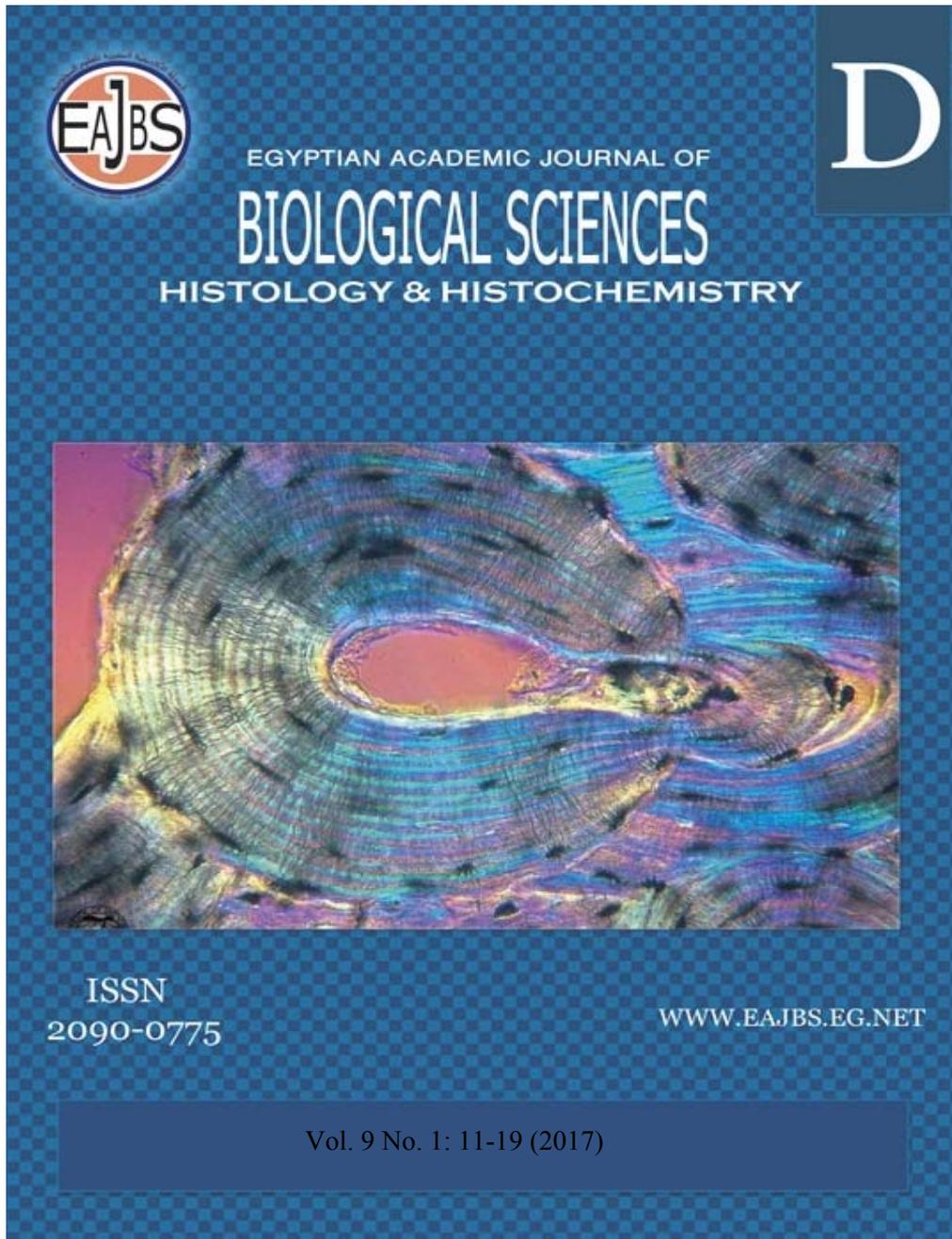


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## Histological and Histochemical Studies on the Protective Effect of Fennel Oil on the Formaldehyde-Induced Hepatotoxicity in Male Rabbits.

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### ABSTRACT

This study aimed to elucidate the protective role of fennel oil on in hepatic tissue, restoration of total protein and glycogen content formaldehyde induced hepatotoxicity in male rabbits by histological, histochemical, immunohistochemical and biochemical investigations . Twenty-four rabbits were divided into 4 groups (6 rabbits each). G1: This group was served as normal controls, G2: This group was administrated with fennel oil (0.3 mg/kg / ip ) for 14 days . G3: this group was administrated with formaldehyde (10mg/kg/ip ) for 14 days. G4: This group administrated with fennel oil then after 3 hours was injected with formaldehyde. In comparison with control group, histological results showed that formaldehyde induced many histopathological alterations as leucocytic infiltration, blood venues congestion, proliferation of bile duct and hemorrhage. Histochemical demonstrations showed that formaldehyde induced marked depletion in both hepatic total proteins and glycogen contents. PCNA-positive stained nuclei were significantly increased in formaldehyde – treated group. Biochemical investigations of serum liver function markers indicate that; formaldehyde induced significant elevation of AST, ALT, ALP and bilirubin; while albumin level was decreased. Co-treatment of fennel oil and formaldehyde induced a marked degree of improvement and marked decrease of PCNA-positive stained nuclei. Moreover, serum levels of ALT, AST, ALP and bilirubin were significantly decreased, and albumin level was increased.

### INTRODUCTION

Formaldehyde (HCHO) is very reactive toxic chemical, it a colorless, flammable gas with a pungent, suffocating odor. The physical properties like colorlessness and well solubility in water make it more dangerous. It is commonly used in anatomy and histopathology laboratories as formaldehyde is one of the most common fixatives. Moreover, it is also found in cigarette smoke, automobile exhaust, industrial, cosmetic products, and building materials (Abdulqader and Mustafa, 2014; Gerin *et al.*, 2016). Chronic inhalation of formaldehyde causes hepatotoxicity, nephrotoxicity, and induces oxidative stress through increasing lipid peroxidation and formation of reactive oxygen species (ROS) (Abdulqader and Mustafa 2014). In addition, formaldehyde induces histological and physiological alterations in the respiratory, digestive, and reproductive and nervous systems. Formaldehyde have also allergic properties (Pekmez *et al.*, 2008).

The liver injuries are one of the world problems to be solved. Using conventional drugs in the treatment of hepatic disorders are sometimes inadequate and can induce serious side effects.

Therefore many researchers have been focused on alternative drugs, natural antioxidants and medicinal plants. (Rabeh and Aboraya, 2014).

Fennel (*Foeniculum vulgare*, family Umbelliferae) is a medicinal plant with anti-inflammatory and antioxidant properties (Hassaan and Soltan, 2016). Essential oil of fennel has a great medical importance, as it widely used in the treatment of bacterial, microbial and fungal infectious diseases (Duško *et al.*, 2006). Fennel is used as anti-diabetic agent. (Sushruta *et al.*, 2007). The phytochemical studies have shown that the major chemical components of fennel essential oil are phenylpropanoid derivatives and monoterpenoids (Hassaan and Soltan, 2016).

In this publication, we report the hepatoprotective effects of fennel oil against toxicity induced by formaldehyde.

## MATERIALS AND METHODS

### Rabbits and Experimental Design:

Twenty-four male New Zealand healthy rabbits (*Oryctolagus cuniculus*), of mean body weights 2.1 kg, were used in this investigation. Rabbits were housed under hygienic conditions, at a room temperature of  $25 \pm 2^\circ\text{C}$ , regular light and dark cycles, and provided with food and water ad libitum.

Rabbits were divided into 4 groups (6 rabbits each) as follows:

Group 1: Animals of this group were served as normal controls.

Group 2: Animals of this group were administrated with fennel oil (0.3 mg/kg / ip) for 14 days.

Group 3: Animals of this group were administrated with formaldehyde (10mg/kg/ip) for 14 days.

Group 4: Animals of this group were administrated injected with fennel oil (0.3mg/kg) then after 3 hours was injected with Formaldehyde (10mg/kg) for 14 days.

Chemicals:

1- Formaldehyde: Purchased from Sigma Chemical Company (St. Louis, Missouri, USA).

2- Fennel oil (extraction and preparation):

Fresh fennel seeds were dried at  $35^\circ\text{C}$  and crushed to fine powders using a grinder. Dry seeds were powered using a Clevenger-type apparatus to produce essential oil by hydrodistillation. The essential fennel oil was separated, dried over anhydrous sodium sulfate and kept in dark bottle till using. (Hassaan and Soltan, 2016).

### Methods:

#### Histological Examinations:

All Rabbits were dissected out and their livers were removed, and then fixed in 10% neutral formalin, dehydrated, cleared and embedded in paraffin wax. Paraffin sections of 5 microns thickness were prepared and stained with routine haematoxylin and eosin stain (Drury and Wallington, 1980).

#### Histochemical Examination:

##### Total proteins:

For histochemical demonstration of Total proteins; Paraffin sections of 5 microns thickness were prepared and stained with Mercury bromophenol blue method (Mazia, *et al.*, 1953).

##### Carbohydrates:

For histochemical demonstration of glycogen; paraffin sections were stained with Periodic acid Schiff's (PAS) stain. (Hotchkiss, R.D., 1948)

3- Immunohistochemical (PCNA): Demonstration of proliferating cell nuclear antigen (PCNA) immunoreactivity in liver sections of

normal and treated rabbits was performed according to the method described by Eldridge and Goldsworthy (1996)

#### 4- Serum biochemical analysis

Sera were obtained by centrifugation of the blood samples and stored at 20°C until assayed for the biochemical parameters. Serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT), albumin and bilirubin were assayed according to the method of Schumann and Klauke (2003) using reagent kit purchased from Biosystems (Spain).

#### 5- Statistical analysis

Statistical analysis was performed using SPSS v.16. Results were articulated as mean  $\pm$  standard deviation (SD) and all statistical comparisons were made by means of one-way ANOVA test.

A P value  $<0.05$  was considered significant.

## RESULTS AND DISCUSSION

### Histopathological Results:

Fig. (1) and Table (1) showing Histopathological alterations observed in

livers of rabbits among all experimental groups. Microscopic examination of sections control rabbits' livers showed the classical hepatic lobules with centrally placed central vein and radiating hepatic cords formed of normal hepatocytes with intact cell wall and central rounded nucleus, free sinusoids with Kupffer cells. (Fig. 1 a).

Treating rabbits with fennel oil didn't cause any histopathological alterations as the hepatic lobules were well formed (Fig. 1 b).

Formaldehyde treated group revealed marked histopathological changes when compared with control group. Hepatic architecture was deformed with abnormal arrangement of hepatocytes, Kupffer cells were activated, and abnormal sinusoids disappeared, widened or congested with blood. In addition, most of blood vessels were congested, severe hemorrhage was observed and perivascular aggregation inflammatory cells (Fig. 1 c). Moreover, formaldehyde induced bile duct proliferation (Fig. 1d) and leucocytic infiltration (Fig. 1 e).

**Table 1: Histopathological alterations observed in livers of rabbits.**

	G1	G2	G3	G4
Deformation in in hepatic cords	+	++	++++	+++
Activated Kupffer cells	+	+	++++	++
Leucocytic infiltration	Ø	Ø	+++	+
Proliferation of bill duct	Ø	Ø	++	Ø
Hemorrhage	Ø	Ø	++++	+
congested blood vessels	Ø	Ø	++++	+
Abnormal sinusoids	+	++	++++	++

Ø, absent; +, mild; ++, moderate; ++++ severe . (The livers of six animals in every group were examined).

Our results are agreed with many investigations, as formaldehyde has proven to induce several histological changes. Pekmez *et al.* (2008), used the same dose and the same treatment period of our investigation, and they found that, formaldehyde caused enlarged sinusoids congested with blood, periportal cellular infiltration, congestion of blood vessels. Furthermore, some hepatocytes revealed vacuolation of cytoplasm, while some

had pyknotic nuclei. Moreover, formaldehyde causes disorganization of hepatocytes, activation of Kupffer cells, leucocytic infiltration, hemorrhage, apoptosis and necrosis (Abdulqader and Mustafa 2014), Gerin *et al.* (2016).

Co-treatment with both fennel oil and formaldehyde induced advanced degree of improvement of hepatic tissue when compared with formaldehyde treated group. The hepatic lobules

appeared well formed; all the inflammatory features grades were minimal (Fig. 1f).

Similarly, fennel essential oil ameliorated the hepatic histopathological alterations induced by CCl<sub>4</sub> in albino rats, as ballooning degeneration, apoptosis, and periportal leucocytic infiltration were diminished. Furthermore, cirrhotic nodules induced by CCl<sub>4</sub> were not observed in CCl<sub>4</sub>+ fennel oil treated group (Özbek *et al.*, 2004).

### Histochemical results

#### Total protein:

Microscopic examination of the liver sections of both control rabbits and fennel-treated rabbits showed normal distribution of proteinic content in the hepatocytes which appeared as small bluish dense bodies distributed randomly in a weak to moderate stained cytoplasm. In addition the cell wall and the nuclear membrane were well stained with bromphenol blue (Fig. 2 a). In comparison with control group, formaldehyde treated rabbits; examination showed diminution of the proteinic content and the most of hepatocytes appeared devoid of protein, especially, the degenerated, vacuolated and necrotic hepatocytes which were slightly stained (Fig. 2b). Examination of liver sections of rabbits treated with both formaldehyde and fennel oil showed marked increase of proteinic material in hepatocytes and relatively appeared normal, when compared to formaldehyde treated group (Fig. 2c).

Many authors studied the histochemical alterations induced by hepatotoxins and the possible protective role of medicinal herbs or natural antioxidants. Abdul-Hamid *et al.* (2017) studied the Cypermethrin-induced histochemical changes in hepatic total protein content and the protective role of propolis and curcumin. Cypermethrin administration induced a marked reduction of proteins in the most of

hepatocytes. Intake of Propolis plus Cypermethrin and curcumin plus Cypermethrin increased the intensity of blue color of protein when compared with Cypermethrin treated group (Abdul-Hamid *et al.*, 2017).

#### General Carbohydrates:

Sections stained by periodic acid Schiff (PAS) demonstrated the glycogen and showed a strong positive reaction (magenta color) in the cytoplasm of the hepatocytes in control group and fennel oil treated group (Fig. 3a). Formaldehyde treated rabbits showed marked glycogen depletion, thus a weak positive reaction in the cytoplasm of hepatocytes when compared to corresponding control animals (Fig. 3b). Co-treatment of formaldehyde and fennel oil induced a marked restoration of glycogen in hepatocytes when compared with formaldehyde treated rabbits (Fig. 3c). Our results are similar with those of Pekmez *et al.* (2008), as they investigated the histochemical effects of formaldehyde on glycogen content by PAS stain, and they recorded that, formaldehyde induced marked glycogen depletion.

Many natural antioxidants and medicinal herbs can treat the glycogen depletion-induced by various hepatotoxins in hepatic tissue. Abdul-Hamid *et al.*, (2017) reported that intake of Propolis and / or curcumin plus Cypermethrin restored the intensity glycogen content in hepatocytes when compared with Cypermethrin treated group which showed marked reduction of glycogen.

#### Immunohistochemical Results:

Examination of the hepatic sections of control and Fennel oil groups stained for proliferating cell nuclear antigen (PCNA) antibodies showed few week positive nuclear immune reaction indicating the cell divisions of few hepatocytes (Fig. 4a). However, section of rabbits treated with formaldehyde showed strong positive nuclear immune

reaction in disrupted hepatocytes (Fig. 4b). The hepatocytes of fennel oil and formaldehyde treated group demonstrated a marked reduction of positive stained nuclei, but few hepatocytes still positively stained (Fig. 4 c). Mahboub and Arisha (2015), reported that, hepatotoxicity induced by diazinon caused marked increase of the number of the PCNA positive staining cells in hepatocytes. While co-treatment of both diazinon and *Ocimum basilicum* extract induced a decrease of PCNA positive staining cells in hepatocytes.

#### Biochemical results:

Data shown in Table (2) explain the effects of formaldehyde -induced hepatotoxicity and the protective effects

of fennel oil, on serum markers of liver function. Treating rabbits with fennel oil didn't cause any alteration of liver function markers levels when compared with the corresponding control group.

Formaldehyde induced significant ( $P < 0.05$ ) elevation of Serum activity of the liver function markers (ALT, AST, ALP and bilirubin). Conversely, albumin was significantly decreased in formaldehyde -administered rabbits compared with control group. Similar to our findings, Gerin *et al.* (2016) reported that formaldehyde induced significant elevation ( $p < 0.05$ ) of ALT, AST, ALP and bilirubin and decreased albumin levels in serum.

**Table 2: Changes in serum markers of liver function among experimental groups.**

Groups / Enzymes	ALT (U/L)	AST (U/L)	ALP (U/L)	T.Bil (mg/dl)	Albu. (mg/dl)
G1(control)	29.8 ±3.1	43.4±3.8	66.0± 4.1	0.43±0.03	3.8±0.23
G2 (FO)	30.5±3.3	46.8±3.87	67.8 ±5.3	0.45±0.02	3.7±0.13
G3 (FD)	98.6±4.24*	112.0±5.5*	147.0 ± 4.4*	1.31±0.03*	2.1±0.12*
G4 (FO+FD)	44.2±3.11**	58.4 ± 3.3**	83.4 ± 3.0**	0.53±0.03**	3.1 ± 0.17**

(\*) Significant increase at  $P < 0.05$  compared with control group

(\*\*) Significant decrease at  $P < 0.05$  compared with formaldehyde group

On the other hand, Oral administration of fennel oil with formaldehyde significantly decreased ALT, AST, ALP and bilirubin, while albumin was increased when compared with formaldehyde treated group (Table 2).

Rabeh and Aboraya, (2014) investigated the hepatoprotective effect of fennel oil on CCl<sub>4</sub>-induced hepatotoxicity and they recorded that co-administration of fennel oil and CCl<sub>4</sub> induced significant decrease ( $p < 0.05$ ) of ALT, AST, ALP, bilirubin and increased albumin serum levels, when compared with CCl<sub>4</sub> treated group.

Formaldehyde was found to disturb the oxidant-antioxidant balance in hepatic tissue and cause oxidative stress through increasing lipid peroxidation and formation of reactive oxygen species

(ROS) in the liver tissue and induce oxidative damage (Gerin *et al.*, 2016). Chromatographic analysis of fennel seeds showed that it contains d-limonene and  $\beta$ -myrcene which have strongly affected liver function. D-limonene increases the concentration of reduced glutathione (GSH) in the liver and improves the antioxidant statuses. On the other hand,  $\beta$ -myrcene increases the levels of apoproteins CYP2B1 and CYP2B2, which are subtypes of the P450 enzyme system that consists of a superfamily of hemoproteins that catalyse the oxidative metabolism of a wide variety of exogenous chemicals (Rabeh and Aboraya, 2014).

#### CONCLUSION

The presented study showed that certainly formaldehyde caused

histological, histochemical, immunohistochemical and biochemical alterations in liver. Fennel oil showed a protective role against this formaldehyde-induced hepatotoxicity.

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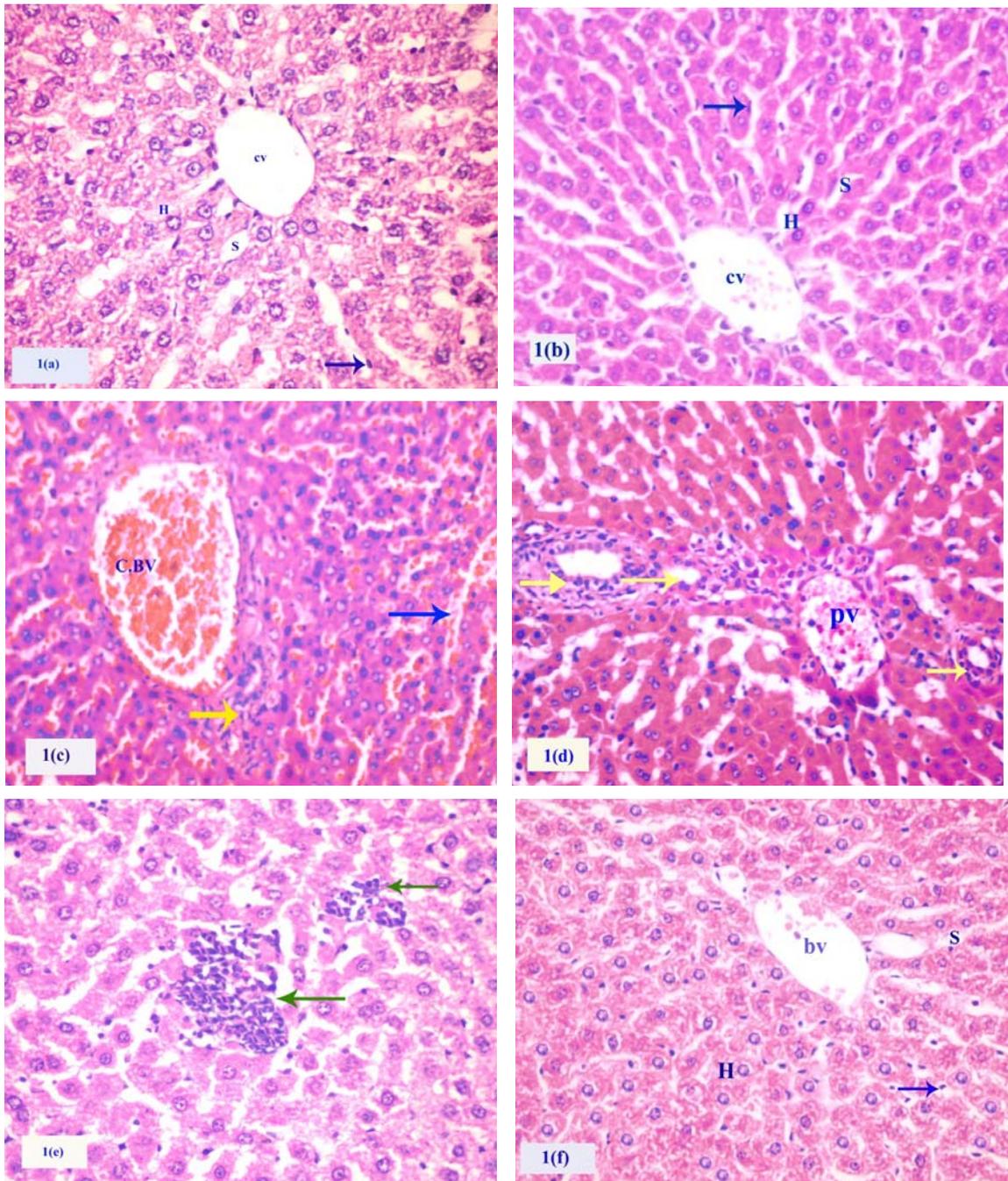


Fig. 1: Photomicrograph showing histological alterations in sections of hepatic tissue among the experimental groups. (H&E, X400). (a) : Control rabbit showing well-formed hepatic lobules with centrally placed central vein (CV) radiating hepatic cords with average thickness formed of normal hepatocytes (H) with free sinusoids (s) with Kupffer cells (arrow). (b) Rabbit treated with fennel oil showing no histopathological alterations. (c) Rabbit treated with formaldehyde showing congested blood vessel (C.Bv), hemorrhage (blue arrow) and perivascular aggregation inflammatory cells (yellow arrow). (d) Rabbit treated with formaldehyde showing congestion of portal vein (PV) and bile duct proliferation. (e) Rabbit treated with formaldehyde showing leucocytic infiltration (arrows). (f) Rabbit treated with both formaldehyde and fennel oil showing marked improvement of hepatic tissue, normal blood vessel (bv) , vein and the hepatocytes (H) and sinusoids (s) and Kupffer cells (arrow) appeared more or less like normal.

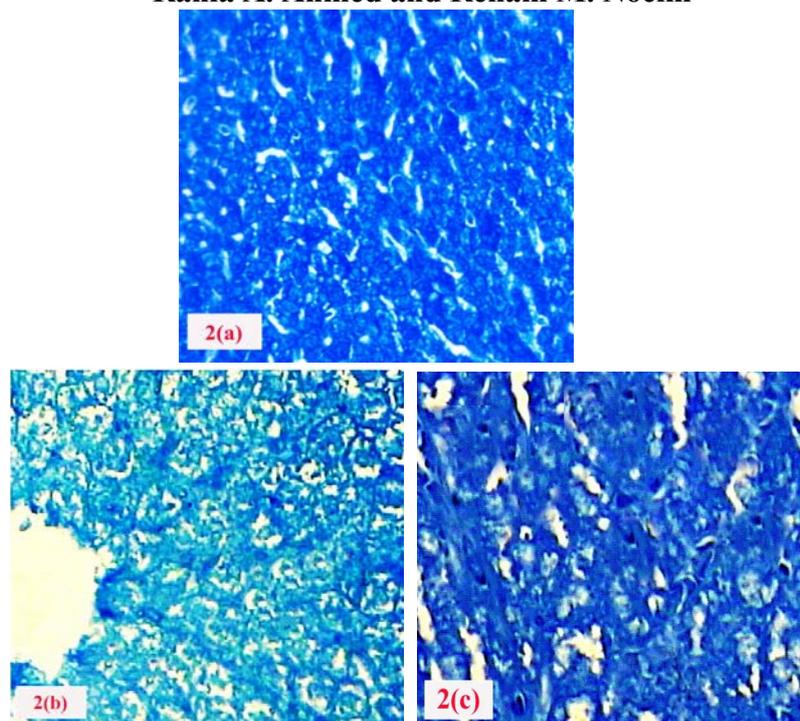


Fig. 2: Photomicrograph showing histochemical alterations of total protein content in sections of hepatic tissue among the experimental groups. (Bromphenol blue, X400). (a). Control rabbits showing normal distribution of proteinic content in the hepatocytes . (b) Formaldehyde-treated rabbits, showing diminution of the proteinic content and the most of hepatocytes appeared devoid of protein. (c) Rabbits treated with both formaldehyde and fennel oil showed marked increase of proteinic material in hepatocytes and relatively appeared normal.

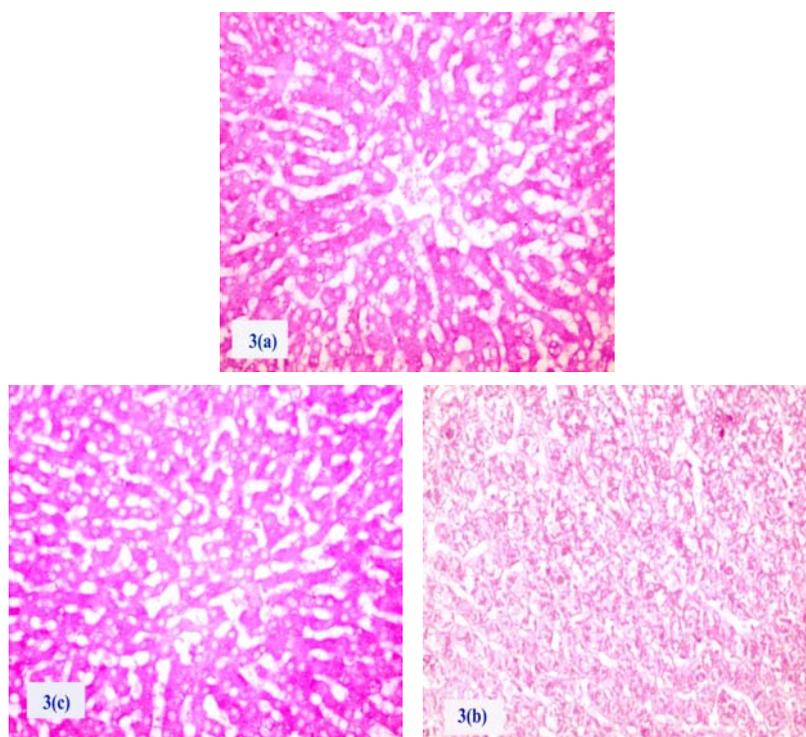
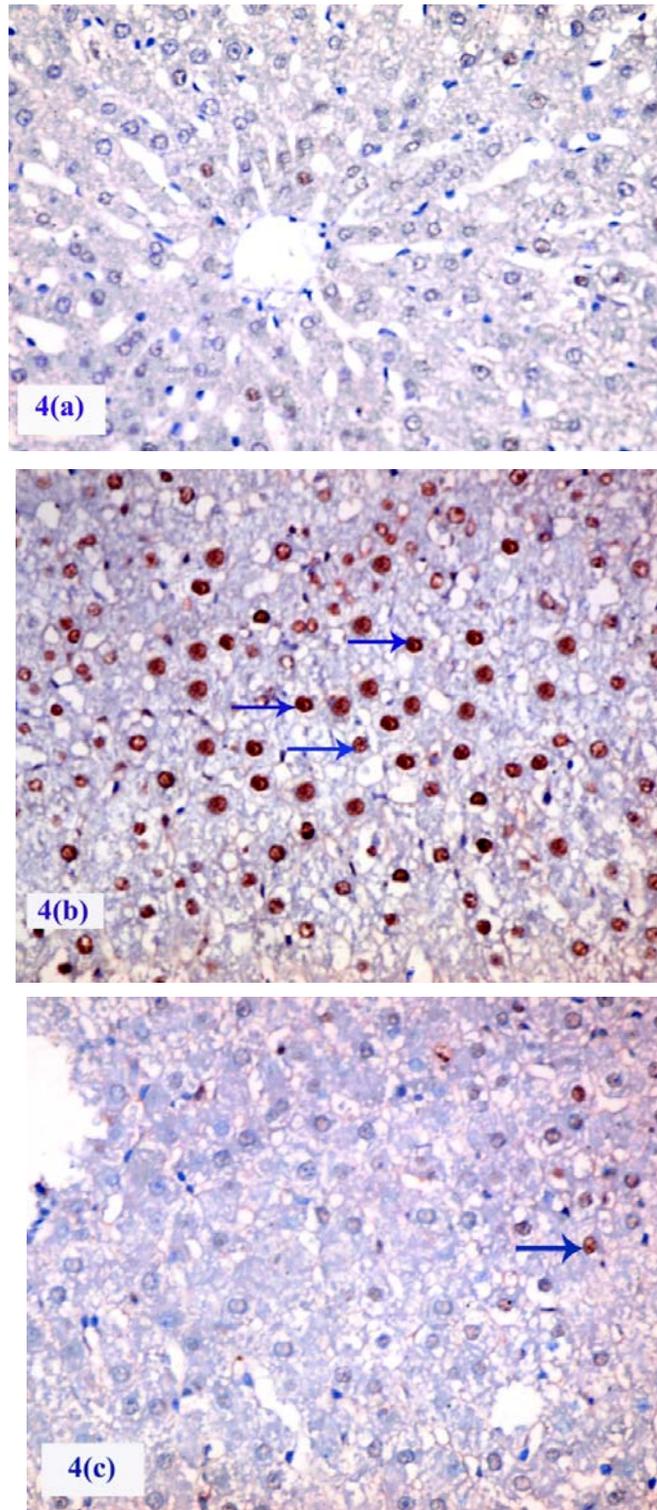


Fig 3: Photomicrograph showing histochemical alterations of general carbohydrates content in sections of hepatic tissue among the experimental groups. (PAS X400). (a) Section in liver of control rabbit showing a strong positive reaction (magenta color) in the cytoplasm of the hepatocytes. (b) Formaldehyde-treated rabbit showing marked glycogen depletion, and weak positive reaction in the cytoplasm of hepatocytes. (c) Section in liver of a rabbit treated with formaldehyde and fennel oil induced a marked restoration of glycogen in hepatocytes.



**Fig 4:** Photomicrograph showing immunohistochemical alterations PCNA immunostain in sections of hepatic tissue among the experimental groups. (PCNA X400). (a) Control rabbit showing few weak positive nuclear immune reaction. (b) Rabbit treated with formaldehyde showing strong positive nuclear immune reaction in the most of hepatocytes (arrows). (c) Rabbit treated with fennel oil + formaldehyde demonstrated a marked reduction of positive stained nuclei, but few hepatocytes still positively stained (arrow) (fig 4 c).

## RABIC SUMMERY

دراسات نسيجية و كيمونسيجية على التأثير الوقائي لزيت الشمر على السمية الكبدية المحدثة بالفورمالدهيد في ذكور الأرانب.

رانيا عبد الكريم إبراهيم احمد ١ و ريهام محمد نعيم  
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هدفت هذه الدراسة إلى شرح الدور الوقائي لزيت الشمر ضد السمية الكبدية المحدثة بالفورمالدهيد في ذكور الأرانب و ذلك من خلال دراسات نسيجية و نسيجوكيميائية و نسيجوكيميائية مناعية و كيموحيوية . أجريت هذه الدراسة على عدد اربعة و عشرون أرنب مقسمة الى أربع مجموعات . ج ١ : المجموعة الضابطة . ج ٢ : تم حقن هذه المجموعة بزيت الشمر . ج ٣ : تم حقن هذه المجموعة بالفورمالدهيد . ج ٤ : تم حقن هذه المجموعة بكل من زيت الشمر و الفورمالدهيد . أظهرت النتائج الهيستولوجية انه بالمقارنه بالمجموعة الضابطة فإن المعالجة بالفورمالدهيد قد انتجت العديد من التغيرات الهيستوباثولوجية في النسيج الكبدي مثل تخلل الخلايا الإلتهابية و احتقان الاوعية الدموية . كذلك اظهرت النتائج النسيجوكيميائية نقص ملحوظ في محتوى البروتينات الكلية و الجليكوجين . اما النتائج الكيموحيوية فقط اظهرت تسبب الفورمالدهيد في ارتفاع ملحوظ في نسب انزيمات الكبد و الصفراء و انخفاض في نسبة الألبومين . بمقارنة المجموعة الرابعة المعالجة بكل من الفورمالدهيد و زيت الشمر ، مع المجموعة الثالثة المعالجة بالفورمالدهيد فقط ، فقد اظهرت النتائج تحسن ملحوظ في تركيب النسيج الكبدي حيث ان بعض القطاعات كانت اقرب للمجموعة الضابطة ، كذلك زيادة في كمية البروتين و الجليكوجين . كما حدث انخفاض ملحوظ في نسب انزيمات الكبد و الصفراء و ارتفاع في نسبة الألبومين .