Original Article

Role of Myrrh Oil in Attenuating Carbon Tetrachloride-Induced Hepatic Injury in Adult Male Albino Rat. Histological and Immunohistochemical Study

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ABSTRACT

Introduction: Carbon tetrachloride (CCl4) is an environmental hepatic toxin that is usually used to induce hepatic injury in experimental animals. Myrrh essential oil is one of the main components of myrrh tree that has been widely used in folk medicine to treat different diseases.

Aim of the Work: This work was conducted to evaluate the possible role of myrrh oil in attenuating CCl4 induced hepatic injury in adult male albino rat employing histological and immunohistochemical methods.

Materials and Methods: Thirty adult male albino rats were divided into 4 groups; control group, myrrh oil-treated group (orally administered 50 mg/kg body weight once daily for four weeks), CCl4-treated group (injected intraperitoneally with 1ml/kg CCl4 dissolved in olive oil in 1:1 ratio twice a week for four weeks) and both myrrh oil and CCl4- treated group. Liver specimens were prepared for light microscopic examination using H&E & Masson's trichrome stains. Immunohistochemical study was performed using active caspase-3, α-SMA and PCNA antibodies.

Results: CCl4-treated group revealed disturbed hepatic architecture. Most of hepatocytes had vacuolated cytoplasm and small darkly stained nuclei. The central veins and portal veins were markedly dilated and congested with intense mononuclear cellular infiltrations in addition to bile duct proliferation and excessive collagen fiber deposition. The immunohistochemical study results showed a significant increase in the active caspase-3, α-SMA and PCNA immunoreaction. On the other side, negligible changes were detected in rats concomitantly treated with myrrh oil and CCl4 with a non-significant change in the immunohistochemical reactions.

Conclusion: Myrrh oil could be beneficial in attenuating CCl4-induced hepatic injury.

Received: 21 June 2021, **Accepted:** 08 August 2021

Key Words: α-SMA; active caspase-3; carbon tetrachloride; myrrh oil; PCNA.

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ISSN: 1110-0559, Vol. 45, No. 4

INTRODUCTION

The liver is considered an important complex organ in the body that is responsible for many physiological functions, such as excretory and secretory functions, protein synthesis, storage of nutrients and maintenance of homeostasis in addition to its defensive role against toxic drugs and xenobiotics[1]. So, this organ is at high risk of damage as it deals with most of chemicals, different drugs and environmental pollutants entering the body. Liver diseases have become a global health problem that causes high rate of deaths year by year[2].

Carbon tetrachloride (CCl4) is an environmental hepatic toxin that is almost used to induce hepatic injury in experimental animal models[3]. Occupational exposure to CCl4 may occur in chemical industries or laboratories, through inhalation, ingestion or by dermal contact[4].

The treatment of liver diseases with conventional medications may be associated with many side effects, so there is a rising demand and great interest in natural herbal medicines that usually have multi-target and multi-component characteristics making them more beneficial in the treatment of various diseases with quite low negative effects[5].

Myrrh is a reddish brown sap like substance extracted from a thorny tree Commiphora Myrrha, also known as Commiphora Molmol that is common in Africa and the Middle East. Based on its chemical structure, myrrh is composed of resins, gum and essential oils. It has a broad history in traditional medicine[6]. Moreover, myrrh has been accepted by the FDA as a safe flavoring agent in foods and drinks in addition to its use as a fragrance in cosmetics[7]. Furthermore, many scientific studies have proven the beneficial uses of myrrh in medicine[8,9]. In addition, myrrh is used for skin wound healing for many years[10]. Myrrh essential oil is a main component of myrrh obtained by distillation from the oleogum resins. This oil has been widely used in folk medicine to treat different diseases, as it was proved to possess various pharmacological effects including anti-inflammatory[11], antioxidant[12], antiparasitic[13], antifungal[14], antibacterial[15], antihyperlipidemic[16] as well as anticancer effects[17,18]. While myrrh is an essential medicinal herb having powerful antioxidant and anti-inflammatory activities, its

 DOI: 10.21608/ejh.2021.80815.1506

hepato-protective action has not been fully illuminated. Therefore, this study was performed by using different histological and immunohistochemical methods to assess the possible role of myrrh oil in attenuating CCl4 induced hepatic injury in rats.

MATERIALS AND METHODS

Animals

After the permission of the Local Ethics Committee of Faculty of Medicine, Tanta University, Egypt, a total of thirty adult male albino rats of average weight 180–200 grams each were employed in this study. The rats were randomly grouped in clean properly ventilated plastic cages with free accessibly to water and food ad libitum throughout the experimental period. The rats were adapted for 2 weeks before the experiment, and were kept on a 12h light/12h dark cycle before the experiment and throughout the experimental period.

Animals grouping and experimental procedures

Thirty adult male albino rats were randomized into four groups:

Group I (Control group): Twelve rats were subdivided into two subgroups of six rats each; subgroup IA were kept without intervention, subgroup IB was intraperitoneally administered 1ml/kg olive oil (vehicle for CCl4) twice a week for four weeks.

Group II (myrrh oil-treated group): Six rats were orally administered myrrh oil at dose of 50 mg/kg once daily for four weeks[19]. Myrrh oil was purchased from Sakkara Company for essential oils, Giza, Egypt.

Group III (CCl4-treated group): Six rats were intraperitoneally injected with 1ml/kg CCl4 dissolved in olive oil in 1:1 ratio twice a week for four weeks. This dose of CCl4 was selected according to previous studies[20]. CCl4 was purchased from El Nasr Pharmaceutical and Chemical Company, Cairo, Egypt.

Group IV (myrrh oil & CCl4 group): Six rats were administered concomitantly both myrrh oil and CCl4 (at the same doses and duration as in groups II and III, respectively).

At the end of the experimental period, the animals were deprived of food overnight before being euthanized by intraperitoneal injection of pentobarbital (40 mg/kg)[21]. The livers were then dissected out, cleaned and prepared for light microscopic study.

For light microscopic study

Briefly, the liver specimens after being immersed in 10% neutral-buffered formalin, were washed, dehydrated, cleared and then embedded in paraffin. 5µm thick sections were stained with haematoxylin & eosin (H&E) stain. Masson's trichrome stain was used for detection of collagen fibers[22].

For immunohistochemical staining

Briefly, about 5 µm thick sections were dewaxed, rehydrated and washed with phosphate buffered saline (PBS), then they were incubated with 10% normal goat serum in PBS. The sections were then incubated with the primary antibodies; rabbit polyclonal anti- activated caspase-3 antibody (1:100 dilution) (ab2302; Abcam, Cambridge, Massachusetts, USA), mouse monoclonal anti-alpha smooth muscle actin (α-SMA) antibody (1:500 dilution) (ab7817; Abcam, Massachusetts, USA) and rabbit polyclonal anti-proliferating cell nuclear antigen (PCNA) antibody (1:500 dilution) (ab152112, Abcam, Cambridge, Massachusetts, USA). Then, the sections were incubated with biotinylated IgG at room temperature for 60 min, and then with a streptavidin–biotin–peroxidase conjugate for additional 60 min. As a chromogen, diaminobenzidine (DAB) was added to the slides. As a counterstain, Mayer's haematoxylin was used. The negative control sections were performed without the primary antibodies[23]. For the positive controls and according to the product data sheets, camptothecin-treated Jurkat cells were used for active caspase-3, human cervical carcinoma tissue was used for PCNA and human breast ductal carcinoma tissue was used for α-SMA. Positive cells for active caspase-3 showed brown cytoplasmic/or nuclear coloration. Positive cells for α-SMA brown cytoplasmic coloration, while positive cells for PCNA showed brown nuclear coloration.

Morphometric analysis

A Leica light microscope (DM500, Swizterland) was used to attain images, and image analysis was done by using the software "ImageJ" (version 1.48v National Institute of Health, Bethesda, Maryland, USA). From each slide, randomly selected ten non-overlapping fields were examined at a magnification power of 400 to measure:

1. The mean area percentage of collagen fibers.
2. The mean area percentage of positive active caspase-3 immunohistochemical reaction.
3. The mean area percentage of positive α-SMA immunohistochemical reaction.
4. The mean number of PCNA-immunopositive hepatocytes.

Statistical analysis

One-way analysis of variance (ANOVA) was used for data analysis followed by Tukey's procedure to compare between the study groups. Values were presented as mean ± standard deviation. Probability value $p < 0.05$ considered significant and $p<0.001$ considered highly significant[24].

RESULTS

In the present study, no deaths were recorded during the whole study period.

Histological Results

H&E staining

Sections from control group I (including subgroups IA &IB) as well as myrrh oil treated group II showed

nearly the same findings. They revealed the normal lobular architecture of hepatic tissue that showed cords of hepatocytes that appeared branching and anastomosing. These hepatocytes cords were radiating from the central vein at the center toward the portal tract areas at the periphery. The hepatocytes were polygonal in shape having acidophilic cytoplasm and central vesicular rounded nuclei with prominent nucleoli. Some hepatocytes appeared bi-nucleated. In between the hepatocytes cords, the hepatic sinusoids appeared as irregular vascular spaces lined with flat endothelial cells. The portal tract areas contained branches of portal vein, hepatic artery, bile duct and lymphatic vessels that were all supported by minimal connective tissue (Figures 1,2,3).

Sections from CCl4 treated group III showed disturbed hepatic architecture as most of hepatocytes had vacuolated cytoplasm, and small darkly stained (pyknotic) nuclei, others appeared ballooned with cytoplasmic debris around the nuclei. In addition, massive dilatation and congestion of central veins with injured endothelial lining and intense perivascular mononuclear cellular infiltrations were observed. Moreover, the hepatic blood sinusoids appeared dilated and congested in some sections. Furthermore, the portal veins appeared massively dilated and congested with perivascular mononuclear cellular infiltrations. Moreover, bile duct proliferation was noticed in some sections, and some oval cells with pale stained nuclei appeared starting to migrate to hepatic parenchyma (Figures 4,5,6,7).

On the other hand, sections from myrrh oil & CCl4 treated group IV showed most of hepatocytes more or less normal having acidophilic cytoplasm and vesicular nuclei, while few hepatocytes had small darkly stained nuclei The portal areas showed apparently normal portal vein but with minimal mononuclear cellular infiltrates. The central veins appeared quietly normal in some sections, while being mildly dilated and congested in others (Figures 8,9,10).

Masson's trichrome staining

The examined sections of control group I and myrrh oil treated group II revealed scanty collagen fibers around the central veins and at portal tract areas (Figure 11A,11B). While in CCl4 treated group III, the deposition of collagen fibers was apparently markedly increased and abundant collagen fibers were observed around the central veins, the portal vessels as well as the proliferating bile ducts at the portal tract areas. Moreover, some collagen fibers were observed in-between hepatocytes (Figure 12A,12B,12C). In contrast, myrrh oil and CCl4 treated group IV revealed few collagen fibers around central veins and at the portal tract areas compared to the CCl4 treated group III (Figures 13A,13B).

Statistical analysis of the mean area percentage of collagen fibers illustrated a high significant increase in CCl4-treated group III compared to control group I. On the other side, myrrh oil and CCl4 group IV expressed a non-significant difference when compared to control group (Table 1, Histogram 1 A).

Immunohistochemical Results

Immunohistochemical staining for active caspase-3

The examined sections from control group I and myrrh oil treated group II revealed only few hepatocytes exhibiting faint positive active caspase-3 immunoreaction (Figure 14A). While hepatic sections of CCl4 treated group III showed most of hepatocytes showed strong positive cytoplasmic immunoreaction for active caspase -3 (Fig. 14B), however myrrh oil treated group IV revealed few hepatocytes having weak positive active caspase-3 immunoreaction (Figure 14C).

Statistical analysis of the mean area percentage of active caspase-3 immunoreaction illustrated a high significant increase in CCl4-treated group compared to control group, whereas myrrh oil & CCl4 group expressed a non-significant difference when compared to control group (Table 1, Histogram 1 B).

Immunohistochemical staining for α-SMA

The examined sections from control group I and myrrh oil treated group II showed that the positive immunostaining for α- SMA was minimal and restricted to the vascular smooth muscle cells in the wall of the central vein (Figure 15A), and the blood vessels in the portal area (Figure 15B). While in CCl4 treated group III, this positive immunostaining was more extensive being demonstrated in vascular smooth muscle cells, peri-sinusoidal and around proliferating bile ducts. (Figures 16A,16B). On the other side, myrrh oil & CCl4 treated group IV demonstrated a limited α- SMA staining that appeared in the vascular smooth muscle cells and within few cells between hepatocytes compared to CCl4 treated group III (Figures 17A,17B).

Statistical analysis of the mean area percentage of α- SMA immunoreaction illustrated a high significant increase in CCl4-treated group compared to control group, whereas myrrh oil & CCl4 group expressed a non-significant difference when compared to control group (Table 1, Histogram 1 C).

Immunohistochemical staining for PCNA

The examined sections from the control group I and myrrh oil treated group II showed few faint brown PCNA positive nuclei among few hepatocytes (Figure 18A). While in CCl4 treated group III, an intense positive PCNA nuclear immune reaction appeared in most of hepatocytes as well as in the nuclei of proliferating bile ducts (Figure 18B). On the other hand, myrrh oil & CCl4 treated group IV demonstrated few PCNA positive nuclei compared to CCl4 treated group (Figure 18C).

Statistical analysis of the mean number of PCNA immunopositive nuclei illustrated a high significant increase in CCl4-treated group III compared to control group, whereas myrrh oil & CCl4 group IV expressed a non-significant difference when compared to control group (Table 1, Histogram 1 D).

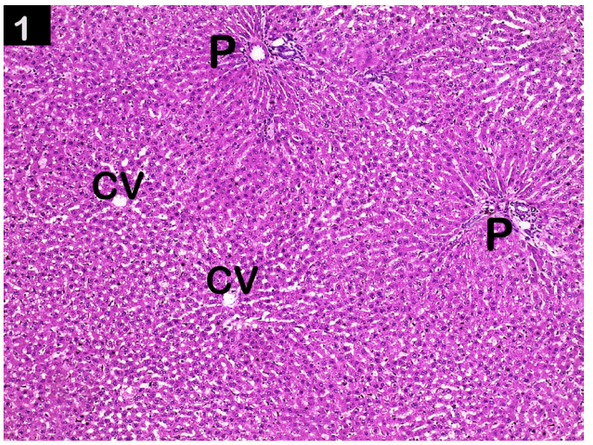


Fig. 1: H&E staining of control group showing the normal lobular architecture of hepatic tissue with central veins (CV) at the center and portal tract areas (P) at the periphery. (Magnification X 100)

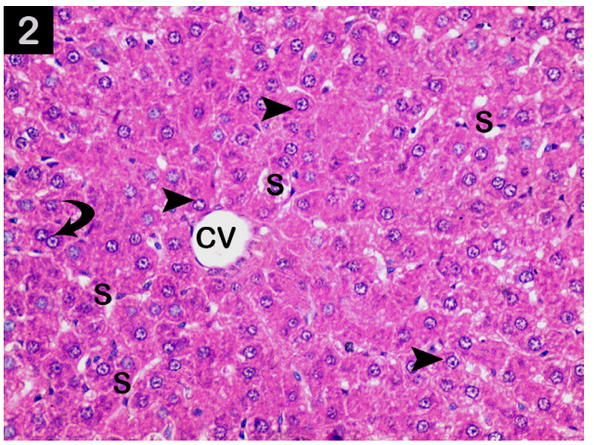


Fig. 2: H&E staining of control group showing branching and anastomosing cords of hepatocytes radiating from the central vein (CV). The hepatocytes cords are separated by hepatic sinusoids (S) which are irregular vascular spaces lined with flat endothelial cells. The hepatocytes are polygonal in shape having acidophilic cytoplasm and central vesicular rounded nuclei with prominent nucleoli (arrow head). Notice some hepatocytes are bi-nucleated (curved arrow) (Magnification X 400)

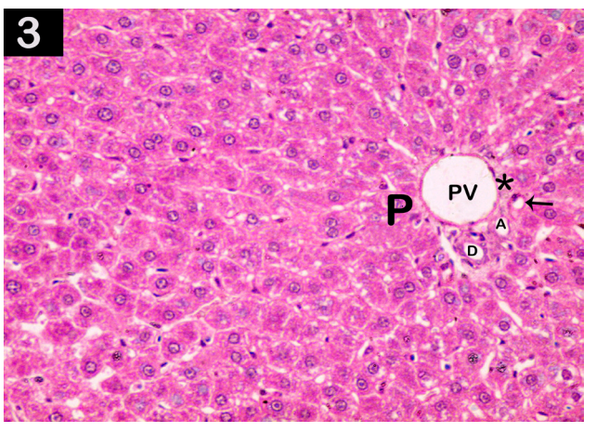


Fig. 3: H&E staining of control group showing the portal tract areas (P) contains branches of portal vein (PV), hepatic artery (A), lymphatic vessel (arrow) and bile duct (D) supported by minimal connective tissue (asterisk). (Magnification X 400)

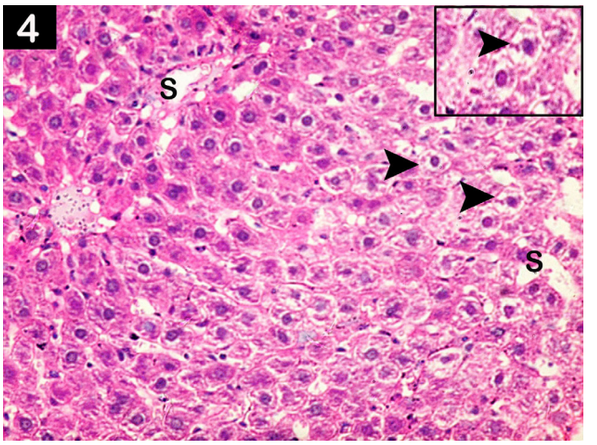


Fig. 4: H&E staining of CCl4 treated group showing disturbed hepatic architecture as most of hepatocytes appear with vacuolated cytoplasm and small darkly stained (pyknotic) nuclei (arrow head). Notice the dilated blood sinusoids (S) (Magnification X 400)

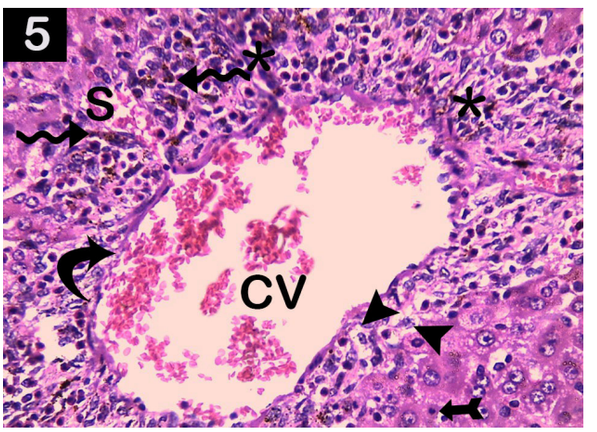


Fig. 5: H&E staining of CCl4 treated group showing massive dilatation and congestion of central vein (CV) with injured endothelial lining (curved arrow) and intense perivascular mononuclear cellular infiltration (asterisk). Notice the degenerated hepatocytes that appear ballooned with cytoplasmic debris around the nuclei (arrow head). Other hepatocytes had small darkly stained (pyknotic) nuclei (notched arrow). The hepatic blood sinusoids (S) are dilated and congested. Note: Kupffer cells (wavy arrow) engulfing brownish pigment granules are seen. (Magnification X 400)

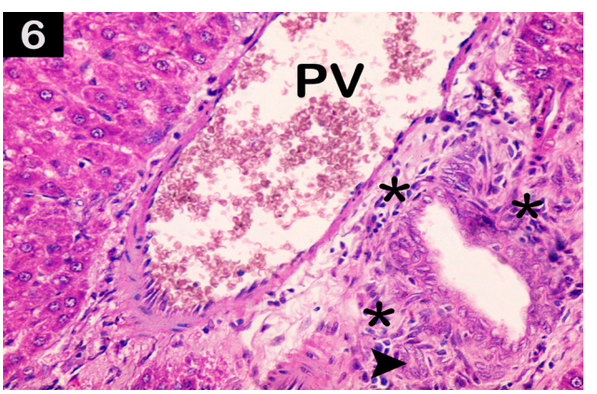


Fig. 6: H&E staining of CCl4 treated group showing massively dilated congested portal vein (PV) with perivascular mononuclear cellular infiltration (asterisk). Note: Oval cells having pale stained nucleus (arrow head) can be seen and they appear starting to migrate to hepatic parenchyma (Magnification X 400)

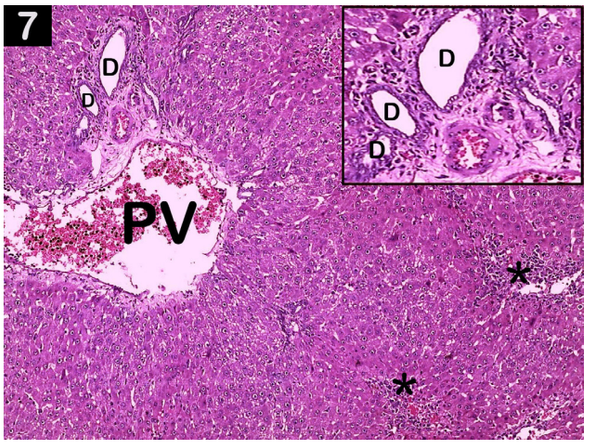


Fig. 7: H&E staining of CCl4 treated group showing markedly dilated and congested portal vein and proliferation of bile ducts (D) in the portal area (P). Notice the areas of mononuclear cellular infiltration (asterisk). (Magnification X 100, Inset X 400)

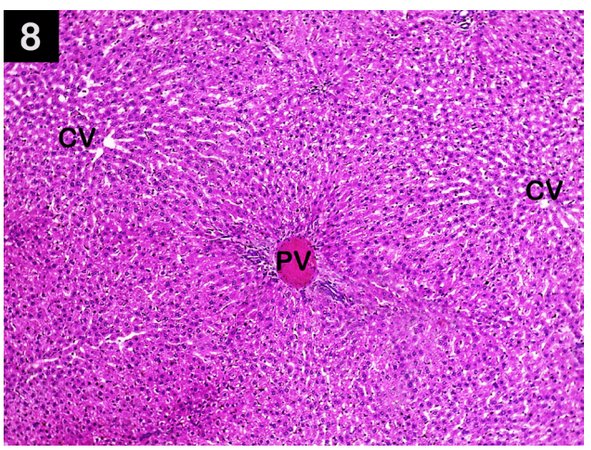


Fig. 8: H&E staining of myrrh oil & CCl4 treated group showing more or less normal hepatic lobular architecture with apparently normal central veins (CV). The Portal area shows mildly dilated and congested portal vein (PV) (Magnification X 100)

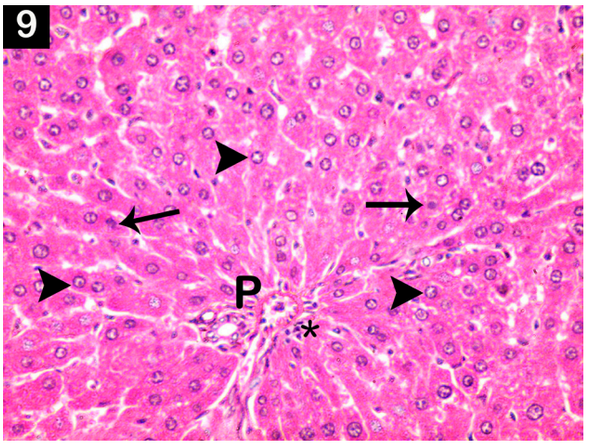


Fig. 9: H&E staining of myrrh oil & CCl4 treated group showing most of hepatocytes more or less normal having acidophilic cytoplasm and vesicular nuclei (arrow head). Few hepatocytes appear having small darkly stained nuclei (arrow). Portal areas (P) shows apparently normal portal vein (PV) with minimal mononuclear cellular infiltrates (asterisk) (Magnification X 400)

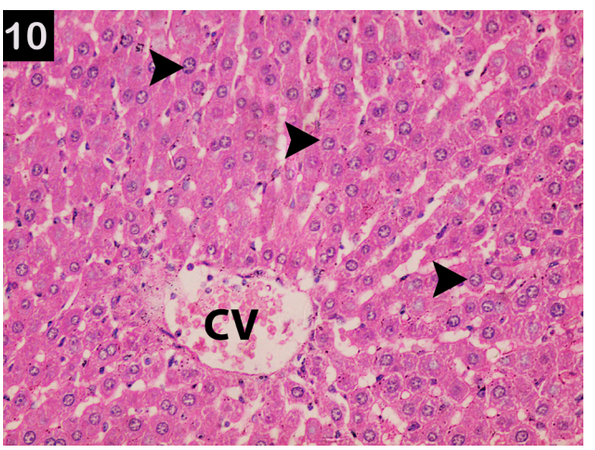


Fig. 10: H&E staining of myrrh oil & CCl4 treated group showing mildly dilated and congested central vein (CV). Note: Most of hepatocytes appear quietly normal exhibiting acidophilic cytoplasm and vesicular nuclei. (Magnification X 400)

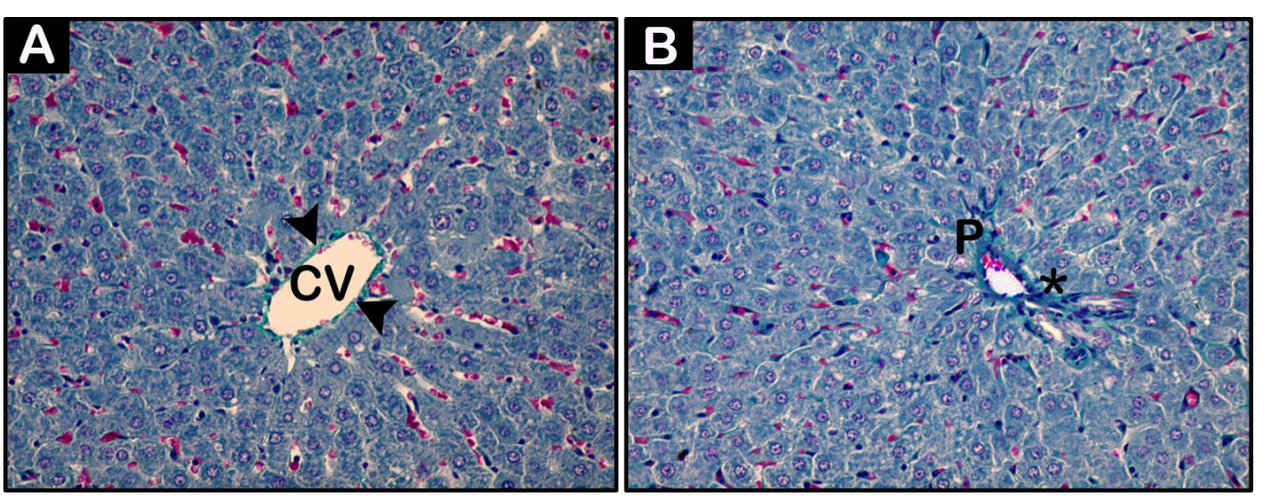


Fig. 11: Masson's trichrome stained sections of control group. A: showing scanty collagen fibers (arrow head) around the central vein (CV). B: showing scanty collagen fibers (asterisk) at portal tract areas (P). (Magnification X 400)

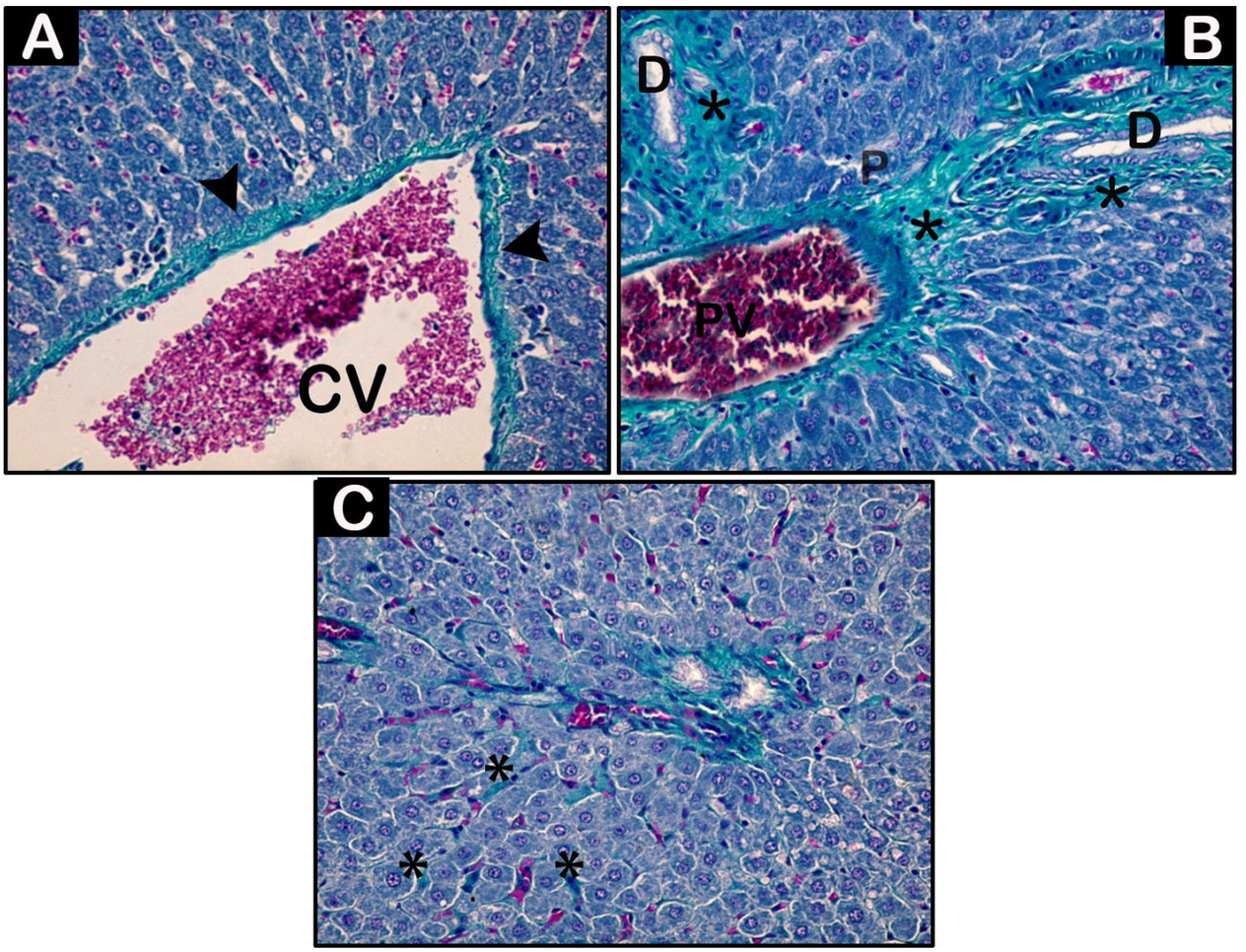


Fig. 12: Masson's trichrome stained sections of CCl4 treated group. A: showing abundant collagen fibers (arrow head) around the central vein (CV). B: showing abundant collagen fibers (asterisk) around the portal blood vessels and around the proliferating bile ducts (D) at portal tract areas (P). C: showing some collagen fibers (arrow head) in-between the hepatocytes. (Magnification X 400)

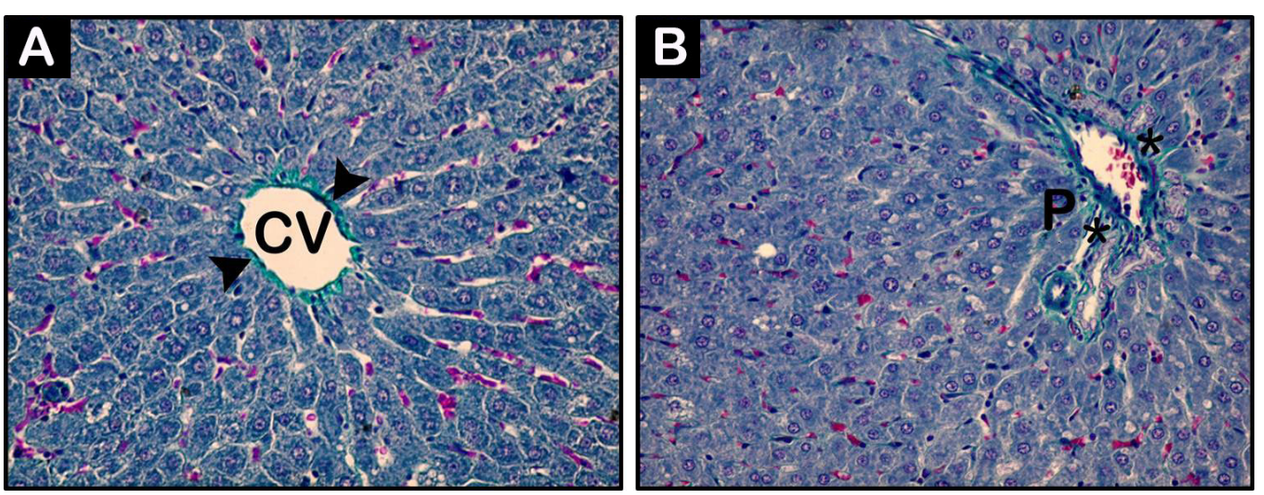


Fig. 13: Masson's trichrome stained sections of myrrh oil and CCl4 treated group A: showing few collagen fibers (arrow head) around the central vein (CV). B: showing few collagen fibers (asterisk) at portal tract area (P). (Magnification X 400)

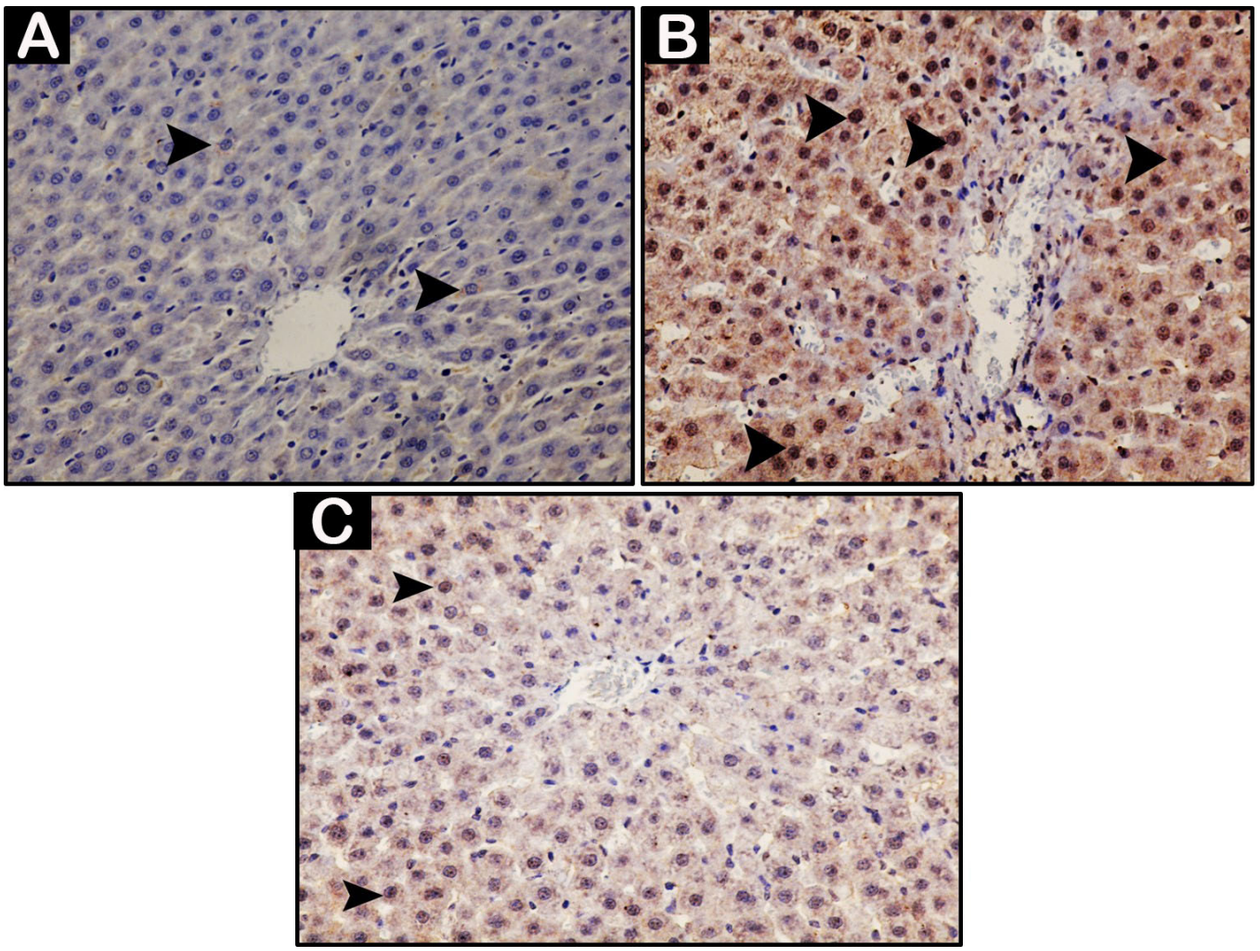


Fig. 14: Active caspase-3 immunostained sections. A: control group showing few hepatocytes with faint positive cytoplasmic immunoexpression (arrow head). B: CCl4 treated group showing most of hepatocytes with strong positive cytoplasmic and nuclear immunoexpression (arrow head). C: myrrh oil and CCl4 treated group showing few hepatocytes with weak positive cytoplasmic immunoexpression (arrow head). (Magnification X 400)

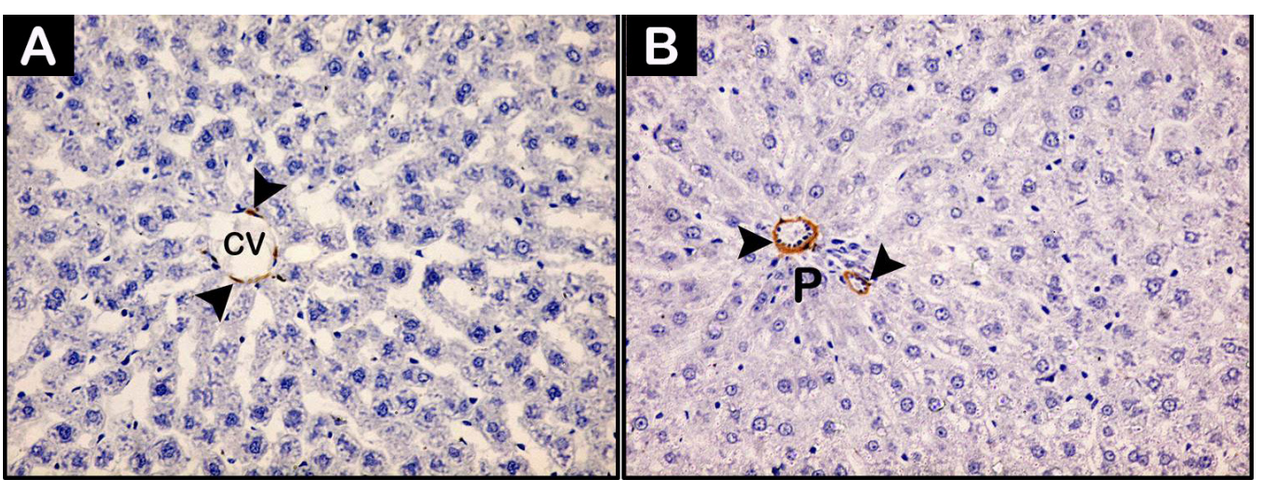


Fig. 15: α-SMA immunostained sections of control group. A: showing a minimal positive immune-staining for α- SMA in the vascular smooth muscle cells (arrow head) in the wall of central vein (CV). B: showing a minimal positive immune-staining for α- SMA in the vascular smooth muscle cells (arrow head) of the wall of blood vessels in the portal area (P). (Magnification X 400)

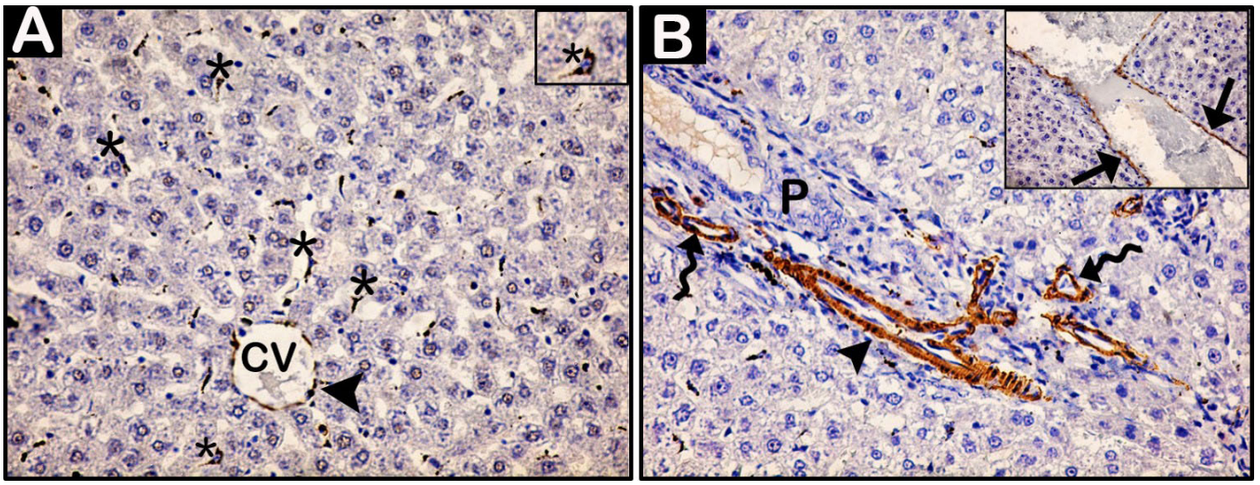


Fig. 16: α-SMA immunostained sections of CCl4 treated group. A: showing many intense positive α-SMA stained cells (asterisk) in between the hepatocytes. These cells appear as spindle shaped cells having fine processes in addition to positive immune-staining (arrow head) in the wall of central vein (CV). B: showing an intense positive immune-staining in the wall of blood vessels (arrow head) of portal area (P) as well as around the proliferating bile ducts (wavy arrow). The inset shows an intense positive α-SMA staining around the blood sinusoids (arrow) (Magnification X 400)

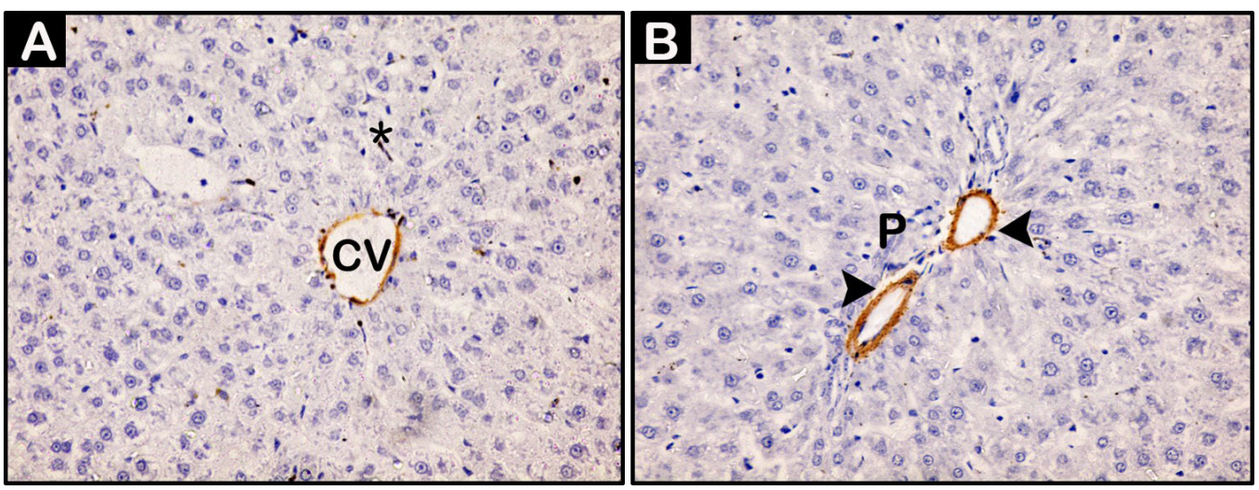


Fig. 17: α-SMA immunostained sections of myrrh oil & CCl4 treated group A: showing a limited positive α- SMA immune-staining that appears only in wall (arrow head) of the central vein (CV). Note: Few positive α-SMA stained cells (asterisk) are seen in-between hepatocytes. B: an apparent reduction in α- SMA immune-staining that appears only in the wall of blood vessels (arrow head) in the portal area (P). (Magnification X 400)

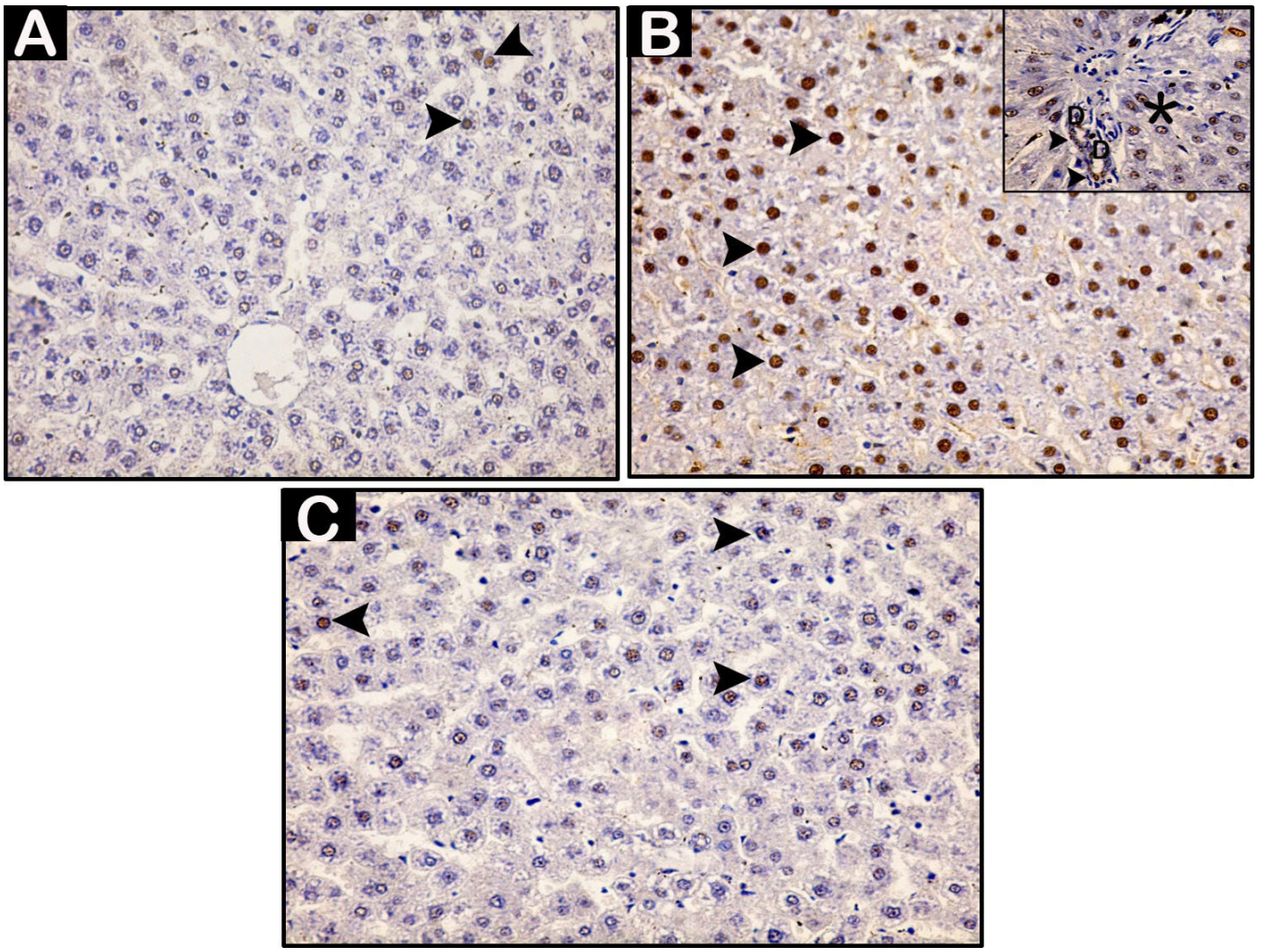
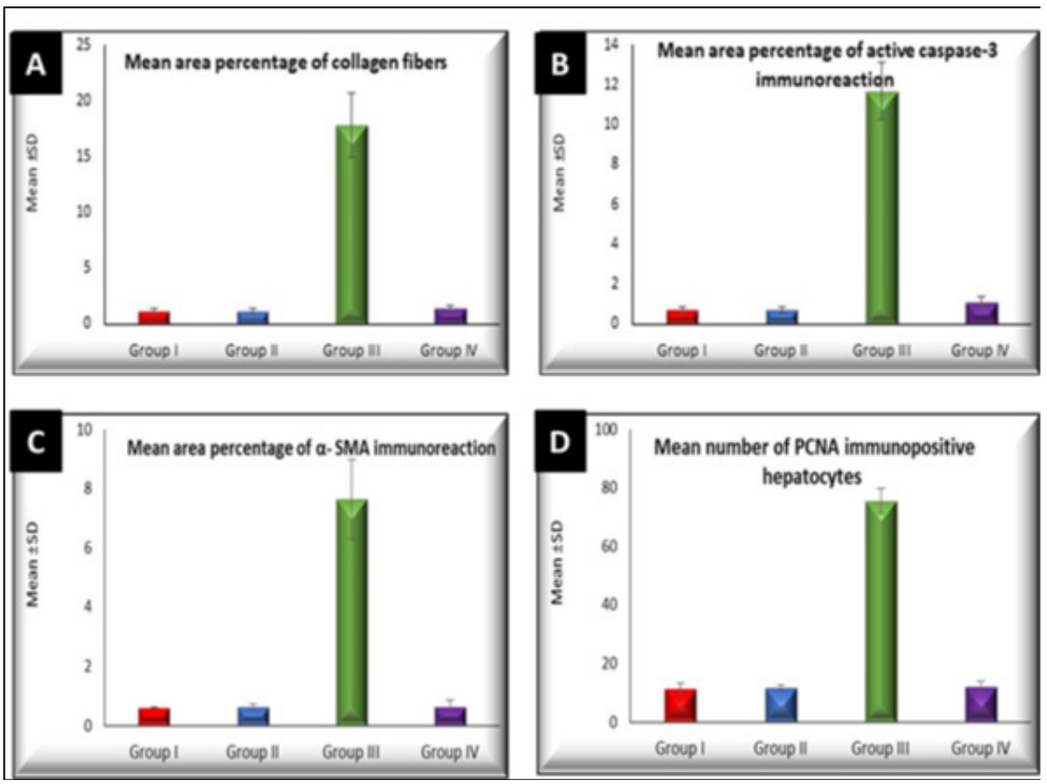


Fig. 18: PCNA immunostained sections. A: control group showing few faint PCNA positive nuclei of hepatocytes (arrow head). B: CCl4 treated group showing an intense positive PCNA nuclear immune reaction in most of hepatocytes (arrow head) as well as in the nuclei of proliferating bile ducts (D). Note: PCNA positive cells are seen in the periportal area (asterisk). Notice the vacuolated cytoplasm of the affected hepatocytes. C: myrrh oil & CCl4 treated group showing few faint PCNA positive nuclei of hepatocytes (arrow head). (Magnification X 400)

Table 1: Statistical analysis of hepatic specimens in the different study groups

Parameters	Group I	Group II	Group III	Group IV
Mean area percentage of collagen fibers	1.2±0.24	1.19±0.21	17.8±2.85**	1.43±0.28
Mean area percentage of active caspase-3 immunoreaction	0.72±0.14	0.74±0.16	11.7±1.4**	1.12±0.3
Mean area percentage of α- SMA immunoreaction	0.6±0.02	0.61±0.12	7.64±1.33**	0.63±0.24
Mean number of PCNA immunopositive hepatocytes	11.35±1.92	11.68±1.06	75.47±4.16**	11.98±1.88

** indicates highly significant vs control.



Histogram 1: Morphometric analysis of different groups. A: The mean area percentage of collagen fibers. B: The mean area percentage of active caspase-3. C: The mean area percentage of α- SMA immunoreaction. D: The mean number of PCNA immunopositive hepatocytes.

DISCUSSION

The liver is the key organ involved in metabolism and detoxification of many toxins in the human body[25]. Recently, herbal medicines have attained wide popularity for treatment of hepatic diseases because of their efficacy and safety and lower cost compared to chemical drugs[26,27]. Myrrh essential oil is one of the herbal products widely used for treatment of signs and symptoms of different diseases[28]. CCl4 induced hepatic injury is the classic model widely used for screening of hepato-protective drugs[29,30,31]. Thus, this experimental study was done to examine the role of myrrh oil in attenuating CCl4 induced hepatic injury in rats.

The results from the present study, in agreement with other studies[32] confirmed that CCl4 administration induced hepatic injury in rats manifested by disturbed hepatic architecture with evident degenerative changes affecting most of hepatocytes that appeared with vacuolated cytoplasm. This degenerative effect of CCl4 on liver could be explained by several mechanisms. The oxidative stress is reported one of the main mechanisms of CCl4-induced hepatic injury[33]. The liver considers CCl4 a foreign toxin, metabolizing it by cytochrome P450 oxygenase system into trichloromethyl and trichloromethyl-peroxyl free radicals causing oxidative stress induced membrane damage depending on the dose and exposure time[34]. The vacuolated cytoplasm of the affected hepatocytes might be due to failure of regulating mechanism of cell to pump out water from the inside[35]. Others suggested that these cytoplasmic vacuoles might contain accumulated fat secondary to CCl4 induced hepatic fatty degeneration[36].

Furthermore, the hepatic tissue of CCl4 treated group of the present study demonstrated different inflammatory signs that were in the form of dilated congested central veins, portal veins and blood sinusoids in addition to heavy cellular infiltrations. These changes could be attributed to the induction of inflammatory cascades which is considered as one of the mechanisms of CCl4 induced hepatic injury[37]. Both inflammation and oxidative stress were considered the most important mechanisms for various hepatic diseases[38].

Proliferation of bile ducts was noticed in CCl4 treated animals. Similar results were recorded in other studies[39]. Some investigators in their clinical studies reported that in patients with chronic hepatic disease, the hepatocyte regeneration was related to bile duct proliferation; a process named as ductular reaction (DR), and they added when this hepatocyte regeneration is defective, bile ductular cells can migrate outwards from the portal tracts and then differentiate into hepatocytes. These biliary cells are called hepatic oval cells or oval cells. the bile ductular cells can differentiate into hepatocytes suggesting their function as progeny of hepatic stem cells[40]. In accordance with these data, our results revealed the presence of biliary

cells that were oval cells having pale nuclei and they appeared starting to migrate to hepatic parenchyma, and this might suggest the role of bile ductular cells as hepatic stem cells. Moreover, DR is not only associated with bile duct proliferation but also with other liver reactions, such as inflammatory cellular infiltration in the hepatic portal areas[41]. In addition, many studies illustrated the strong association between DR and hepatic fibrosis resulting from hepatic injury because of close relationship between the hepatic stellate cells (HSCs) and biliary cells[42].

The present study revealed that CCl4 administration led to increased fibrous tissue deposition as evidenced by the statistically highly significant increase the area percentage of collagen fibers in CCl4 treated group in comparison to control group. Similar findings were recorded in previous studies who concluded that CCl4 administration produced an extensive hepatic fibrosis[43,44]. The hepatic fibrosis could be attributed to the increased collagen fibers synthesis by activated HSCs as well as their decreased degradation. Upon hepatic injury, The HSCs can differentiate into myofibroblasts and expressing alpha smooth muscle actin (α-SMA)[45]. In agreement with this hypothesis, this study displayed a significant increase in area percentage of the expression of α-SMA (a unique marker for HSC)[46], in peri-sinusoidal spaces indicating activation and migration of HSCs. Moreover, there is growing evidence indicating that the proliferating bile ducts may stimulate the chemo-attraction of HSC[42]. In agreement with this concept, this study demonstrated an increased expression of α-SMA around proliferating bile ducts. In hepatic fibrosis, both inflammatory mediators and oxidative stress play a key role in HSCs activation that enhance inflammation and reactive oxygen species (ROS) generation and also inhibit the antioxidant defense[47].

In addition, Kupffer cells engulfing brownish pigment granules are seen among the inflammatory cellular infiltrates in CCl4 treated group of the present study. Several studies have depicted the role of macrophages in hepatic fibrogenesis, as they reported that inflammation stimulates Kupffer cells to release pro-inflammatory mediators and fibrogenic factors that causes cellular apoptosis and HSCs activation[48].

Apoptosis is a pivotal incident in toxin-induced hepatic damage[49]. The results of the present work supported this concept and revealed that CCl4 induced hepatocytes apoptosis that appeared having pyknotic nuclei, and this was confirmed by the immunohistochemical results that revealed that CCl4 induced apoptosis through up-regulation of active caspase-3 expression as manifested by the significant increase in the area percentage of active caspase-3 positive immunoreaction in CCl4-treated group when compared to control group. This goes in line with other studies that explained the CCl4- induced apoptotic cell death through active caspase-3 activation[50]. Another study[51] explained this to be owing to CCl4 induced oxidative stress and inflammatory process.

The current study demonstrated that CCl4 increased the proliferation of hepatocytes, and this was manifested by the highly significant increase in the mean number of PCNA-immunopositive hepatocytes when compared to control. PCNA is a key marker of proliferating cells[52] whose increased expression indicates an enhanced proliferative activity allowing for the regeneration of injured cells. This goes in line with other studies that reported stronger replicative activity of hepatocytes of other hepatic fibrosis models[53]. In contrary, this was against the results of other investigators who reported that the hepatocytes replicative activity decreases in progressive hepatic cirrhosis[54], but this discrepancy might be due to the shorter duration used in the current study than was needed to induce chronic hepatic cirrhosis.

In the present study, myrrh oil was administered to assess its ability to attenuate CCl4 induced hepatic injury. The results of the current study showed that myrrh oil attenuated the changes induced by CCl4 administration as manifested by the noticeable improvement in the different histological and immunohistochemical results.

In the present study, treatment with myrrh oil attenuated the signs of hepatic inflammation. This was in agreement with many previous experimental studies that have reported the beneficial role myrrh in ameliorating inflammation in different animal models such as lipopolysaccharide (LPS)-induced hepatic injury[55], chemically induced hepatocarcinogenesis[56]. Moreover, myrrh anti-inflammatory effect was also proved in a model of ulcerative colitis induced by acetic acid in rats[57] by significantly declined lipid peroxidation and nitric oxide (NO) levels. Moreover, some investigators[58] reported the anti-inflammatory effect of myrrh in a mouse model of carrageenan induced paw edema and attributed this effect to the inhibitory effect of myrrh against prostaglandin (PGE2) production. All these studies together with the current one prove the concept that the myrrh oil anti-inflammatory effect might be one of its hepato-protective mechanisms.

In addition, other studies attributed the hepatoprotective effect of myrrh oil to its powerful antioxidant and free radical scavenging effects[59,60]. This antioxidant property could be contributed to its bioactive components that have powerful antioxidant effects, such as furanosesquiterpenes, m-cresol, limonene, cuminic aldehyde, terpenoids, commiphoric acids, eugenol and pinene[61]. Furanosesquiterpenes are well-known to possess many pharmacological actions including antifungal, antibacterial, local anesthetic[62] and neuroprotective activities[63].

The present study demonstrated that myrrh oil attenuated hepatic fibrosis as indicated by decreased collagen fibers deposition as well as decreased expression of a-SMA fibrotic marker. Until now, there are no confirmed anti-fibrotic drugs despite the extensive knowledge of the mechanisms underlying hepatic fibrosis, therefore, it was reported that agents exhibiting anti-inflammatory or anti-

oxidant effects could be promising for attenuating hepatic fibrosis. Moreover, inhibition of the a-SMA signaling pathway have been reported to attenuate liver fibrosis[64].

Notably, treatment with myrrh oil significantly downregulated the active caspase-3 expression. These results suggest that myrrh oil could reduce CCl4 induced hepatic injury might be through downregulation of hepatic cellular apoptosis. In addition, the antiapopotic activity of myrrh oil have also been reported in recent experimental studies that declared the anti-apoptotic activity by decreasing apoptotic markers in induced myocardial infarction rat model[19].

This study had suggested the anti-proliferative effect of myrrh oil as indicated by the decreased expression of PCNA proliferative marker, and this effect might be due to its anti-fibrotic and anti-apoptotic effects. It was reported that the anti-proliferative effect mediated by decreased PCNA expression could be helpful in the treatment of various fibrosis models[65,66].

CONCLUSION

The outcomes of the current study suggest the ability of myrrh oil in attenuating the CCl4-induced hepatic injury in rats, may be through its antioxidant, anti-inflammatory, anti-fibrotic, anti-proliferative, and anti-apoptotic effects. Accordingly, incorporation of myrrh oil a hepatoprotective agent might be a good candidate for clinical trials. Moreover, further investigations are recommended to determine the precise underlying protective mechanisms of myrrh oil on CCl4-induced hepatic injury.

CONFLICT OF INTERESTS

There are no conflicts of interest.

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الملخص العربى

دور زيت المر في تخفيف الإصابة الكبدية التي يسببها رابع كلوريد الكربون في ذكر الجرذ الأبيض البالغ. دراسة هستولوجية وهستوكيميائية مناعية

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مقدمة: يعد رابع كلوريد الكربون سم كبدي بيئي يستخدم عادة للحث على إصابة الكبد في حيوانات التجارب. يعتبر زيت المر العطري أحد المكونات الرئيسية لشجرة المر التي تم استخدامه على نطاق واسع في الطب الشعبي لعلاج الأمراض المختلفة.

الهدف من البحث: تم إجراء هذا العمل لتقييم الدور المحتمل لزيت المر في التخفيف من الإصابة الكبدية التي يسببها رابع كلوريد الكربون في ذكر الجرذ الأبيض البالغ.

المواد وطرق البحث: تم تقسيم ٣٠ من ذكور الجرذان البيضاء البالغة إلى أربع مجموعات؛ المجموعة الضابطة, مجموعة زيت المر(تم إعطاؤها عن طريق الفم ٥٠ مجم / كجم من وزن الجسم مرة واحدة يوميًا لمدة أربع), مجموعة رابع كلوريد الكربون (تم حقنها داخل الصفاق ١ مل / كجم رابع كلوريد الكربون مذاب في زيت الزيتون بنسبة ١: ١ مرتين في الأسبوع لمدة أربع أسابيع) و مجموعة زيت المرو رابع كلوريد الكربون. تم تحضيرعينات الكبد للفحص بالميكروسكوب الضوئي باستخدام صبغات H&E و Masson'n trichrome. وقد أجريت دراسة هستوكيميائية مناعية باستخدام أجسام الأجسام المضادة النشطة لـ caspase-٣ و α-SMA و PCNA.

النتائج: كشفت المجموعة المعالجة برابع كلوريد الكربون عن بنية كبدية مضطربة. احتوت معظم خلايا الكبد على سيتوبلازم مفرغ وأنوية صغيرة داكنة. كما كانت الأوردة المركزية والأوردة البابية متسعة ومحتقنة بشكل ملحوظ مع وجود تسلل شديد لخلايا أحادية النواة بالإضافة إلى تكاثر القناة الصفراوية والترسب المفرط لألياف الكولاجين. وأظهرت نتائج الدراسة الهستوكيميائية المناعية ارتفاعا ذو دلالة إحصائية في التفاعل الهستوكيميائي المناعي caspase-٣ و α-SMA و PCNA. علي الجانب الاخر لوحظت تغيرات ضئيلة في الفئران التي تمت معالجتها بزيت المر مصاحبا برابع كلوريد الكربون مع وجود تغيرات غير ملحوظه في التفاعل الهستوكيميائي المناعي.

الإستنتاج: زيت المر يمكن أن يكون مفيدًا في التخفيف من إصابة الكبد الناجمة عن رابع كلوريد الكربون.