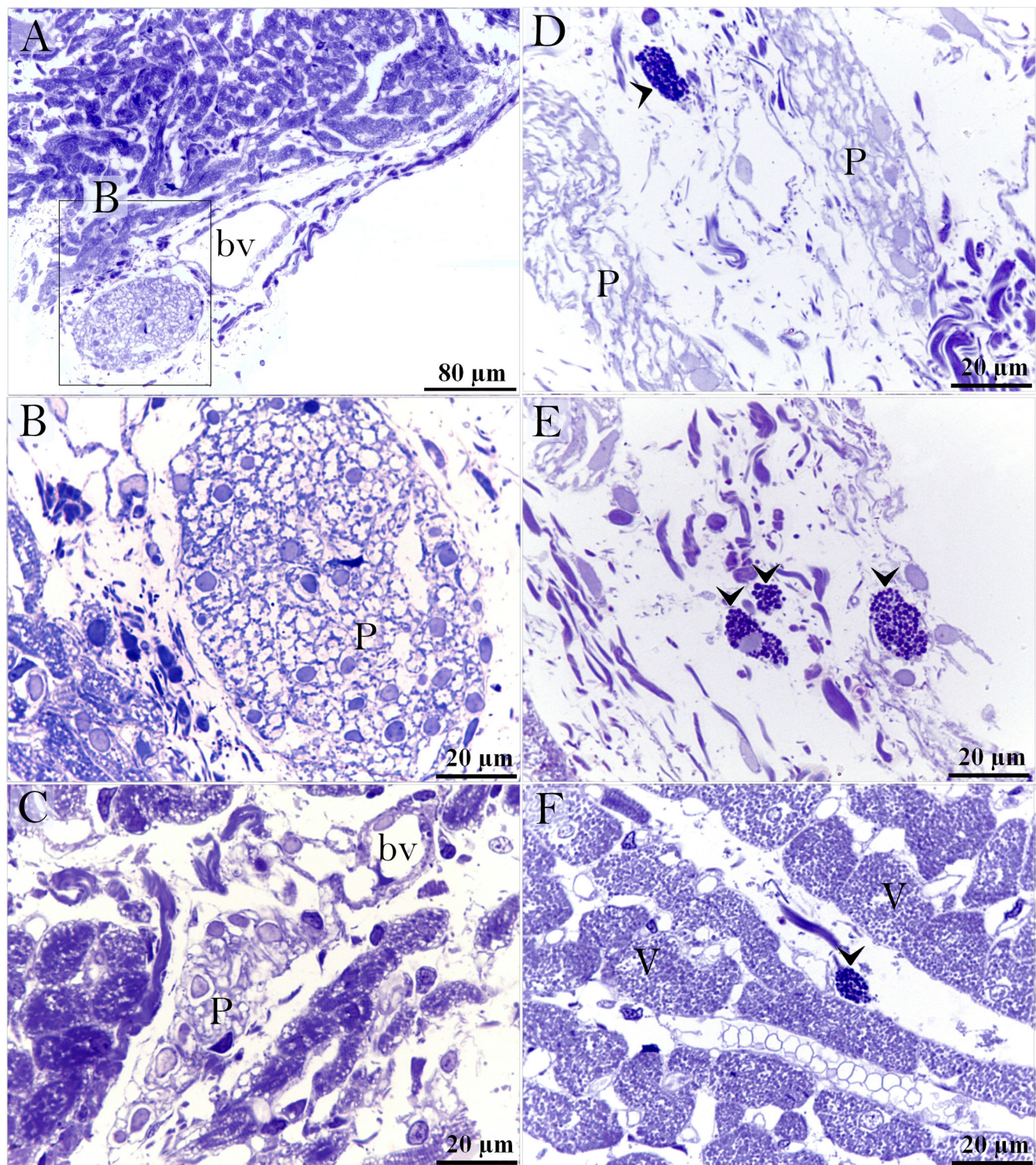


Fig. 9 (heart specimen): Effect of the low dose of the ginkgo Biloba on the ventricular conducting system of the rat hearts. A, B: degeneration of the Purkinje cell fibers (p) in the subepicardial regions. C: degeneration of the Purkinje cell fibers (p) in the intramuscular region. D: degeneration of the Purkinje cell fibers (p) of the subendocardial. localization of mast cell (arrowhead) in the subendocardium. E: numerous mast cells (arrowheads) in the subendocardium. F: mast cell (arrowhead) between cardiomyocytes. Mild vacuolation (v) of the cardiomyocytes.

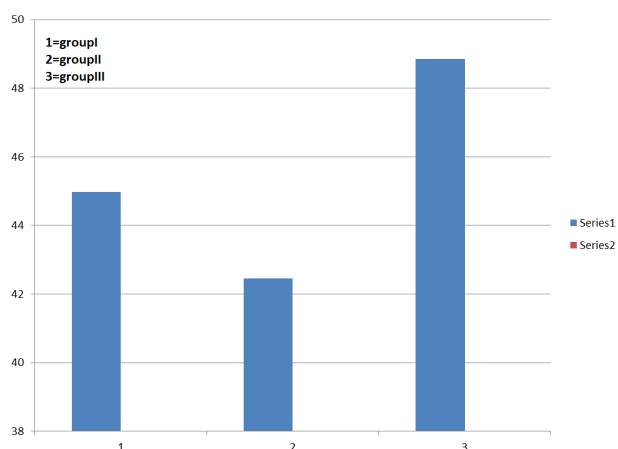


Fig. 10 (Graphical presentation): graphical presentation showing varieties in diameters of central vein in hepatocytes of different groups

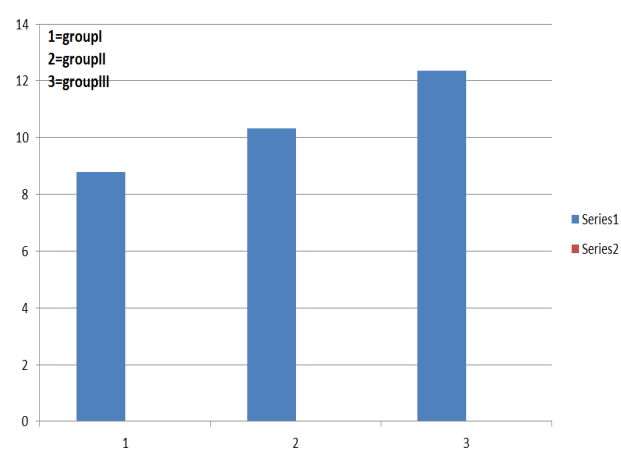


Fig. 11 (Graphical presentation): graphical presentation showing varieties in the transverse diameter of muscle fibers of different groups

Table 1: Mean values (in micron) of the diameters of central vein of hepatocytes in different groups

	(Number)	(Minimum)	(Maximum)	(Mean)	(Std. Deviation)
group I	8	28.85	69.53	44.9737	14.92859
group III	8	32.50	55.28	42.4588**	7.10737
group III	8	34.60	80.62	48.8525***	16.57495

Table 2: Mean values (in micron) of the diameters of different types of muscle cells in different groups

	(Number)	(Minimum)	(Maximum)	(Mean)	(Std. Deviation)
group I	14	7.01	10.22	8.7829	.85910
group III	14	7.69	13.88	10.3350*	1.74201
group III	14	8.24	15.74	12.3693***	1.99734

DISCUSSION

Incidence of liver fibrosis is growing as a result of the widespread occurrence of chronic hepatitis (predominantly type C). It has been observed that the reversibility of hepatic fibrosis dependent on the cause, in alcoholic liver disease, may show reversible hepatic fibrosis in the early-stage and withdrawal of affecting agent. Medicinally some plants have made a significant effect on current medical practice and traditional Chinese herbs are used in medical cases as a protective agent^[8]. GbE has been used to improve blood circulation without ill effects for centuries in traditional Chinese medicine. GbE contains two groups of major components: flavonoid glycosides and terpenoids. GbE has the property of inactivating oxo-ferryl radical species, which are more efficient oxidative agents than classical hydroxyl radicals^[9]. In our study, we observed mild degenerative changes of the hepatic parenchyma treated by the low dose of Ginkgo biloba. However, there is marked degenerative changes, vascular changes and fibrotic changes with a high dose. Lisa L. von Moltke *et al* showed that there was no significant change in liver parenchyma with Ginkgo Biloba in hepatic fibrosis rats. It is possibly attributed to its effect of inhibiting the expression of tissue inhibitor of metalloproteinases (TIMP-1) and promoting the apoptosis of hepatic stellate cells. Also, the biochemical and histological protocol demonstrated that GbE^[10], administrated at a safe dosage

with minimal side effects, effectively prevented both the biochemical and histological changes associated with liver fibrosis. A primary consideration in the assessment of the efficacy of a potential therapeutic agent for hepatic fibrosis is its effect on liver histology. GbE administration in rats with liver fibrosis accelerated the reversion of liver fibrosis and lowered the high levels of serum ALT and AST activity, indicating that GbE was also effective in reversing liver cirrhosis. Hepatic fibrosis, regardless of the cause, is characterized by an increase in extracellular matrix (ECM) constituents. There is now overwhelming evidence suggesting that the hepatic stellate cells (HSC), lying in the space of Disse beneath the endothelial cell layer, are the principal cells involved in hepatic fibrogenesis. Thus, to prevent or reverse liver fibrosis depends greatly on controlling the hepatic stellate cells (HSC)^[11-12]. These cells are usually quiescent, with a low proliferation rate. A variety of liver lesions occurred in rats and mice following Ginkgo biloba extract administration^[12-13] retrospectively analyzed the relative predictive value after taking Ginkgo biloba extract which concluded that there was an increase in liver weight, centrilobular hepatocytic hypertrophy, minimal focal necrosis (male mice), or fatty change of hepatocytes (male rats). These findings are consistent with changes associated with hepatic enzyme induction. Unfortunately, we observed marked degeneration of liver cells after taking Ginkgo biloba with a high dose. There

was hepatocyte injury, activation, and hypertrophy of the Kupffer cells which contained phagocytic inclusions, metachromatic granules of the mast cells in the blood sinusoids, and fibrotic tissue. It suggests that GbE could enhance the allergic reaction on liver cells and we suggested that there is a hypersensitive reaction from Ginkgo Biloba.

In contrast to our results,^[14] revealed that Ginkgo Biloba extract (GBE) promotes cardioprotection via activation of cholinergic signaling in a model of isoproterenol-induced cardiac hypertrophy. In the present study, degeneration of the cardiomyocytes as Cytoplasmic condensation and atrophy of the cardiomyocytes was found, however^[7] confirmed that GBE could ameliorate the cardiac remodeling, the mechanism was partly involved in regulating the expression of transforming growth factor beta superfamily of cytokines (TGF- β 1), matrix metalloproteinases (MMP-2), and matrix metalloproteinases (MMP-9), and finally attenuating the extracellular matrix deposition.

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CONFLICT OF INTERESTS

There are no Conflicts of Interest.

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الملخص العربي

التأثير النسيجي للجينكة بيلوبا على الكبد والقلب لدى ذكور الجرذان البيضاء البالغة

علي عبدالرحمن غويل^١، سهي سليمان^٢، مها عبدالباقي احمد^٢^١قسم الامراض المتوطنة، ^٢قسم التشريح الادمي وعلم الاجنه، كلية الطب، جامعة جنوب الوادي^٢قسم الهستولوجي، كلية الطب البيطري، جامعة جنوب الوادي

الخلفية والأهداف: الجينكو بيلوبا هو مكمل غذائي يستخدم لمختلف الفوائد العلاجية المزعومة حيث يحتوي على مواد قوية يمكن أن تساعد كمضادات الأكسدة في مكافحة الالتهاب وتسبب اضطرابات نفسية وتحسن وظائف المخ. هناك بعض الباحثين يوصى باستخدام الجينكو بيلوبا كعامل وقائي وتهدف الدراسة إلى تقييم التأثير النسيجي للإعطاء المزمن بجرعات مختلفة من الجينكو بيلوبا علي الكبد والقلب من ذكور الجرذان البيضاء البالغة.

المواد والاساليب: أجريت الدراسة على عدد إجمالي يبلغ ٣٠ جردًا بالغًا من ذكور الجرذان البيضاء. وزنها ١٧٠-٢٠٠ جم. قسمت الجرذان إلى ثلاث مجموعات متساوية (١٠ فئران لكل مجموعة) وعولجت مرة واحدة في اليوم. المجموعة الأولى (مجموعة التحكم وعددها = ١٠) ، تلقى كل واحد من المجموعة ماء مقطر (١ مل / يوم). المجموعة الثانية (مجموعة الجرعات المنخفضة وعددها = ١٠): تم تناول الجينكو بيلوبا بجرعة ١٠٠ مجم / كجم من وزن الجسم. المجموعة الثالثة (جرعة عالية ؛ وعددها = ١٠): تلقيت جرعة من الجينكو بيلوبا ٢٠٠ ملجم / كجم من وزن الجسم عن طريق الفم يوميا لمدة ٤ أسابيع. تم التضحية بالجرذان وتجهيزها لعمل دراسة نسيجية.

النتائج: في العينات الخاصة بنسيج الكبد ، كانت هناك تغيرات تنكسية طفيفة. في جرعة منخفضة ، كانت التغييرات في شكل التفريغ السيتوبلازمي والتكثيف النووي. في الجرعة عالية من الجينكو بيلوبا ، كانت هناك تغييرات تنكسية اختلفت من درجة خفيفة إلى درجات واسعة. في عينات أنسجة القلب ، كان هناك الفجوات السيتوبلازمية وضمور خلايا عضلة القلب خاصة في المجموعة الثالثة ، وكان هناك تغيرات تنكسية في الشريان التاجي وانحطاط ألياف خلايا بركنجي في نظام التوصيل البطيني بأكمله.

الاستنتاج: إن تناول الجينكو بيلوبا بيلوبا له آثار تنكسية على الكبد والقلب خاصة في الجرعات العالية.