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### Study the Physiological and Histological Changes that Induced by Ethanol in Mice Liver and the Role of *Trigonella foenum - graecum* Seeds Extract to Treatment

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#### KEYWORDS

Ethanol in mice liver, *Trigonella foenum – graecum*, seeds extract.

#### A B S T R A C T

The purpose of this study to evaluate the seed extract (aqueous) on the mice liver lesions that caused by ethanol. Twenty albino mice were randomly assigned to five groups A, B, C, D and E. (each group consist 4 mice), the first group was control group administrated only normal diet and water, the second group administrated with ethanol for fifteen days, the third group administrated with ethanol for twenty one days, the fourth group administrated with ethanol for fifteen days and treated with seeds extract for 10 days, the fifth group administrated with ethanol for twenty one days and treated with seeds extract for 10 days. The GOT and GPT levels were increased and showed high significant changes ( $P < 0.01$ ) in groups that administrated ethanol only compared with control group and also the liver tissue showed different lesion including necrosis, degeneration, infiltration, but when these groups treated with seed extract, the GOT and GPT levels return to the normal ranges and the liver tissue became normal and repaired completely. It was concluded from this study that aqueous extract of Fenugreek has amply good effect on the weight on liver enzymes and tissue.

#### Introduction

*Trigonella foenum - graecum* belonging to the family Papilionaceae commonly known as Fenugreek is a aromatic, 30-60 cm tall, annual herb, cultivated throughout the country (Kirtikar and Basu; The Ayurvedic Pharmacopoeia of India). Fenugreek (*Trigonella foenum-graecum*) found in nature and is cultivated in India and Pakistan is a well known medicinal plant having

properties of reducing blood sugar level (Alarcon-Aguilara *et al.*, 1998; Raghuram *et al.*, 1994), antibacterial, anthelmintic (Bhatti *et al.*, 1996), antimicrobial (Alkofahi *et al.*, 1996), anti-inflammatory and antipyretic (Ahmadiani *et al.*, 2001).

The seeds are hot, with a sharp bitter taste; tonic, antipyretic, anthelmintic, increase the

apetite, astringent to the bowels, cure leprosy, "vata", vomiting, bronchitis, piles; remove bad taste from the mouth (Prajapati *et al.*, 2003).

The seeds are contains lecithin and choline and vitamins A and D. It also contains neurin, biotin, trimethylamine, lysine, L-tryptophan rich proteins, mucilaginous fiber and other rare chemical constituents such as saponins, coumarin, fenugreekine, nicotinic acid, saponin, phytic acid, scopoletin and trigonelline (Michael *et al.*, 2003). Therefore, the present study deigned to show the protective effects of *Trigonella foenum - graecum* seeds extract against ethanol toxicity in the liver.

## **Materials and Methods**

### **Animal Model**

Twenty adult albino mice (*Mus musculus*), (wt 25-28 g) obtained from the Public company of medicines manufacture and requirements medicals - Samara, Iraq, and kept on standard pellet diet and water.

### ***Trigonella foenum - graecum* Seeds Extract**

Seed were collected from the Kirkuk market, were dried for 24 hours at 37°C in oven. Exposure to sunlight was avoided to prevent the loss of active components. Dry seed were then ground in a grinding machine; 50gm of dried ground seed were taken in a non-metallic jar and one liter of hot boiled distilled water were poured on it, this was filtered and the solvent was evaporated by rotary evaporator (at 40-50 °C and 150 rpm) (11).

### **Experimental Design**

In this study 20 albino mice were used and divided for five groups (each group consist four mice) as follow:

**Group A:** control group administrated with normal saline only for seven days, then killed all were euthanized at eighth day.

**Group B:** administrated with ethanol for fifteen days, then killed all were euthanized at sixteenth day.

**Group C:** administrated with ethanol for twenty one days, then killed all were euthanized at twenty second day.

**Group D:** administrated with ethanol for fifteen days. After that, treated with 1ml seeds extract for 10 days, then killed all were euthanized eleventh day after treatment. .

**Group E:** administrated with ethanol for seven days. After that, treated with 1ml seeds extract for 10 days, then killed all were euthanized at eleventh day after treatment.

### **Prepare of Blood Solution**

Subjected mice under anesthesia then later took heart blood and put in test tubs that contain EDTA. This solution was mixed with phosphate buffer, centerfigation 2000 cycle/min for 10 min. Supernated was taken and 1 ml distal water added for it. GPT, GOT in blood extract were analysed with least significant difference in  $p < 0.05$ .

### **Histological Study**

Fresh pieces of liver from each mice was cut out rapidly, fixed in 10% formalin and then dehydrated with ascending grades of ethanol.

Dehydration was then followed by clearing then tissue samples in two changes of xylene before being impregnated with three changes of melted paraffin wax, embedded

and blocked out. Tissue sections thickness (5 um) were stained with haematoxylin-eosin.

### **Microscopic Study and Microscopic Photograph**

The microscopic investigation of liver sections involved the descriptive histology. A light microscope (Motic microscope) was used to perform the microscopic investigations of this study. Microscopic photograph was made using (Optica\Italy) microscope supplied with a special camera prepared for this purpose.

### **Statistical Analysis**

Data were analyzed statistically using a statistical Minitab program under SPSS and Microsoft Excel XP system. The data were presented in simple measure of mean  $\pm$  SD (standard deviation), minimum and maximum values. Results were analyzed statistically using Analysis of Variance (ANOVA) test, in order to evaluate the significance of variability between treated and control groups. Means of data were compared using Duncan's Multiple Range test. Probability levels of more than 0.01 were regarded as statistically non-significant, whereas values less than 0.01 were considered as significant as follows:

$P < 0.01$  highly significant (Beth *et al.*, 2004).

## **Results and Discussion**

### **Biochemical Tests**

#### **GPT Tests**

The results of the present study showed significant changes ( $P > 0.05$ ) in level of GPT between groups. As shown in chart (1), the group B that administrated 0.1% H<sub>2</sub>O<sub>2</sub>

showed significant change compared with control group. Also, group C that administrated 0.15% H<sub>2</sub>O<sub>2</sub> showed significant change compared with control group. But in the groups D and E, (groups that administrated with ethanol and treated seeds extract), showed non-significant change compared with control group.

#### **GOT Tests**

The results of the present study showed significant changes ( $P > 0.01$ ) in level of GOT between groups. As shown in chart (2), the group B that administrated ethanol for fifteen days showed significant change compared with control group. Also, group C that administrated ethanol for twenty one days showed significant change compared with control group. But in the groups D and E, (groups that administrated with ethanol and treated seeds extract), showed non-significant change compared with control group.

### **Histological Examination**

#### **Control Group**

The microscope examination showed normal structure of liver and demonstrated normal central vein, normal arrangement of hepatocytes and sinusoids and normal kupffer cells (Fig.2).

#### **Group Administrated Ethanol for Fifteen Days**

The cross sections that prepared from this group showed thickening wall of central vein and congestion of blood vessels and degeneration of most hepatocytes (Fig. 3).

#### **Group Administrated Ethanol for Twenty One Days**

The histological examination showed thickening wall of central vein and

congestion of blood vessels and degeneration and necrosis of most hepatocytes and infiltration of lymphocytes (Fig.4).

**Group Administrated Ethanol for Fifteen Days and Treated with Seeds Extract**

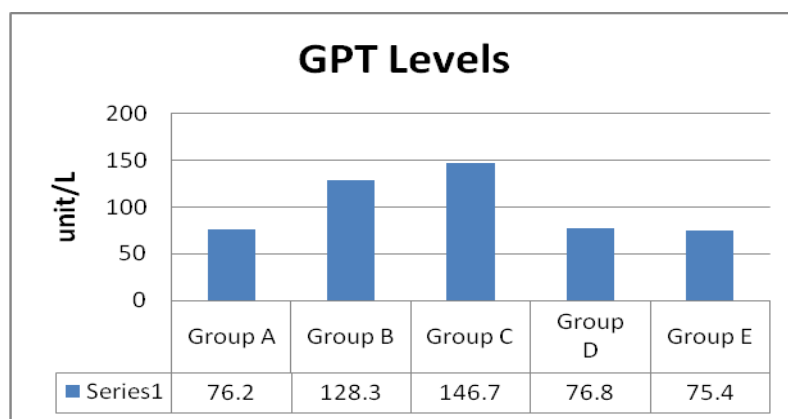
The microscope examination showed more recovery for hepatocytes and normal central

vein without any damage. Also, the kupffer cells and sinusoids appear normal (Fig. 5).

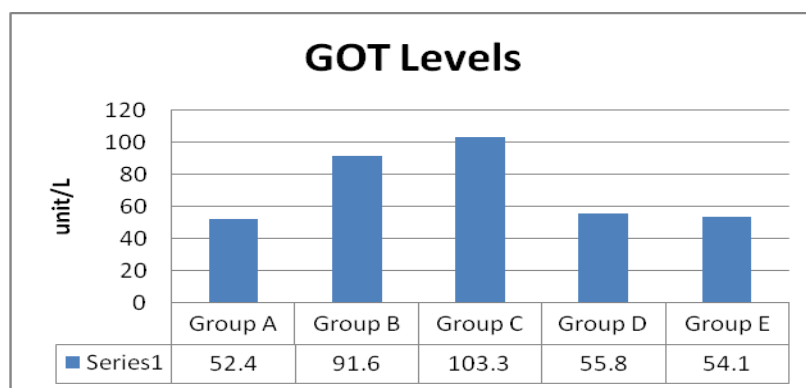
**Group Administrated Ethanol for Twenty One Days and Treated with Seeds Extract**

The microscope examination showed more recovery for hepatocytes and normal central vein without any damage. Also, the kupffer cells and sinusoids appear normal (Fig.6).

**Chart.1 GPT Tests**



**Chart.2 GOT Tests**

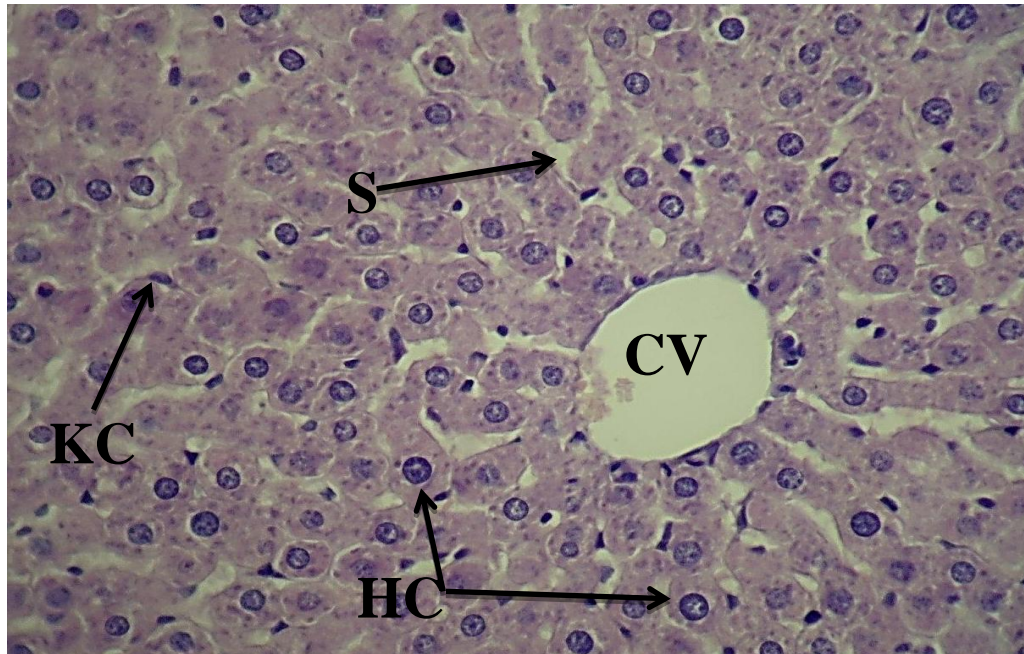


**Figure.1 *Trigonella foenum* seeds**

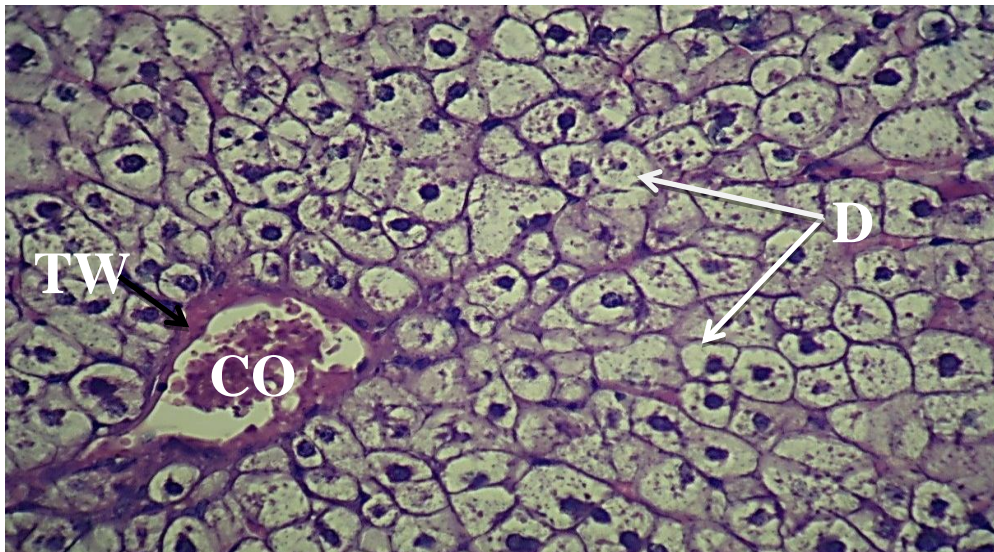
Kingdom: Plantae  
 Division: Magnoliophyta  
 Class: Magnoliopsida  
 Order: Fabales  
 Family: Fabaceae  
 Genus: *Trigonella*  
 Species: *T. foenum-graecum*  
 Botanical name:- *Trigonella foenum-graecum*(10).



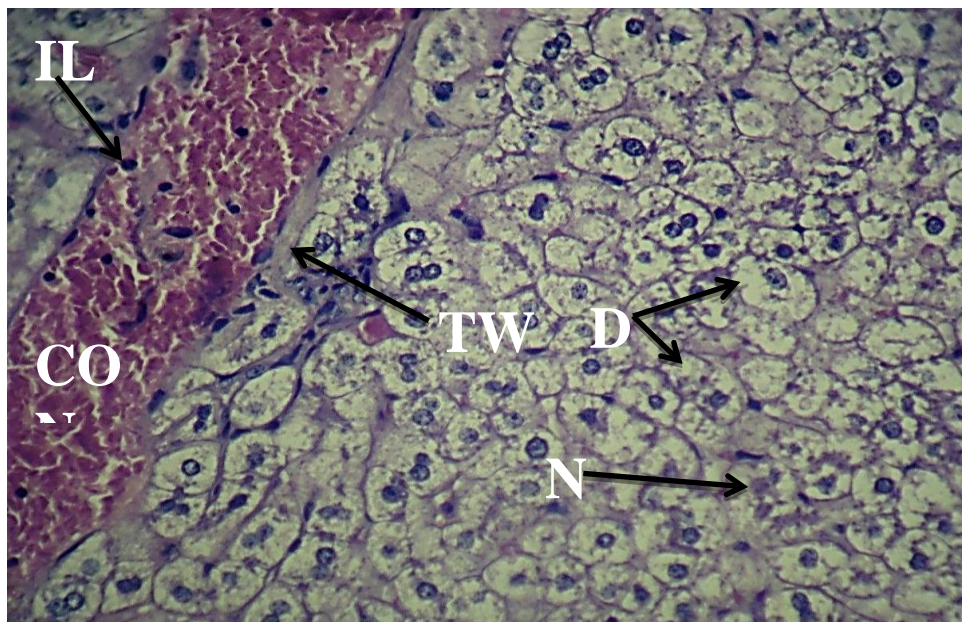
**Figure.2** Liver of control group showed normal central vein (CV), normal hepatocytes (HC), normal sinusoids(S) and normal kupffur cells (KC) H&E 400X.



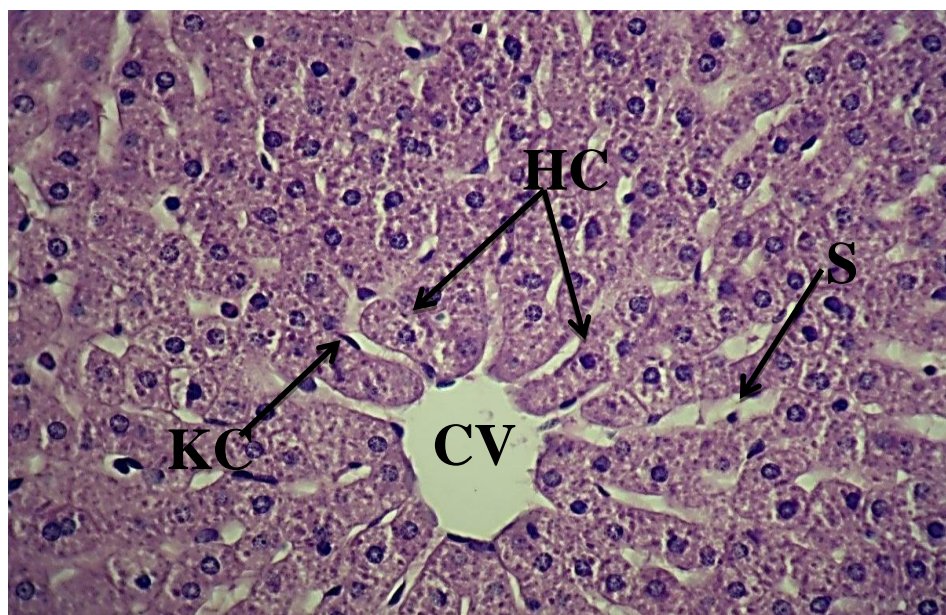
**Figure.3** Liver of ethanol for fifteen days showed thickening wall (TW) of central vein, congestion (CON) and degeneration (D) of hepatocytes H&E 400X.



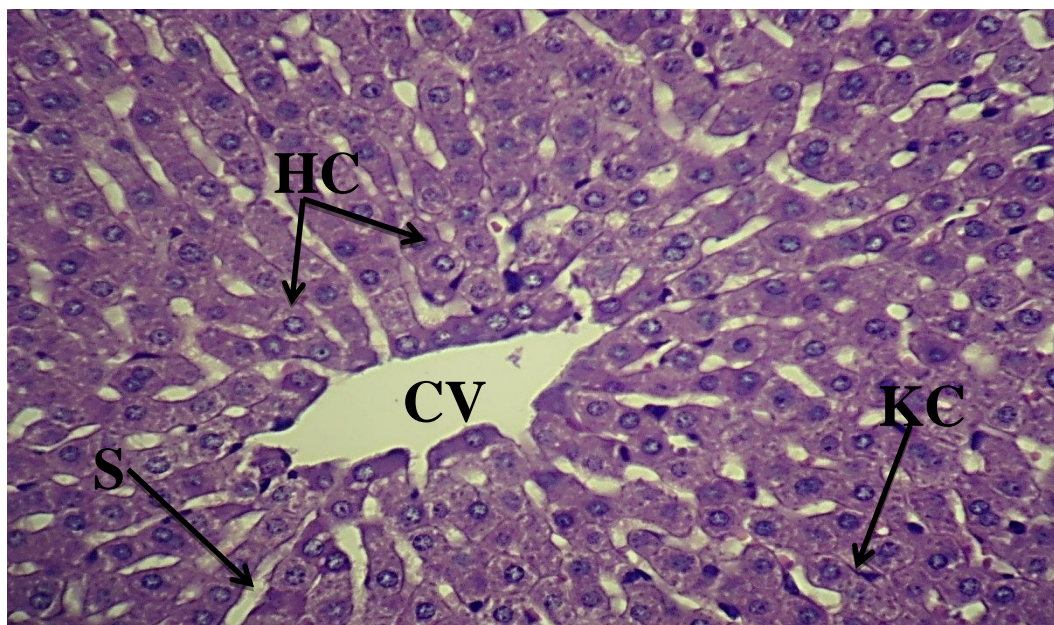
**Figure.4** Liver of ethanol for twenty one days showed thickening wall (TW) of central vein, congestion (CON), degeneration (D) and necrosis (N) of hepatocytes and lymphocytes infiltration (IL) H&E 400X.



**Figure.5** Liver of ethanol for fifteen days and treated with seeds extract group showed normal central vein (CV), normal hepatocytes (HC), normal sinusoids (S) and normal kupffer cells (KC) H&E 400X.



**Figure.6** Liver of ethanol for twenty one days and treated with seeds extract group showed normal central vein (CV), normal hepatocytes (HC), normal sinusoids(S) and normal kupffur cells (KC) H&E 400X.



Nwozo & Babatunji (2011) stated that the serum GOT and GPT was more than normal range in the animals (rat) after administrated ethanol. Also, they found histopathological alterations in liver which including degenerative changes and congestion of blood vessels that is in agreement with the results of the present study.

Wang *et al.*, (2006) referred that the mice treated with ethanol showed increased in serum GOT and GPT levels compare with control group and histopathological changes including degeneration and necrosis of hepatocytes in comparison with control that is in agreement with the results of the present study.

In study of Kaviarasan *et al.*, (2006), they referred that the *Trigonella foenum-graecum* seed extract prevents ethanol-induced toxicity and repaired the changes and lesions

that caused by ethanol in mice liver that is in agreement with the results of the present study.

In other studies, that aimed to showed the ability of *Trigonella foenum-graecum* seed extract to repaired the changes and lesions in liver of experimental animals that caused by different materials. Where, Kumar & Uma (2013) stated that the *Trigonella foenum-graecum* extract has a protective effect on the liver enzymes and tissue and repaired it after the rat administrated with monosodium glutamate. Also, Tohamy *et al.*, (2013) referred that the ability of *Trigonella foenum-graecum* seed extract to protect and repair the mice liver lesion that caused by *Saliva aegyptiaca* which including increased GOT and GPT levels and infiltration, congestion and degenerative changes of hepatocytes. These studies were in agreement with the results of the present

study wherever the ability *Trigonella foenum-graecum* seed extract to repaired the changes and lesions in liver of experimental animals.

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