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Effect of Red Ginseng on Dimethylnitroseamine - Induced Liver Injury in Albino Mice.

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ABSTRACT

Ginseng extracts have shown a wide array of beneficial role in the regulation of liver functions and the treatment of liver disorders of hepatotoxicity, hepatic fibrosis and hepatocellular carcinoma. So, the present study was designed to investigate the positive effect of red ginseng on dimethylnitroseamine (DMN) - induced liver injury in mice. Fifty five Swiss albino mice were randomly categorized into four groups. Group I (n=10) was served as control, group II (n=15) was injected i.p. with DMN (10 mg/kg b.w.) twice weekly for 8 weeks and group III (n=15) was administered red ginseng orally (200 mg/kg b.w.) daily for 2 weeks before injection with DMN. Group IV (n=15) was injected with DMN for 8 weeks then administrated red ginseng for 2 weeks. Histopathological results, liver damage as zonal degeneration, congestion, apoptosis, multinucleated macrophages and fatty degeneration were seen after 8 weeks of receiving DMN in group II. Mild cellular infiltration and regenerated hepatocytes were seen after 8 weeks in group III. Mild edema and congestion, fatty degeneration, apoptosis and necrosis were observed 2 weeks after ginseng in Group IV. Histochemical result; liver sections of group II revealed increased collagen fibers and moderate amyloid deposition as compared with other groups. Immunohistochemical result; strong α-SMA positivity in sinusoids was observed in group II after eight weeks of receiving DMN with higher significant positivity ($P<0.05$) which reduced by ginseng administration. In conclusion, red ginseng exhibited hepatoprotective effects in DMN-induced injury by inhibiting fibrosis and α-SMA, implicating its potential application in clinical intervention.

Keywords: DMN, Liver fibrosis, Ginseng, Collagen fibers, Amyloid and α-SMA.

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INTRODUCTION

Liver fibrosis leads to the development of liver failure which is one of the top ten causes of death in the western world. Patients with liver fibrosis can be a-symptomatic for 15-20 years with morbidity and mortality only occurring after progression to cirrhosis (a risk factor for developing HCC) [1].

In general, liver fibrosis can be regarded as a chronic wound healing process, characterized by increased deposition of connective tissue. On a cellular level, the hepatic stellate cells (HSCs) considered to be the key player in this process. In normal liver, stellate cells encompass approximately 5-8% of the total cell population. Their main functions are the uptake, storage, and release of retinoid, the regulation of sinusoidal blood flow, and the synthesis and degradation of extracellular matrix (ECM) [2]. Stellate cells are the most important producers of ECM.

Dimethylnitrosamine (DMN) is a semi-volatile organic chemical that is highly toxic and is a suspected human carcinogen. In rodents, DMN rapidly absorbed by ingestion or inhalation, was metabolized in the liver and other tissues, and was excreted in urine. Metabolic activation of DMN in the liver of rodents depended on cytochrome P450 2E1 (CYP 2E1) via enzymatic denitrosation and alpha hydroxylation [3].

Cellular damage resulted from formation of nucleic acids and protein adducts caused by methyldiazonium ion alkylation of DNA, RNA, and proteins. Methyldiazonium ions also react with water to form methanol and nitrogen gas (N2) [4, 5].

De Gouville *et al* [6] illustrated that hepatic fibrosis of rats was developed within 3 weeks after exposure to DMN, and the morphological changes were induced such as fibrous septa formation, nodular regeneration, portal hypertension, and development of ascites. Metabolic abnormalities were also documented in rats including hypoproteinemia, hypocholesterolemia, hypophosphatemia, and impaired glucose tolerance with insulin resistance, elevated serum and urinary hydroxyproline and uric acid levels. Also elevated malondialdehyde levels in liver and serum indicating increased lipid peroxidation and oxidative damage [7]. On the other hand activation of HSCs, which is a critical event in the pathogenesis of hepatic fibrosis, was also documented in rats [8]. Ginseng saponins, commonly known as ginsenosides, are principal constituents and have believed to be responsible for multiple ginseng health benefits. To date, approximately 70 kinds of saponin have been isolated from ginseng [9].

Liver fibrosis characterized by the accumulation of collagen and ECM proteins in the space of Disse. These proteins are produced in damaged liver by the stellate cells. Collagen type I, III and IV, fibronectin, laminin, and proteoglicans [10] are the extracellular proteins produced during this process, where collagen types I and III [11] are most abundantly found. Amyloidosis occurs in patients with chronic infectious and inflammatory disease. It is an exceedingly rare complication of cystic fibrosis but should be suspected in patients with unexplained

proteinuria, goitre, or hepatosplenomegaly [12]. Diffuse vascular wall amyloid without parenchymal involvement is frequently seen in the liver [13], and inclusion of such cases may have confounded earlier studies of the natural history of hepatic amyloid [14].

In chronic liver diseases, HSCs undergo a process of activation towards a myofibroblast-like phenotype, migrate, and accumulate at the sites of tissue repair and acquires an increased expression of α-SMA [15].

α-SMA is a good marker for the detection of myofibroblast-like cells. The appearance of α- SMA in liver mesenchymal cells seems closely related to the process of hepatic fibrosis in both rat and man. Cultured myofibroblasts are characterized by stress fibers, containing α-SMA and by supermature focal adhesions (FAs), which are larger than FAs of α-SMA–negative fibroblasts [16].

The ultimate result of chronic injury is the accumulation of ECM and replacement of low density type IV with high density type I collagen within the subendothelial space of Disse [17]. Activated HSCs acquire an increased expression of α-SMA [18]. So the present study was designed to investigate the positive effect of red ginseng on hepatocyte injury under toxic effect of DMN in mice.

MATERIALS AND METHODS

The present study was carried out on fifty five Swiss albino mice weighing 20–25g obtained from Laboratory Animal Unit, Medical Research Institute. Mice were kept under the suitable laboratory conditions from temperature and ventilation and given free access food and water. All ethical protocol of Medical Research Institute animal treatment was strictly followed. Mice were randomly categorized into four groups:

Group I: Ten mice were served as control and received normal saline as vehicle.
Group II: Fifteen mice were injected intraperitonially with DMN (Sigma Co.) at a dose of 10 mg/kg body weight twice weekly for 8 weeks [19]
Group III: Fifteen mice were administered red ginseng (Ginsana, Pharco Co., Egypt) orally at a dose of 200 mg/kg body weight daily for 2 weeks before injection with DMN [20]
Group IV: Fifteen mice were injected with DMN as in group II for 8 weeks then they were administrated red ginseng as in group III for 2 consecutive weeks.

Mice from groups I, II and III were sacrificed under anesthesia by diethyl ether after 2, 4, 8 weeks from the beginning of DMN injection. Mice of group IV were sacrificed at the end of the experiment. Right lobes of liver tissue specimens were immediately removed and divided into 2 parts: The first part was fixed in 10% neutral buffered formalin and the second part was fixed in Bouin's fixative. They were processed for the following studies:

Histopathological Studies:

Hematoxylin and Eosin Stains (H&E)

Fixed liver specimens from all groups were washed in running tap water, dehydrated in ascending ethyl alcohol, cleared in xylene, and then embedded in paraffin wax. 4µm thick sections were prepared, processed, and stained with Hematoxylin and Eosin stains to study the histopathological changes [21].

Histochemical Studies:

Masson's Trichrome Stain for collagen

Deparaffinized sections were processed to distilled water, mordant in Bouin's fixative for 15 minutes at 60^{o}C and wash in running tap water to remove picric acid color. Sections were stained with Weigert's hematoxylin and rinsed in running tap water, then stained in Biebrich scarlet-acid fuchsin solution for 4 minutes and washed in distilled water. Sections were differentiated in phosphomolybdic- phosphotungstic acid solutions for one minute, and then lastly stained with fast green FCF and processed to be mounted [22].

Amyloid by Congo red stain.

Four microns thick sections were deparaffinized and hydrated to distilled water, stained for 2 minutes in Mayer's Hematoxylin. They were rinsed in 3 changes of distilled water, then immersed in solution contains 50 ml of 80% ethyl alcohol saturated in sodium chloride and 50 ml of 1% sodium hydroxide for 20 minutes. Sections were stained for 20 minutes in filtrated solution contained 50 ml of 80% alcohol saturated with Congo red & sodium chloride and 0.5 ml of 1% sodium hydroxide. Finally, sections were dehydrated in 3 changes of absolute alcohol, cleared in xylene and mounted with Canada balsam [23].

Immunohistochemical Study.

Alpha -Smooth Muscle Actin (α-SMA) by immunoperoxidase method.

Sections of 4µ thick were cut and brood onto coated glass slides. They were incubated with methanol-1% hydrogen peroxide to destroy endogenous peroxidase, and blocked with nonspecific staining blocking reagent (Sigma Co.). After overnight incubation at 4^{0}C with mouse monoclonal anti-α-SMA antibody (diluted 1:100; Sigma) sections were processed according to the standard immunoperoxidase method, using a streptavidin biotin peroxidase complex kit (Sigma Co). The immunohistochemical reaction was then developed and stained with diaminobenzidine chromogen solution "DAB" (Sigma). Sections were counter stained with hematoxylin, dehydrated, cleared, and mounted with DPX. Negative control was obtained by omitting primary antibody [24].Morphological measurements of α-SMA positivity were counted by using image analysis (Leica Q-500).

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RESULTS

Histopathological Results:

Hematoxylin and Eosin Stains (H&E)

Control normal liver in group (I) showed preserved hepatic lobular architecture. The polygonal lobule formed of trabeculae of hepatocytes radiating from central vein; with patent hepatic sinusoids lined with Kupffer cells. The majority of hepatocytes have single nucleus with prominent nucleoli and some binucleated cells were also observed in sections (Figure 1). Liver sections of group (II) after two weeks revealed mild steatosis, lymphocytic periportal infiltration and few apoptotic figures were also observed (Figure 2). The alterations were more expressed as the time of receiving DMN was extended. Four weeks showed several changes illustrated as partial loss of normal lobular architecture, marked congestion of portal and central veins as well as in sinusoids and mild degeneration of hepatocytes (Figure 3 A&B). Maximum liver damage and loss of normal lobular architecture were observed after eight weeks interval; zonal degeneration, congestion, numerous apoptotic figures and multinucleated macrophages (Figures 4& 5), as well as fatty degeneration with ballooning hepatocytes (Figure 6). Mice liver sections of group (III) after 2 weeks showed preserved lobular architecture, inflammatory cells infiltration in periportal and with liver parenchyma in addition to few apoptotic figures (Figure 7 A&B). After four weeks, there were mild inflammations in the central veins and mild congestion as well as binucleated cells (Figure 8 A&B). Eight weeks interval showed liver regeneration with more hepatocytes appeared with binucleated forms and mild infiltration of inflammatory cells around portal vein (Figure 9). At the end of receiving ginseng in group (IV), there were mild edema and mild congestion as well as fatty degeneration. Apoptosis in many hepatocytes and area of necrosis were also observed (Figure 10 A&B).

Histochemical Result:

Masson's Trichrome Stain:

Normal control liver section in group (I) showed minimal deposition of connective tissues as thin rims of collagen around central vein and sinusoids (Figure 11).

Two weeks of group (II), liver sections showed moderate deposition of collagen fibers mainly in perivascular area of the periphery of hepatic lobule, around portal vein branches and around sinusoids (Figure 12). Four weeks of DMN administration, showed the collagen fibers deposition ranged from moderate to marked in sinusoids around portal vein and appeared as immature short collagen fibers (Figure 13). Eight weeks revealed increased fibrosis and showed strong thickening of collagen fibers in portal area and in sinusoids (Figure 14).

A common finding in group (III) was the depletion of collagen to reach a weak deposition as compared with their counterparts in group (II) either post two, four or eight weeks.

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There was a little exception where some sections after 4 weeks showed moderate collagen deposition around portal tract and in sinusoids (Figures 15, 16 and 17).

After receiving DMN then two weeks ginseng in group (IV), the collagen deposition was weak around central vein and in sinusoids (Figure 18).

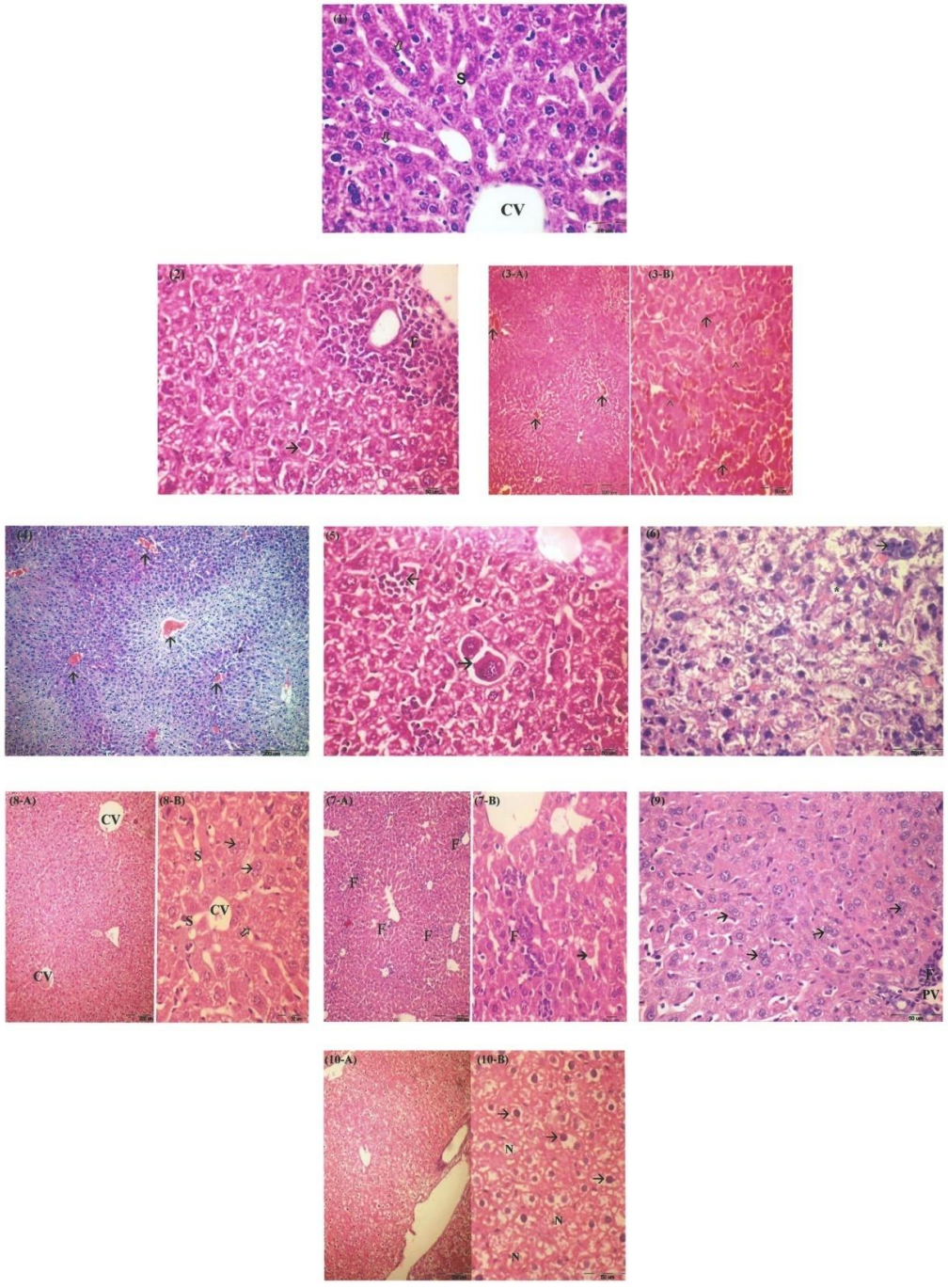

Figure (1): Paraffin section of normal control mouse liver showing normal hepatocytes with central vein (CV) and patent sinusoids (S) lined with kupffer cells(⇒). Note mono and binucleated hepatocytes. (H&E– Bar = 50 µm).

Figure (2): Paraffin section of group II, mouse liver two weeks after receiving DMN showing mild steatosis, periportal acute inflammation (F) and few apoptotic figures (→). (H&E - Bar = 50µm).

Figure (3): Paraffin section of mouse liver four weeks after receiving DMN in group II showing: A- Marked liver congestion in portal and central veins (↑). (H&E - Bar = 200 µm). B- Marked congestion of sinusoids (↑) and mild degeneration of hepatocytes (arrow head). (H&E - Bar = 50 µm).

Figures (4, 5, 6): Eight weeks after receiving DMN, showing central and zonal degeneration and congestion (↑) in figure (4). (H&E - Bar = 200 µm).
Numerous apoptotic figures represented by foci of lytic necrosis (intra parenchymal inflammation) and multinucleated macrophages engulfing apoptotic bodies (→). Fatty degeneration (steatosis), where hepatocytes have ballooned clear cytoplasm (*) and eccentric nuclei and apoptotic figures (→) in figures (5&6). (H&E - Bar = 50 µm).

Figure (7): Two weeks in group III showing: A. Preserved lobular architecture and inflammatory cells (F) in the periportal and parenchymal areas. (H&E - Bar = 200 µm). B. Inflammatory cells within liver parenchyma (F) together with few apoptotic figures (→). (H&E - Bar = 50 µm).

Figure (8): Four weeks in group III showing: A. Mild congestion in central vein (CV). (H&E - Bar = 200 µm). B. Sinusoids (S) opening with the central vein (CV), kupffer cells (⇒) and few binucleated hepatocytes (→). (H&E - Bar = 50 µm).

Figure (9): Eight weeks in group III showing liver regeneration; more hepatocytes with binucleated forms (→) and mild inflammatory cellular infiltration (F) around portal vein (PV). (H&E - Bar = 50 µm).

Figure (10): Group IV showing: A. Mild edema, mild congestion and fatty degeneration with ballooning hepatocytes. (H&E - Bar = 200 µm). B-Numerous apoptotic hepatocytes (→) as well as area of necrosis (N). (H&E- Bar = 50 µm).

Amyloid

Liver sections of normal control mice in group (I) showed very weak deposition of amyloid material around central vein and in sinusoids (Figure 19).

Group II after two weeks of receiving DMN revealed weak deposition of amyloid around portal vein and moderate deposition in hepatocytes and sinusoids (Figure 20), while after four and eight weeks showed moderate amyloid deposition around portal vein, in sinusoids and around some hepatocytes (Figures 21 & 22).

After two weeks in group (III) amyloid deposition was weak around portal vein and moderate in some hepatocytes (Figure 23). The $4^{\underline{th}}$ and $8^{\underline{th}}$ weeks showed only weak deposition of amyloid either in sinusoids around central vein or in hepatocytes (Figures 24 & 25).

Liver sections after receiving DMN then two weeks ginseng in group (IV) revealed portal vein with weak amyloid material and moderate amyloid material around hepatocytes (Figure 26).

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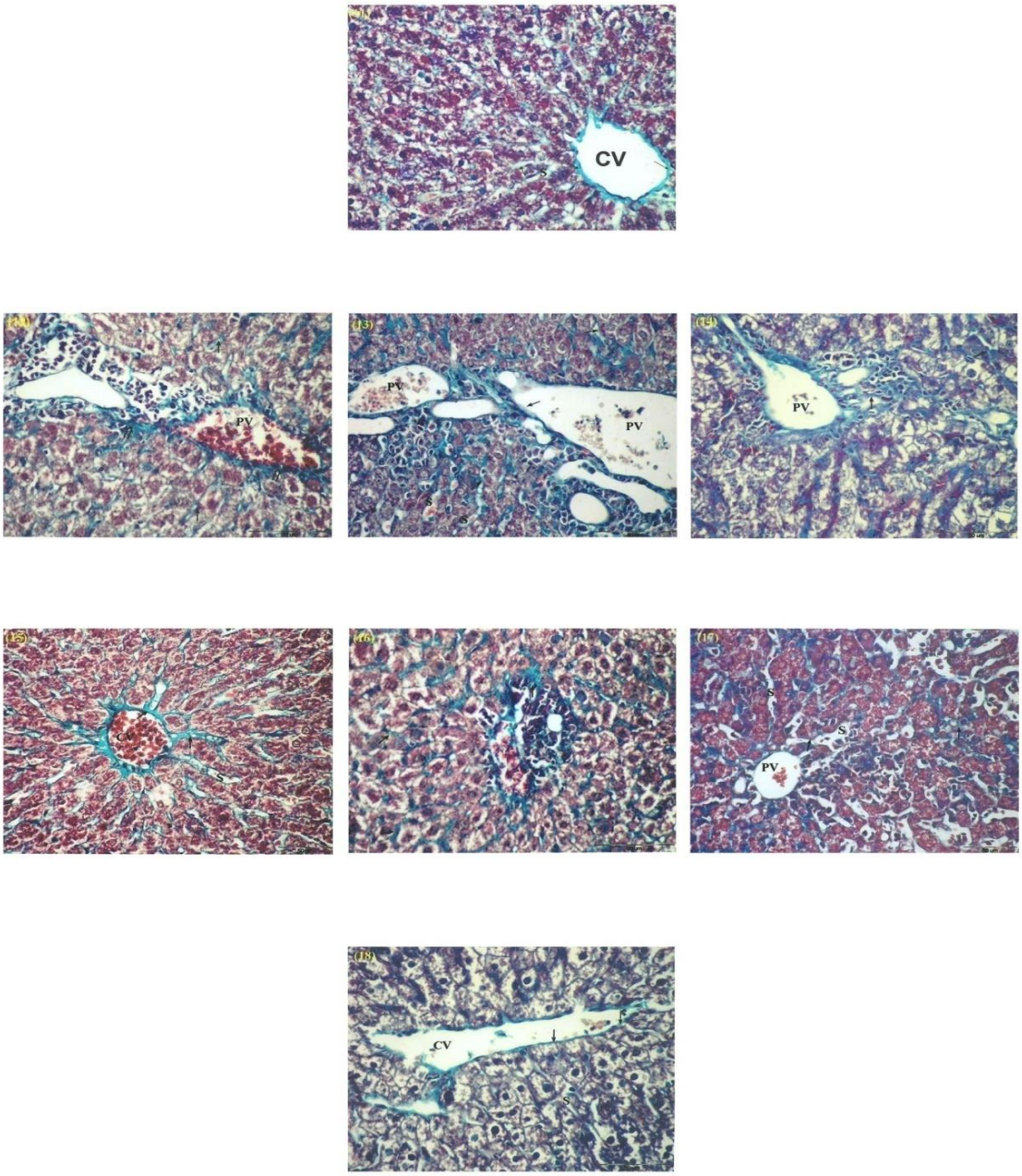

Figure (11): Paraffin section of normal control mouse liver showing thin blue rims of collagen (↑) around central vein (CV) and sinusoids (S). (Masson's Trichrome Stain- Bar = 50 µm)

Figure (12): Two weeks in group II showing moderate deposition (⇒) of collagen fibers in portal area around branches of portal vein (PV) and mild (↑) around sinusoids (S). (Masson's Trichrome Stain - Bar = 50 µm).

Figure (13): Four weeks in group II showing marked collagen deposition (←) around congested portal vein (PV) and moderate collagen deposition in sinusoids (S). (Masson's Trichrome Stain – Bar = 50 µm).

Figure (14): Eight weeks in group II showing strong deposition of collagen fibers (↑) in portal area and in sinusoids. (Masson's Trichrome Stain- Bar = 50 µm).

Figure (15): Two weeks in group III showing weak collagen deposition (↑) around central vein (CV) and sinusoids (S). (Masson's Trichrome Stain- Bar = 50 µm).

Figure (16): Four weeks in group III showing weak to moderate collagen deposition (↑) in hepatic sinusoids and portal tract. (Masson's Trichrome Stain- Bar = 50 µm).

Figure (17): Eight weeks in group III showing weak deposition (↑) of collagen deposition in sinusoids (S). (Masson's Trichrome Stain- Bar = 50 µm).

Figure (18): Group IV showing weak collagen deposition (↓) around central vein (CV) and sinusoids (S). (Masson's Trichrome Stain - Bar = 50 µm).

Immunohistochemical Results:

Alpha Smooth Muscle Actin (α-SMA)

Normal liver sections in group (I) showed mild positive immunostaining of α-SMA around central vein and in sinusoidal linings (Figure 27).

After two weeks in group (II), moderate expression of α-SMA in sinusoidal lining and perivascular zones together with weak expression in periportal area were observed (Figure 28). As four weeks, moderate α-SMA expression in sinusoidal lining area and moderate to strong immunostaining around central veins were observed (Figure 29). After eight weeks strong homogenous positive staining α-SMA in sinusoids, marked proliferation of smooth muscle cells around portal vein with strong positivity of α-SMA was noticed (Figure 30).

Two weeks after receiving ginseng and DMN in group (III), liver sections showed disturbed sinusoidal architecture with moderate α-SMA immunostaining expression and mild expression around central vein (Figure 31). After four weeks, faint expression of α-SMA in sinusoidal lining and around central vein was noted. α-SMA was also observed in the cytoplasm of some hepatocytes as granular cytoplasmic stain (Figure 32). After eight weeks, moderate α-SMA was observed in sinusoidal lining but it was strong around central vein (Figure 33).

Receiving ginseng after DMN in group (IV), liver sections showed moderate α-SMA expression in both sinusoidal lining and perivascular area (Figure 34).

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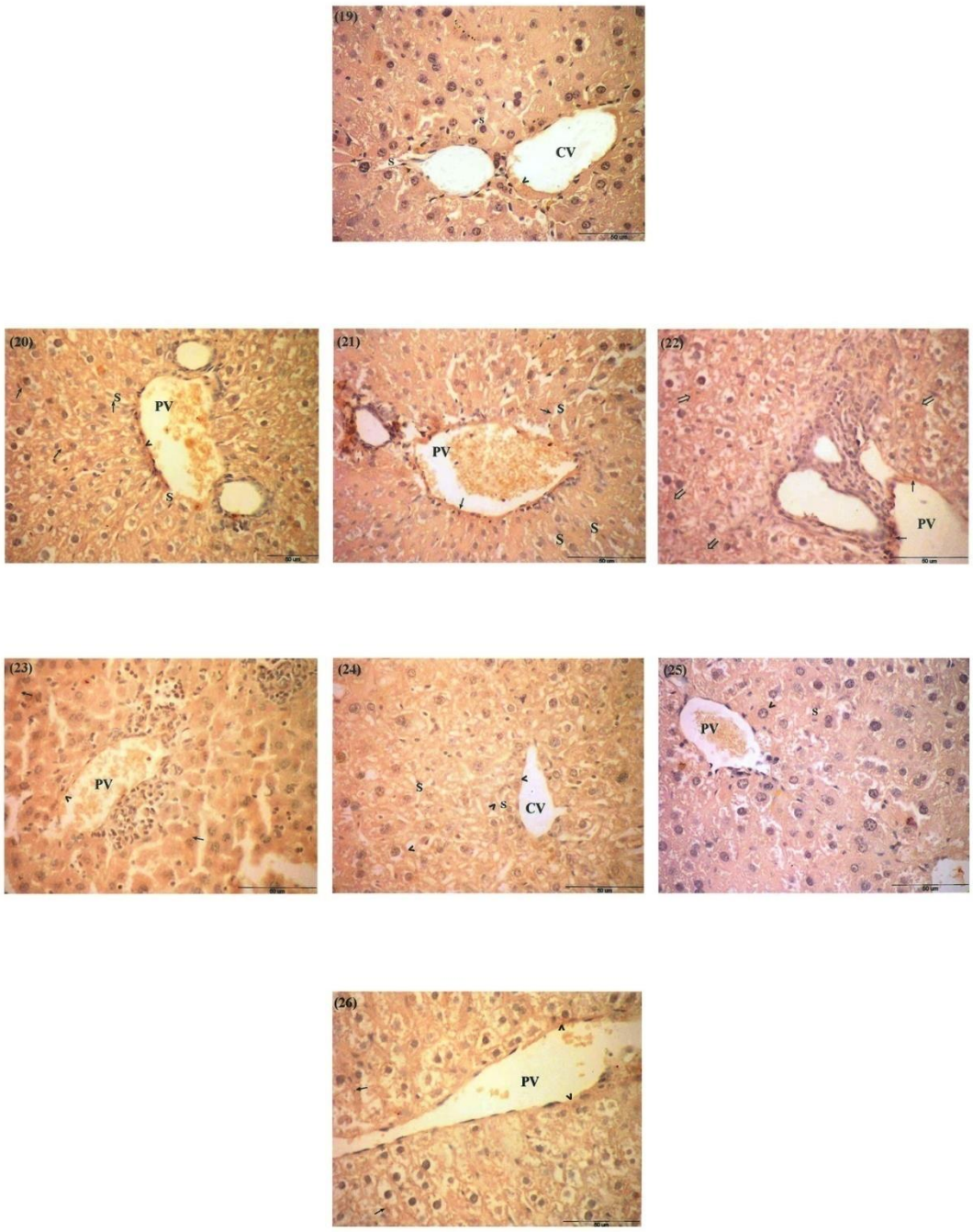

Figure (19): Paraffin section of normal control mouse liver showing very weak deposition of amyloid (^) around central vein (CV) and in sinusoids (S). (Congo red stain- Bar = 50 µm).

Figure (20): Two weeks in group II showing moderate deposition of amyloid (↑) around portal vein (PV) and weak deposition (^) in hepatocytes and in sinusoids (S). (Congo red stain- Bar = 50 µm).

Figure (21): Four weeks in group II showing moderate amyloid deposition (↑) around portal vein (PV) and in sinusoids (S). (Congo red stain- Bar = 50 µm).

Figure (22): Eight weeks in group II show moderate deposition of amyloid (↑) around portal vein (PV) and around some hepatocytes (⇓). (Congo red stain- Bar = 50 µm).

Figure (23): Two weeks in group III showing weak deposition of amyloid (^) around portal vein (PV) and moderate deposition (↑) in some hepatocytes. (Congo red stain- Bar = 50 µm).

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Figure (24): Four weeks in group III showing weak deposition of amyloid (^) in sinusoids around central vein (CV) and in hepatocytes. (Congo red stain- Bar = 50 µm).

Figure (25): Eight weeks in group III showing weak deposition of amyloid (^) in sinusoids and in hepatocytes. (Congo red stain- Bar = 50 µm).

Figure (26): Group IV showing weak deposition of amyloid (^) in sinusoids around portal vein (PV) and moderate around (↑) hepatocytes. (Congo red stain- Bar = 50 µm).

Image analysis

By using software (Leica Q -500), immunohistochemical results were expressed as mean ±SD. Statistical significant differences between groups were estimated with students "t" test. Value of $p \leq 0.05$ was significant and $p < 0.001$ was highly significant while $p > 0.05$ was insignificant.

From the current data we could summarize the results in tables I and II and bar graph. Table II showed that the number of α-SMA positivity in group II (0.63 ± 0.08) was significantly higher ($P < 0.05$) than all other groups {group I (0.52 ± 0.04), group III (0.54 ± 0.11) and group IV (0.58 ± 0.06)}.

Table (I): Comparison between the different studied groups according to the mean number of α-SMA positivity.

	Group I	Group II			Group III			Group IV
		2 weeks	4 weeks	8 weeks	2 weeks	4 weeks	8 weeks	
Min. – Max.	0.48 – 0.57	0.47 - 0.67	0.57 - 0.71	0.63 - 0.74	0.54 - 0.71	0.50 - 0.65	0.40 - 0.42	0.49 - 0.65
Mean ± SD	0.52 ± 0.04	0.57 ± 0.08	0.63 ± 0.05	0.70 ± 0.05	0.62 ± 0.07	0.58 ± 0.07	0.41 ± 0.01	0.58 ± 0.06
Median	0.49	0.57	0.63	0.72	0.63	0.63	0.41	0.56

Table (II): Comparison between the different studied groups according to the mean number of α-SMA positivity.

	Group I (n = 4)	Group II (n = 12)	Group III (n = 12)	Group IV (n = 4)
Mean value				
Min. –Max.	0.48 – 0.57	0.47 - 0.74	0.40 – 0.71	0.49 - 0.65
Mean ± SD	0.52 ± 0.04	0.63 ± 0.08	0.54 ± 0.11	0.58 ± 0.06
Median	0.49	0.63	0.54	0.56
P	0.020*			
P_1		0.117	0.979	0.783
P_2			0.117	0.049*
P_3			0.874	

P: p value for Kruskal Wallis test for comparing between the different studied groups
p_1: p value for Mann Whitney test for comparing between group I and each other group
p_2: p value for Mann Whitney test for comparing between group II and each other group
p_3: p value for Mann Whitney test for comparing between group III and group IV
*: Statistically significant at $p \leq 0.05$

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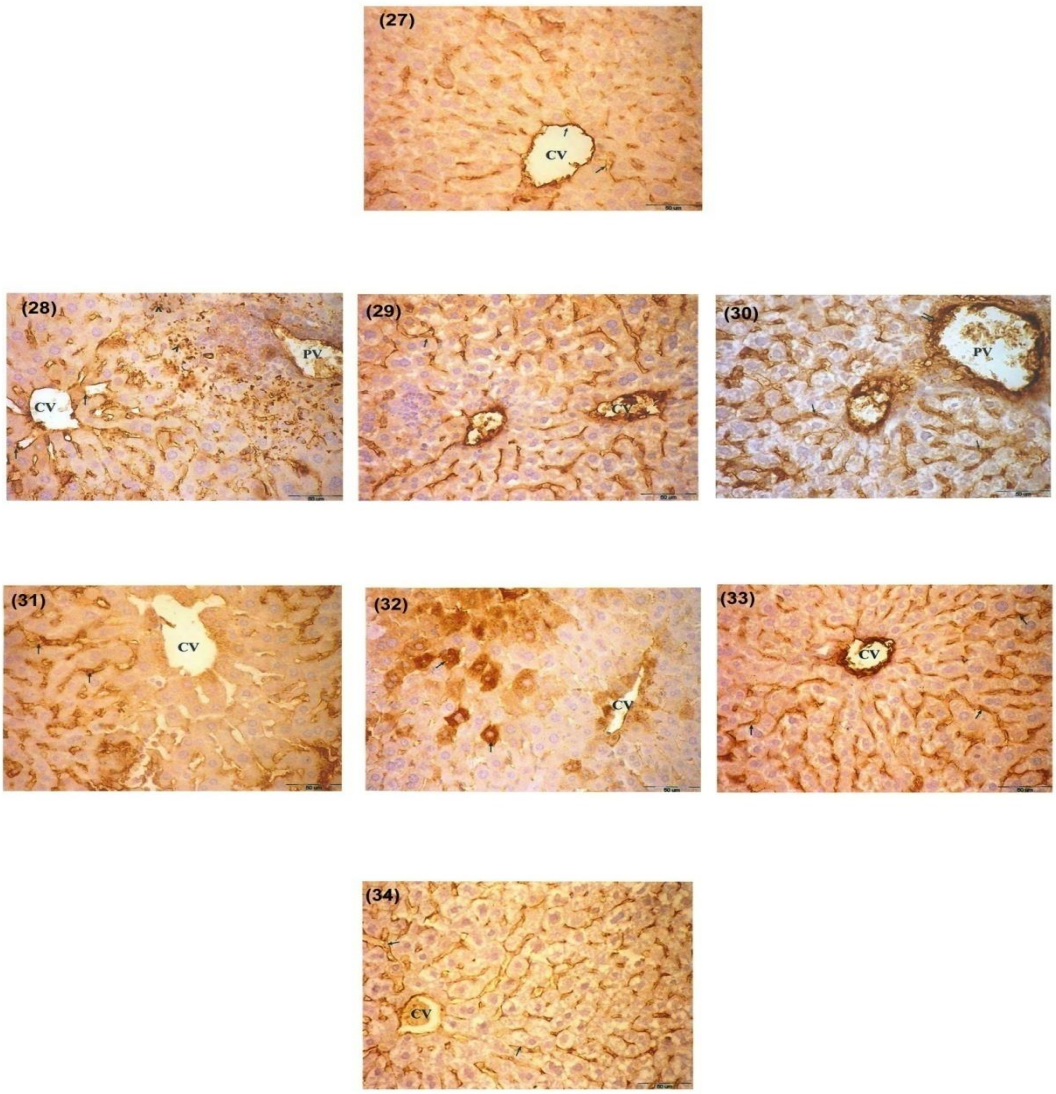

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Figure (27): Control mouse liver section showing faint regular α-SMA expression in sinusoidal lining (↑) and around central vein (CV). (Avidin–biotin complex - Bar = 50 µm).

Figure (28): Two weeks in group II showing moderate α-SMA expression in sinusoidal lining (↑) at perivascular zones around central vein (CV) and dotted expression in periportal region (^). (Avidin–biotin complex - Bar = 50 µm).

Figure (29): Four weeks in group II showing moderate α-SMA expression in sinusoidal lining area (↑) and moderate to strong around central vein (CV). (Avidin–biotin complex - Bar = 50 µm).

Figure (30): Eight weeks in group II showing strong α-SMA expression as homogenous distribution in sinusoids (↑) and around portal vein (PV). Note marked proliferation of smooth muscle cells in perivascular area. (Avidin–biotin complex - Bar = 50 µm).

Figure (31): Two weeks in group III showing moderate α-SMA expression in sinusoidal architecture (↑) and mild expression around central vein (CV). (Avidin–biotin complex - Bar = 50 µm).

Figure (32): Four weeks in group III showing faint expression of α-SMA around central vein (CV). Note: Immunostaining strong expression of α-SMA in cytoplasm in some hepatocytes (↑). (Avidin–biotin complex - Bar = 50 µm).

Figure (33): Eight weeks in group III showing moderate α-SMA expression in sinusoidal lining cells (↑) and strong around central vein (CV). (Avidin–biotin complex - Bar = 50 µm).

Figure (34): Group IV showing moderate α-SMA expression in sinusoidal lining (↑) and around central vein (CV). (Avidin–biotin complex - Bar = 50 µm)

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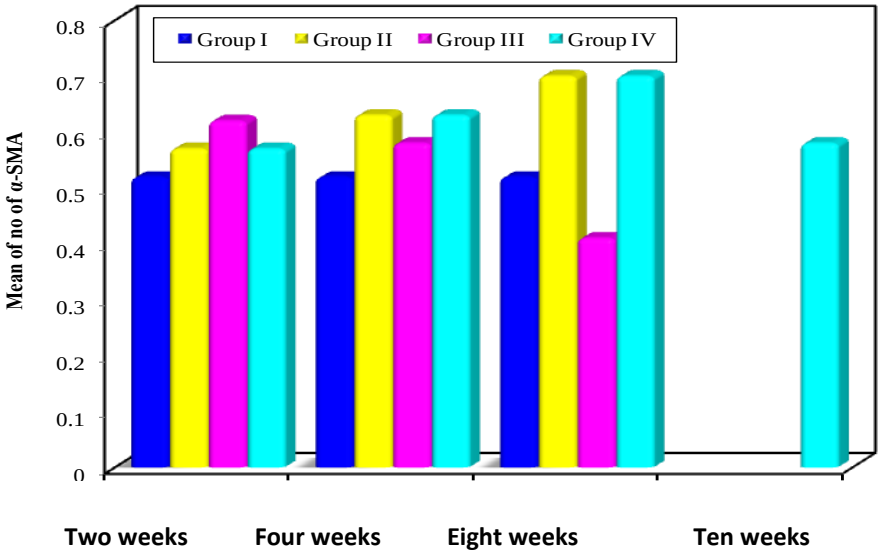

Bar graph: Comparison between the different studied groups according to the mean number of α-SMA positivity.

DISCUSSION

A high single dose of DMN administered in experimental animals caused liver central necrosis, hemorrhage and necrosis at its acute phase similar to human fulminate hepatitis [25] and induced liver fibrosis in a highly reproductive manner by establishing micronodular fibrosis and cirrhosis after three weeks of administration [26].

Histopathologically, De Gouville *et al* [6], illustrated that in rats hepatic fibrosis developed within 3 weeks after exposure to DMN, and the morphological changes were induced such as fibrous septa formation, nodular degeneration, portal hypertension, and development of ascites.

Oryan *et al* [27] showed that DMN-induced histopathological changes such as hemorrhage, necrosis, endothelial cell damage and inflammatory cell infiltration in the liver parenchyma of rats.

Our present result showed that after receiving DMN there were degeneration in liver cells started after two weeks with mild congestion and steatosis, lymphatic infiltrations within portal tract and few apoptotic figures were also observed.

These changes were increased after four weeks till the end of experiment (after eight weeks) where the liver sections showed maximum damage, loss of normal lobular architecture, zonal degeneration, congestion, marked edema, widening in sinusoids, numerous apoptotic figures and fatty degeneration with ballooning hepatocytes were observed.

Several pharmacological activities of ginseng extracts or ginseng dammarane saponin have been reported, including effect on central nervous system, antipsychological action,

tranquillizing effects, protection from stress ulcers, increase of gastrointestinal motility, antifatigue action, endocrinological effects, and enhancement of sexual behavior, accelerating of metabolism, synthesis of carbohydrates, lipids, RNA and proteins [28].

Many researchers have contributed to the accumulation of evidence that ginsenosides are responsible for many biological activities including anti-inflammatory, anti-allergic, and anti-tumor activities seen in cell culture or in vivo studies following intraperitonial or intravenous injection of experimental animals [29].

Perez *et al* [30] showed that panax ginseng inhibited the development of liver cirrhosis in thioacetamide (TA) treated rats. They also concluded that the mechanism of action was associated with decreased oxidative stress and hepatic necroinflammation. Also panax ginseng was found to exert a stimulatory effect on DNA repair synthesis and had an inhibitory effect on mutagencity and cellular transformation [31].

Lee *et al* [32] stated that ginseng might reduce cell damage induced by toxic substances and acted to stabilize cell membranes by providing protection against toxic agents induced tissue injury.

Red ginseng was found to inhibit development of DMN-induced liver cancer in rats [33] and skin cancer in mice [34]. Also ginseng was shown to have a strong inhibitory effect of rat mammary adenocarcinoma induced by methyl-N-nitrosourea as well as development of experimental uterine cervix and vaginal tumors [35].

Li *et al* [36] observed that, dietary administration of red ginseng suppressed preneoplastic lesions in the colon of rats induced by 1,2 dimethylhydrazine.

Ginseng significantly reduced the histopathological changes of liver cells and accelerated liver regeneration especially its effect on myofibroblasts that are thought to be deeply involved with liver fibrosis seems to be useful for repair of liver injury, improvement of fibrotic changes of the liver and promoting regeneration in liver injured rats. Thus improvement included the suppression of activation of myofibroblasts in the liver, and the improvement of retinoid storage in the cells.

Two weeks post ginseng which was received at the end of DMN injection showed mild edema and mild congestion as well as fatty degeneration, apoptosis and area of necrosis. These findings suggest that ginseng had a role in protecting the hepatocytes from injury caused by DMN thereby.

Hepatic fibrosis is a wound-healing process in livers with chronic injury and is characterized by the excess production and deposition of ECM components which resulted in excessive collagen deposition in the liver [37] and the replacement of low density type IV with high density type I collagen within the subendothelial space of Disse [17]. Bataller & Brenner [1]

concluded that liver fibrosis reflects imbalance between the synthesis and breakdown of extracellular connective tissue components of the liver.

The present results showed that after DMN injection moderate deposition of collagen fibers (mainly in perivascular area, around portal veins and around sinusoids), to strong thickening of collagen fibers were observed. While after ginseng these collagen deposits were mild especially around blood vessels and in sinusoides.

The increase of total liver collagen observed in the present study was coincided with the previous investigations on DMN-induced hepatic fibrosis in rats [38]. The ginseng significantly suppressed the increase of collagen accumulated in the DMN induced liver injury.

Lo *et al* [39] suggested that ginsenoside Rb1 which was the modern active component purified from ginseng and has been considered to be hepatoprotective exerted an antifibrotic effect by inhibiting the expression of collagen (mRNA expression of type I and III collagen).

Active inflammatory disease causing persistently high serum concentrations of serum amyloid A protein is associated with progressive AA amyloidosis [40] whereas occasional cases of clinical improvement suggesting amyloid regression have been reported in patients in whom the underlying disease has remitted.

Mandelstam [41] showed that there was net accumulation of AA amyloid in the liver and elsewhere in most patients whose underlying inflammatory disorder remained active, and that parenchymal liver infiltration was a late sign with a poor prognosis. Abnormalities of liver function tests were common but non-specific, and proteinuria was almost universal whether hepatic amyloid was present or not.

Accordingly our present study showed that after receiving DMN moderate deposition of amyloid in portal areas and increase deposition around some hepatocytes whereas after ginseng amyloid material showed weak deposition around portal veins and moderate deposition around hepatocytes.

Frequent estimation of the plasma serum amyloid A protein is highly desirable [42]. Substantial and fairly rapid regression of amyloid occurs in about one half of patients whose underlying acute phase response is controlled [43]. However the rate of amyloid regression is highly variable, and there is a poor correlation between the quantity of amyloid and the degree of resulting organ dysfunction. Similarly, in amyloidosis, chemotherapy regimens that reduce monoclonal immunoglobulin light chain production have been used with some success [44].

In the damaged hepatocytes, the membrane components, metabolites of toxic agents, and infiltrating inflammatory cells are the activators of Kupffer's cells, which release a number of soluble agents, including cytokines, reactive oxygen species (ROS), and other factors [45].

α-SMA is an actin isoform and a specific marker for smooth muscle cell differentiation [17]. The appearance of α-SMA positive cells has been reported to be associated with the process of liver fibrosis and the formation of liver nodules in rats [46].

Lo *et al* [39] showed that ginsenoside Rb1 at 5 - 40 mg/ml significantly reduced α-SMA levels in rats. The current study showed that the expression of α-SMA in sinusoids was strong homogenous positive staining, while marked proliferative of smooth muscle cells around portal vein was seen in group II after eight weeks. Receiving ginseng showed moderate expression of α-SMA in sinusoidal lining and perivascular area.

Our present data showed that DMN increased the content of α-SMA in the liver and this proliferation was suppressed by red ginseng administration. These findings suggested that the anti-fibrotic effect of red ginseng in turn led to hepatoprotective effect.

In conclusion, ginseng significantly reduced the histopathological changes of liver cells and accelerated liver regeneration. The ginseng significantly suppressed the increase of collagen accumulated in the DMN induced liver injury. Red ginseng suppressed the increased content of α-SMA induced by DMN. These findings supported the anti-fibrotic effect of red ginseng which in turn, led to hepatoprotective effect.

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