

Study of Biochemical and Histological effects of fennel seeds (*Foeniculum vulgare*) on Liver in Male Rats.....

Noori Mohammed Luaibi , Ayser Abdullah Shafiq

## **Study of Biochemical and Histological effects of fennel seeds (*Foeniculum vulgare*) on Liver in Male Rats**

**Noori Mohammed Luaibi      Ayser Abdullah Shafiq**

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### **Abstract**

Consumption of foods enriched with herbs and herbal medicine products are highly popular. Their current popularity renders the assessment of their safety an urgent necessity, they must figure prominently in nutritional surveys as possible factors in cancer and some other diseases, as they may contain highly toxic chemicals and heavy metals in addition to natural organic toxins, made the present study aims to investigate the side effects of fennel seeds *Foeniculum vulgare*, in male rats, on the weights, histological changes and some of the physiological parameters of the liver. For this purpose, 60 Spargue-Dawley albino adult male rats were daily treated orally with fennel seed powder mixed with rat diet in three different doses (50, 100, 200)gm/kg bodyweight(bw) in three different periods of time (10, 20, 30) days. Adult male rats were divided into 12 groups each of five rats, as following : (Group 1, 2, 3) normal control rats that were fed with chow pellet only for (10, 20, 30) days subsequently, (Group 4, 5, 6) experimental treated groups that respectively received fennel pellet in three doses of (50, 100, 200) gm/kg for 10 days, (Group 7, 8, 9) experimental treated groups that respectively received fennel pellet in three doses of (50, 100, 200) gm/kg for 20 days, (Group 10, 11, 12) experimental treated groups that respectively received fennel pellet in three doses of (50, 100, 200) gm/kg for 30 days.

The end of each experiment was followed by weighing the animals, blood sample of each animal was collected by heart puncture then directly centrifuged and the serum was kept at -80 °C for biochemical analysis and some histological standards, the animals were dissected, then the liver was excised and fixed in neutral buffered 10% formalin for histological preparation.

Increased doses of fennel consumption and treatment duration statistically caused:

- ❖ Highly significant ( $p < 0.01$ ) decrement in liver weights of fennel treated groups ( 7, 8, 9, 10, 11, 12) in comparison to control groups.

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- ❖ Highly significant increment ( $p < 0.01$ ) in Glutamate oxaloacetate transaminase (GOT) and Glutamate pyruvate transaminase (GPT) serum levels in treated groups (5,6,7,8,9,10,11,12), while Alkaline phosphatase (ALP) serum levels showed significant increment ( $p < 0.05$ ) in fennel treated groups (10, 11, 12) compared to the control groups.
- ❖ liver sections showed sinusoidal dilatation with mild degenerative, inflammatory cells infiltration, congestion in blood vessels and necrosis. Fennel, liver, doses, necrosis.

### Introduction :

Medicinal herbs are good alternative to chemical drugs as they have low side effect compared to chemical drugs , one of the important oldest spice plants is fennel (*Foeniculum vulgare* Mill) which is used in the pharmaceutical, food, cosmetic and healthcare industries (Abe and Ohtani, 2013). Fennel has been used in traditional medicine for a wide range of disorders related to digestive, endocrine, reproductive, and respiratory systems (Badgular *et al.*, 2014).

The dried, aromatic fruits are vastly used in culinary preparations for flavoring bread and pastry, in candies, and in alcoholic liqueurs, also used in cosmetic and medicinal preparations (Farrell, 1985). The oil yield (2.5 - 5%) varies according to origin and variety and the highest concentration of fennel oil is found in seeds ranging between 2 - 7%, fennel volatile oil is a mixture of different chemicals and the main ingredients are: trans- anethole (40 - 70%), fenchone (1 - 20%) and estragole (2 - 9%), other compounds ( $\alpha$ -pinene, chavicol, dipentene,  $\alpha$ -limonene etc.) are present in concentration usually less than 1% (Bernath *et al.*, 1996; Cosge *et al.*, 2008).

Fennel fruits consist 10 to 12 % of oil that is stored in the cotyledons of seeds Oil obtained from the fennel fruit has 4% palmitic acid, 22% oleic acid, 14% linoleic acid and 6% petrocyclic acid. The aromatic property of fennel is because of the essence that has value of 4 to 6% its essence and combine ingredients vary according to the location of plant growth (Ahmadi *et al.*, 2007). *F. vulgare* known as a culinary herb also useful for pharmaceutical industry for its high content in 1,8-cineole, linalool, fenchone and estragol (Özcan and Chalchat, 2010).

Healthy importance of fennel comes from its numerous chemical compounds, such as volatile compounds, flavonoids, phenolic compounds, amino acids , and fatty acids, hence it has been used for abundant types of disorders (Badgular *et al.*, 2014). Fennel shows antispasmodic activities (Ostad *et al.*, 2001). And as curative in infantile colic (Alexandrovich *et al.*,

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2003). Different techniques carried out the antifungal and antioxidative potentials of fennel(Singh *et al.*,2005). Also can be used to reduce the potential of lung cancer, asthma and prevent thrombosis and atherosclerosis (Vardavas *et al.*, 2006). As it improves the milk supply of a breast feeding mother so it has been used as a galactagogue that occurs due to the presence of phytoestrogens present in fennel which promote growth of breast tissue (Agarwal *et al.*, 2008). In a study on experimental rats by Koppula and Kumar, (2013)improved that fennel has an anti-stress, memory enhancing and antioxidant effects due to phytoestrogens a chemical compound in fennel. Fennel exhibit antibacterial activity due to compounds such as, linoleic acid, undecanal, 1, 3-benzenediol, oleic acid and 2,4-undecadienal, also it 5-hydroxy-furanocoumarin that plays an important role in antibacterial activity of this plant( Parejo *et al.*,2004). A study demonstrated that fennel has an anti-fungal activity due to anethole that had the strongest antifungal activity among chemical compositions of the plant extract( Naeini *et al.*, 2011) Due to the anetholes antiplatelet activity, clot destabilising effect and vaso-relaxant action ,it has been reported that anethole is a safe antithrombotic agent (Tognolini *et al.*, 2007) Of the pharmacological effects of fennel plant, anti-inflammatory activity can be noted, results in a research has shown that the fennel methanol extract had anti-inflammatory activity dependent on the central and peripheral mechanisms (Choi and Hwang, 2004). Phyto constituents of *F. vulgare* demonstrated notable insecticidal activities against *Sitophilus oryzae*, *Callosobruchus chinensis*, and *Lasioderma serricorne*. This activity was examined using direct contact application and fumigation methods. The biologically active components are phenylpropenes (E)anethole and estragole, and the monoterpene (+)-fenchone were characterized from *Foeniculum* fruit. By using a filter paper diffusion test, estragole caused 91% mortality to *S. oryzae* within 1 day after treatment whereas fenchone and(E)-anethole gave over 90% mortality at 2 and 4 day after treatment, respectively ( Kim and Ahn, 2001).

### **Aims of the study :**

The present study was undertaken to evaluate the effects of fennel seeds (*Foeniculum vulgare*) in male rats on :-

- ❖ Function of liver GOT, GPT, ALP
- ❖ Weight and histological changes in Liver.

### **Materials and Methods**

#### **Laboratory Animals**

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Male Spargue-Dawley albino rats (*Rattus norvegicus*) were used in the present study as a mammalian model. Mature males (2-3 months) were purchased from National Center for Drug Control and Research (NCDRC), Ministry of Health and housed in the animal house of the College of Science/ Al-Mustansiriyah University. They were kept in standard plastic cages with a metal network cover under climate controlled conditions of the animal house with temperature  $25\pm 2^{\circ}\text{C}$  and 12:12 light and dark cycle. Rats were provided with water and food *ad libitum*.

The daily consumption of diet for each rat of the control and treated groups was 18-20 gram, within the US Constitution for medical herbs (PDR For Herbal Medicines, 2000).

## The plant

Fennel seeds were purchased from the local markets in the Ishtariyah Street of Al-Baya'a/ Baghdad. They were obtained as a fennel herb for culinary use, then they were prepared to be used for the experiment.

## Fennel pellet preparation

Fennel seeds about (2100 kg) were powdered in a seed grinder, (15,900kg) of pellet which contained (20% soya, 10% protein of fish powder, 20% American protein, 40% corn, 10% (wheat flour and additives such as fish oil and antioxidants) was powdered as well by the grinder, then the components were mixed and kneaded by addition of tap water and distributed into three groups as followed:

1. group of 50 grams fennel powder + 950 gram pellet powder.
2. group of 100 grams fennel powder + 900 gram pellet powder
3. group of 200 grams fennel powder + 800 gram pellet powder .

Small cylinder blocks were made from this dough similar to the normal rodent pellet. These blocks were separated gently and dried under the sun temperature for 48 hours. The resulted pellet represented the fennel pellet.

## Animals Groups

In this experimental study, after an adaptation period of one week, sixty male rats were randomly divided into twelve groups of five rats each, described as following :

Group 1, 2, and 3 : (control) did not receive any dose they were fed with rat chow pellet only for 10, 20, and 30 days subsequently.

Group 4, 5, and 6 : (the experimental groups) that respectively received 18- 20 g fennel pellet in three doses of (50, 100, and 200)g/kg every 24 hours for 10 days.

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Group 7, 8, and 9 : (the experimental groups) that respectively received 18- 20 g fennel pellet in three doses of ( 50, 100, and 200)g/kg every 24 hours for 20 days.

Group 10, 11, and 12 : (the experimental groups) that respectively received 18- 20 g fennel pellet in three doses of ( 50, 100, and 200)g/kg every 24 hours for 30 days.

## **Collection of Blood Samples**

The end of each experiment was followed by weighing the animals, they were fully anaesthetized by diethyl ether for several minutes and blood samples were obtained by heart puncture. 5 ml of blood collected from each rat, 1 ml of the blood was examined by hemoanalyzer (Genex Laborarores Company from U.S.A) in Histological laborator of college of science in Al-Mustasiriya University which is computerized, highly specialized machine that counts the number of different types of red and white blood cells in a blood sample (Orathai & Surapon, 2008), 4 ml of the blood was used to obtain sera (0.5-1.0)ml separated by centrifugation 3000 rpm for 5 min, then sera kept in -20°C until the time for using.

## **Collection of Organs :**

The animals were dissected and their livers were excised, washed with normal physiological saline 0.9% (NaCl), blotted with filter paper, weighed and kept in the fixative solution (neutral buffered 10% formalin) for histological study.

## **Measurement of the Level of ALP**

ALP was measured by using a colormetric method (Kind and King, 1954) for the determination of alkaline phosphatase levels in serum according to bioMê rieux company.

## **Measurement of the Level of GPT**

GPT was measured by using a colormetric method (Reitman and Frankel) for determination of serum alanine aminotransferase levels in serum (Reitman and Frankel., 1957), according to Randox company kit.

## **Measurement of the Level of GOT**

GOT was measured by using a colormetric method (Reitman and Frankel) for determination of serum aspartate aminotransferase levels in serum (Reitman and Frankel., 1957), according to Randox company kit.

## **Histopathological Study**

The Preparation for histological sections was performed according to the method of (Humason, 1979).

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### Results and Discussion :

#### Liver weight and liver functions

❖ The statistical analysis of the present study for fennel effects on liver weights (gm) in the figure (1) showed that:

Consumption of fennel for 10 days showed non-significant decrease in liver weights of the experimental treated groups with concentrations (50, 100, 200)gm/kg fennel compared to control group, while results revealed a significant decrease ( $p < 0.05$ ) in liver weights at 20 days experimental treated groups of (50,100, 200)gm/kg fennel ( $6.57 \pm 0.83$ ), ( $6.00 \pm 0.31$ ), ( $5.65 \pm 0.11$ ) (gm) respectively comparing with the control group ( $8.31 \pm 0.26$ ). As well there was significant decrease ( $p < 0.05$ ) in liver weights at 30 days feeding with fennel between control ( $8.32 \pm 0.29$ ) (gm) and all the experimental treated groups with concentrations (50, 100, 200)gm/kg ( $5.97 \pm 0.31$ ), ( $5.92 \pm 0.47$ ), ( $5.72 \pm 0.51$ ) (gm) respectively. Significant decrement ( $p < 0.05$ ) in liver weights was observed in the concentrations (50,100,200)gm/kg of fennel consumption with the increment of the treatment duration.

❖ Statistical analysis of the present study for fennel effects on liver enzymes that included GOT, GPT and ALP in the figures (2),(3),(4) revealed that:

- Fennel consumption for 10 days duration showed highly significant increment ( $p < 0.01$ ) in GOT level (U/L) in experimental groups with concentration (100,200) gm/ kg ( $13.00 \pm 1.00$ ), ( $14.40 \pm 1.40$ ) (U/L) respectively comparing with control group ( $8.00 \pm 1.00$ ) (U/L), as well there was highly significant increment ( $p < 0.01$ ) in GOT level at 20 days period of time fennel feeding in experimental groups with concentration (100,200) gm/kg ( $16.49 \pm 1.65$ ), ( $18.19 \pm 1.83$ ) (U/L) respectively comparing with control group ( $10.00 \pm 1.26$ ), even a 30 day treatment with fennel showed highly significant increment ( $p < 0.01$ ) in GOT level in all experimental groups with concentrations (50,100,200)gm/kg ( $12.00 \pm 1.00$ ), ( $17.00 \pm 1.00$ ), ( $20.00 \pm 1.00$ ) (U/L) respectively comparing with control group ( $8.00 \pm 1.00$ ). Statistical analysis revealed non-significant increment due to fennel consumption on GOT level related with treatment duration when concentrations (50, 100)gm/kg bw were fixed factors in all treated groups, but there was significant increment ( $p < 0.05$ ) in GOT level during 30 days treatment in concentration (200)gm/kg between all treated groups.as shown in figure (2)

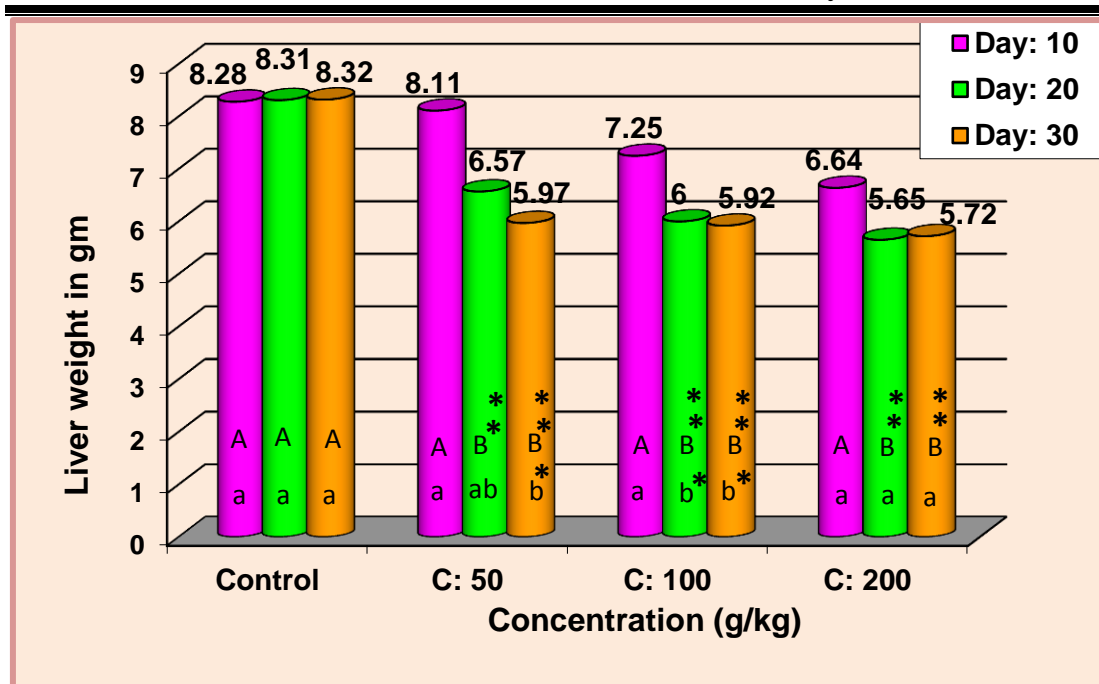
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- The present study showed findings about fennel consumption effect on GPT level (U/L) in treated rats in comparison with control groups similar to the results of GOT level with some exceptions. Fennel administration in 10 days duration showed highly significant increment ( $p < 0.01$ ) in GPT level in experimental groups with concentrations (100,200)gm/kg ( $37.00 \pm 1.00$ ), ( $42.00 \pm 1.00$ ) (U/L) respectively comparing with control group ( $29.20 \pm 2.87$ )(U/L), also a 20 day period with fennel administration showed highly significant increment ( $p < 0.01$ ) in GPT level in all experimental groups with concentrations (50,100,200) gm/kg ( $32.00 \pm 1.58$ ), ( $38.00 \pm 1.58$ ), ( $45.00 \pm 1.58$ ) (U/L) respectively comparing with control group ( $27.00 \pm 0.71$ )(U/L) and so did a 30 day period treatment with fennel illustrated highly significant increase ( $p < 0.01$ ) in GPT level in all experimental groups with concentrations (50,100,200) gm/kg ( $46.00 \pm 1.78$ ), ( $62.00 \pm 1.78$ ), ( $88.00 \pm 1.78$ )(U/L) respectively comparing with control group ( $28.20 \pm 2.35$ )(U/L). There was highly significant increment due to fennel consumption on GPT level related with treatment duration when concentration was a fixed factor observed only at 30 days period of time in treated groups with concentrations (50,100)gm/kg bw ( $46.00 \pm 1.78$ ), ( $62.00 \pm 1.78$ ) (U/L) and in 20 period of time in treated group with concentrations (200)gm/kg bw ( $45.00 \pm 1.58$ ) (U/L) while comparing between groups when concentration is a fixed factor, as shown in figure (3).
- Fennel consumption for 10 and 20 days demonstrated non-significant increase in ALP levels (U/L) comparing between controls and experimental treated groups with different concentrations (50, 100, 200)gm/kg, but treatment for 30 days showed significant increase ( $p < 0.05$ ) in ALP levels in concentrations (50,100,200)gm/kg bw ( $703.00 \pm 79.62$ ), ( $766.00 \pm 46.92$ ), ( $797.00 \pm 39.49$ ) (U/L) respectively comparing with control group ( $424.59 \pm 77.98$ ) (U/L), while there was non-significant increase in ALP levels comparing between experimental groups themselves in each concentration (50,100,200)gm/kg bw when duration time is a fixed factor, as shown in figure (4).

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Figure(1): Effect of different concentrations from fennel (50,100,200) gm/kg on liver weights of rats with different periods of time (10,20,30) days in comparison with control groups. (\*) significant decrease ( $P \leq 0.05$ ). (\*\*) highly significant decrease ( $P \leq 0.01$ ). (\*\*\*) highly significant decrease ( $P \leq 0.001$ ).

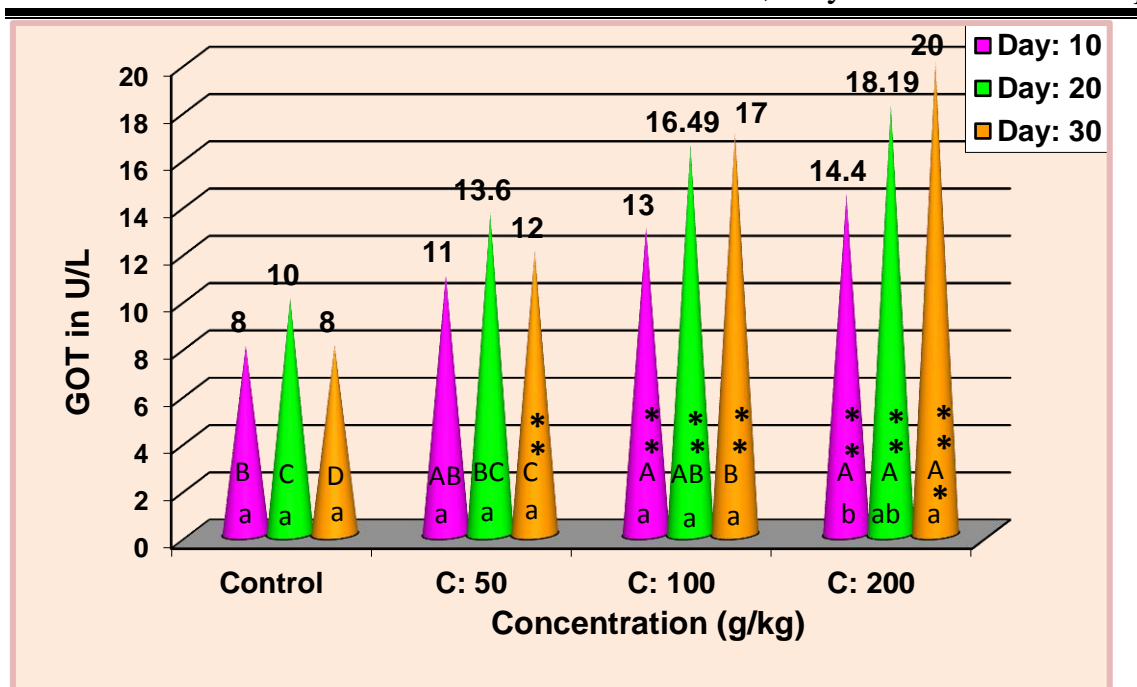
(A,B,C) represents the significant difference between groups with days as a fixed factor and concentrations as a variable factor.

(a,b,c) represents the significant difference between groups with concentrations as a fixed factor and days as a variable factor.



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Figure(4-14): Effect of different concentrations of fennel Figure(2): Effect of different concentrations from fennel (50,100,200)gm/kg on GOT levels of rats with different periods of time (10,20,30) days in comparison with control groups.

(\*) significant increase ( $P \leq 0.05$ ).

(\*\*) highly significant increase ( $P \leq 0.01$ ).

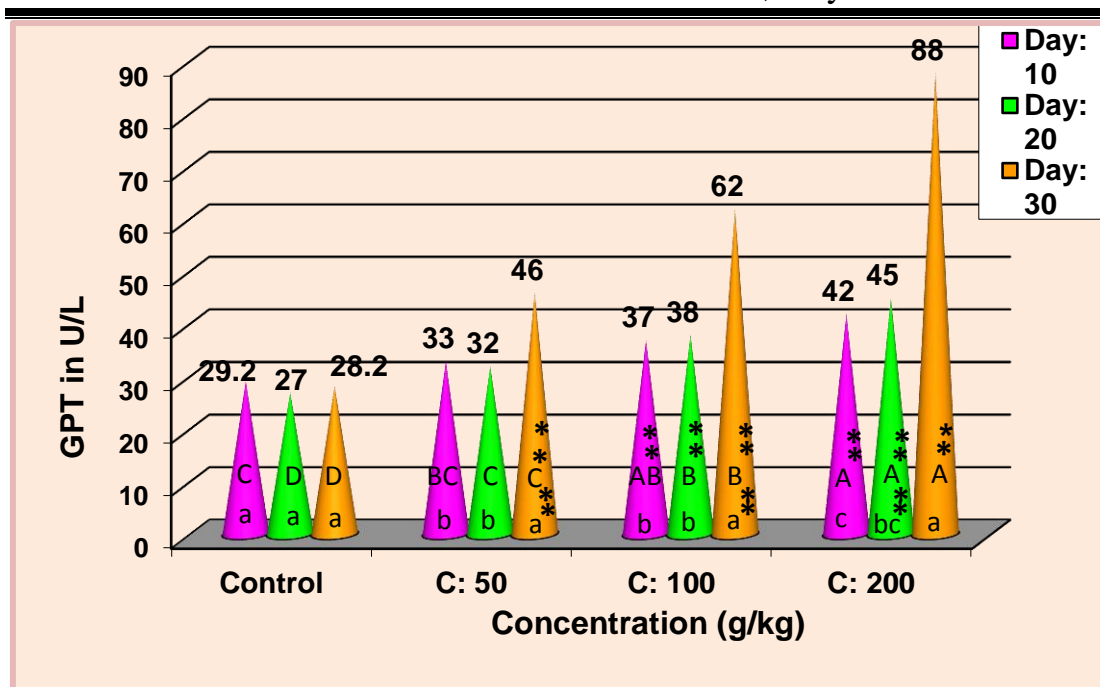
(\*\*\*) highly significant increase ( $P \leq 0.001$ ).

(A,B,C) represents the significant difference between groups with days as a fixed factor and concentrations as a variable factor.

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Figure(3): Effect of different concentrations from fennel (50,100,200)gm/kg on GPT levels of rats with different periods of time (10,20,30) days in comparison with control groups.

(\*) significant increase ( $P \leq 0.05$ ).

(\*\*) highly significant increase ( $P \leq 0.01$ ).

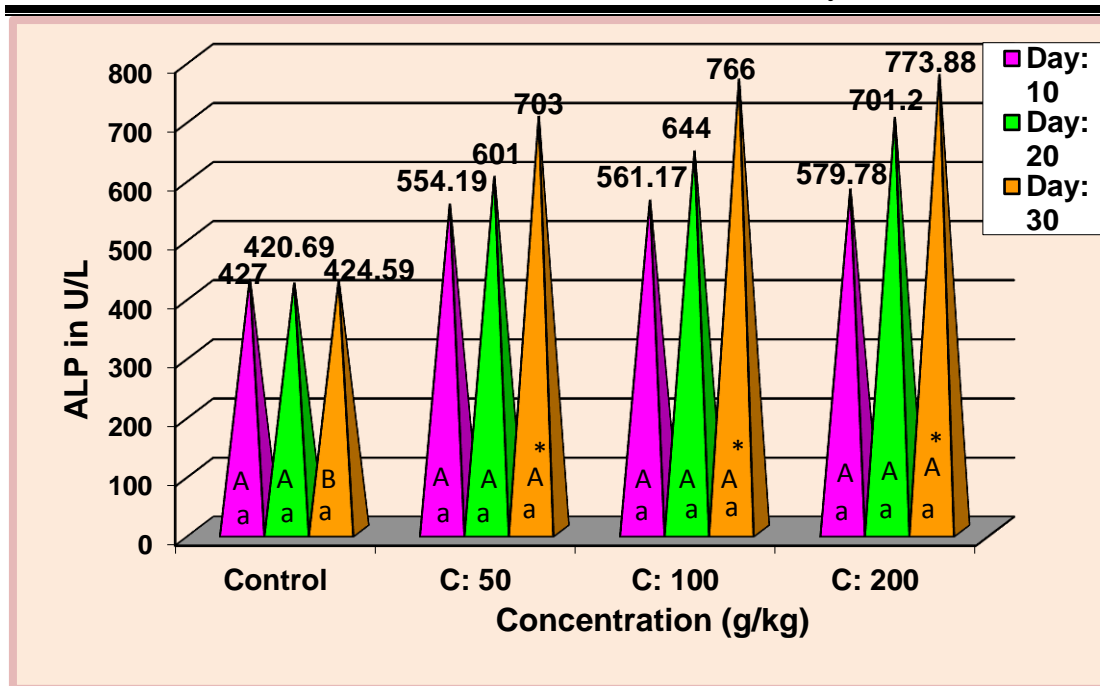
(\*\*\*) highly significant increase ( $P \leq 0.001$ ).

(A,B,C) represents the significant difference between groups with days as a fixed factor and concentrations as a variable factor.

(a,b,c) represents the significant difference between groups with concentrations as a fixed factor and days as a variable factor

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Figure(4-16): Effect of different concentrations of fennel Figure(4): Effect of different concentrations from fennel (50,100,200)gm/kg on ALP levels of rats with different periods of time (10,20,30) days in comparison with control groups.

(\*) significant increase ( $P \leq 0.05$ ).  
 (\*\*) highly significant increase ( $P \leq 0.01$ ).  
 (\*\*\*) highly significant increase ( $P \leq 0.001$ ).  
 (A,B,C) represents the significant difference between groups with days as a fixed factor and concentrations as a variable factor.  
 (a,b,c) represents the significant difference between groups with concentrations as a fixed factor and days as a variable factor.

Results of the present study about the decrement of liver weights due to the fennel consumption are in agreement with previous reports of dietary administration of trans-anethole to groups of 24 male and female mice received (0, 0.1, 0.25, 0.5)% trans-anethole in diet for 22 days, induced statistically significant decrease in mice liver weights that fed (0.25, 0.5)% trans-anethole, while it induced statistically significant increase in mice liver weights that fed (0.1)%, both compared with control group (Reed, 1994). Also in a study by Newberne, (1997) was conducted in male and female rats received doses of (0, 150, 300, 600, 900 and 1200)mg/kg bw per day trans-anethole in diet for 28 days induced statistically significant decrease in liver weights of rats at the intermediate

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and high doses of *trans*-anethole. The findings of Vasavasour,(1999) disagreed with results of the present study, he concluded according to his results that the liver was identified as a target of anethole-induced toxicity in mice and rats; oral administration of (30-900)mg/kg per day anethole to mice and rats showed dose-related increases in liver weights. Also, Gershbein,(1977) indicated that sub- cutaneous injections of oils of fennel and anise for 10 days in rats led to significant liver weight increment. In another study by Rompelberg *et al.*,(1993) they fed male wistar rats with *trans*-anethole (0, 125, 250) mg/kg bw per day for 10 days in corn oil by gavage , liver weights increased significantly for animals fed with *trans*-anethole 250 mg/kg bw per day but there was no effect on GOT or GPT activities. In a study by Mohammed, (2010) whom fed male rats with fennel oil (250mg/kg bw) for 21 days showed insignificant increase in GOT,GPT and alkaline phosphatase when compared with control rats. while different results were obtained from a study by Bitar-Shamas, (2009) in a period of 2 weeks on rat groups fed with fennel ethanol extract (0.6, 1.2, 2.4)%, ALP and GPT levels showed non-significant decrease in groups fed with 1.2% fennel extract but GOT level increased non-significantly in groups fed with (0.6, 2.4 and 4.8)% fennel extract, GPT level increased in groups treated with (2.4 and 4.8)% fennel extract, ALP level increased in groups treated with (0.6 and 2.4)% fennel extract, each group was compared to the control group. Flavonoids in fennel oil thought to be responsible of the activities in fennel (Sheikh *et al.*, 1997). Fennel seed extract has been reported to contain d-limonene and  $\beta$ -myrcene which proved to effect liver function (Rabeh and Aboraya, 2014). In another study by Awe and Banjoko, 2013),they observed toxic effects of parsley leaf extract after 8 weeks of oral treatment to rats of both sexes, the extract caused significant increase in GPT and ALP levels at high doses (1000 mg/kg bw) compared to control groups and led to liver injury that is related to GPT increment, these results indicate that leaf ethanol extract of parsley was hepatotoxic. The current study disagreed with the results of Rabeh and Aboraya, (2014) whom fed fennel oil extract or dill to rats induced toxicity by carbon tetrachloride (CCl<sub>4</sub>), fennel led to significant decrease(p<0.05) in levels of serum GOT and GPT, moreover Dill and Fennel oil supplementation suppressed the increase of ALP activity. Hepatic injury is correlated with distortion of metabolic function, biochemical analysis of serum tests (GOT, GPT and ALP) evaluates hepatic disease (Hyder *et al.*, 2013). Marked elevation of GOT and GPT in the appropriate clinical

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context indicates acute cell necrosis which is caused by drugs, toxins, alcohol, viral infection or ischemia (Burke, 2002).

Serum concentrations of aminotransferases are biochemical markers of hepatocellular necrosis, they increase as a result of cellular membrane damage and leakage to the tissues producing them. Thus the increment in liver enzymes reflects the histological changes and congestion that occurred in hepatic vein in addition to damage of hepatocytes followed by necrosis, these changes demonstrated after consumption to fennel may be because of flavonoids , *d*-limonene and anethole that are present in fennel which led to an imbalance in liver functions and with the increment in period of time exposing to fennel, these changes where more worsen.

### **Histological Changes of Liver**

The main histological changes in all treated rats with fennel seeds on liver tissues in different periods of time compared to control groups is shown as follows:

Liver sections showed different histological changes after treatment with fennel in concentration of 50gm/kg of body weight for 10 days, included sinusoidal dilatation with mild degenerative changes of hepatocytes(Figure 6), these changes have developed in a period of 20 days. and in a period of 30 days, liver sections showed dispersed necrotic cells with very mild inflammatory cells infiltration and sinusoidal dilatation(Figure 7)compared with the histological sections of liver from control groups of rats(Figure 5).

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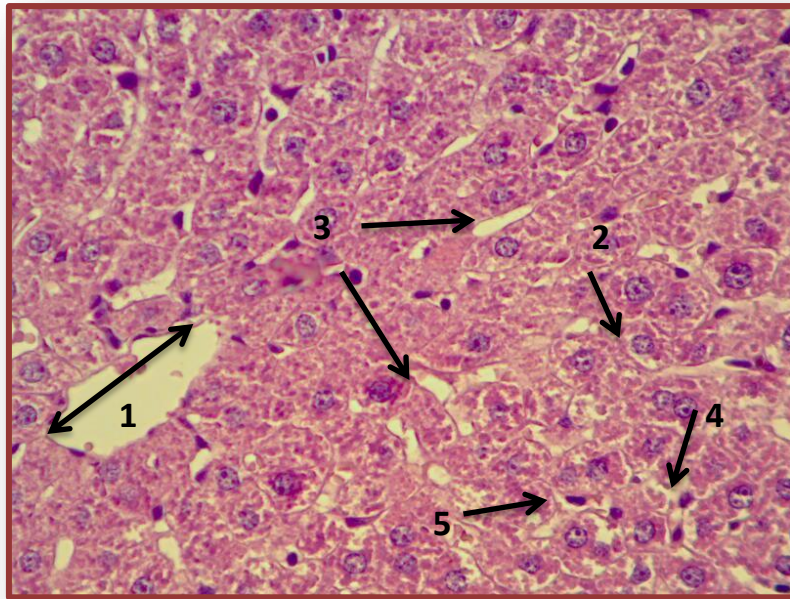


Figure (5) Section of liver from rat control groups showing  
1. Appearance of central vein, 2. Arrangement of hepatocytes as like a cord around central vein, 3. Sinusoids 4. Kupffer cells, 5. Endothelial cell. (400x) H&E.

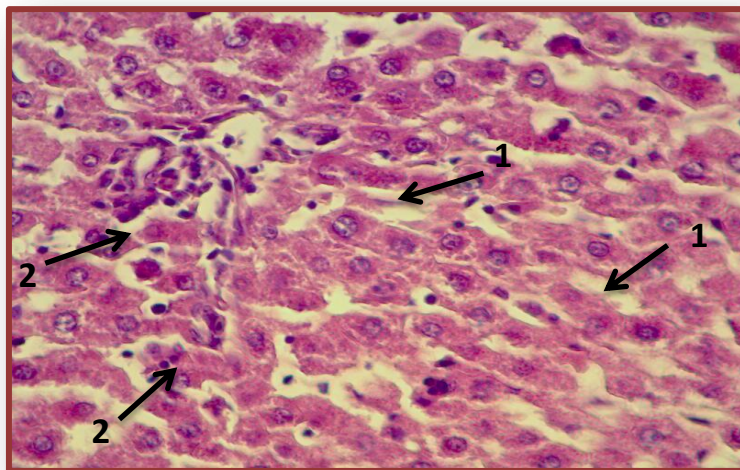


Figure (6) Section of liver from rat groups treated with 50g/kg bw for 10 days, showing: 1. Sinusoidal dilatation, 2. Mild degenerative changes of hepatocytes (400x) H&E.

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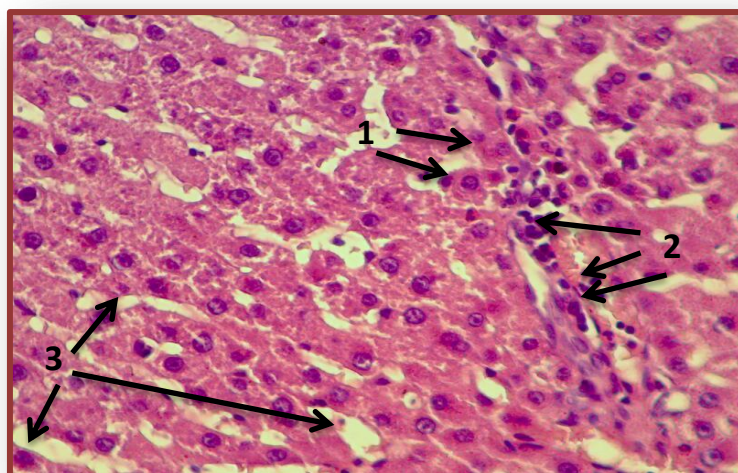


Figure (7) Section of liver from rat groups treated with 50g/kg of bw fennel for 30 days, showing 1. Dispersed necrotic cells, 2. Very mild inflammatory cells, 3. Sinusoidal dilatation, (400x) H&E.

Histological changes after treatment with fennel in concentration of 100gm/kg of body weight for 10 days on liver sections showed dispersed focal area of necrosis of hepatocytes with mild inflammatory cells infiltration and dilatation of sinusoid (Figure 8) but it seems to show further effects in experimental group of 20 days that are nearly identical to the outcomes of 30 days exposing to fennel, while in experimental group of 30 days treatment with fennel illustrated more prominent appearance of inflammation cells infiltration and necrosis with sinusoidal dilatation and congestion in blood vessels (Figure 9). compared with the histological sections of liver from control groups of rats (Figure 5).

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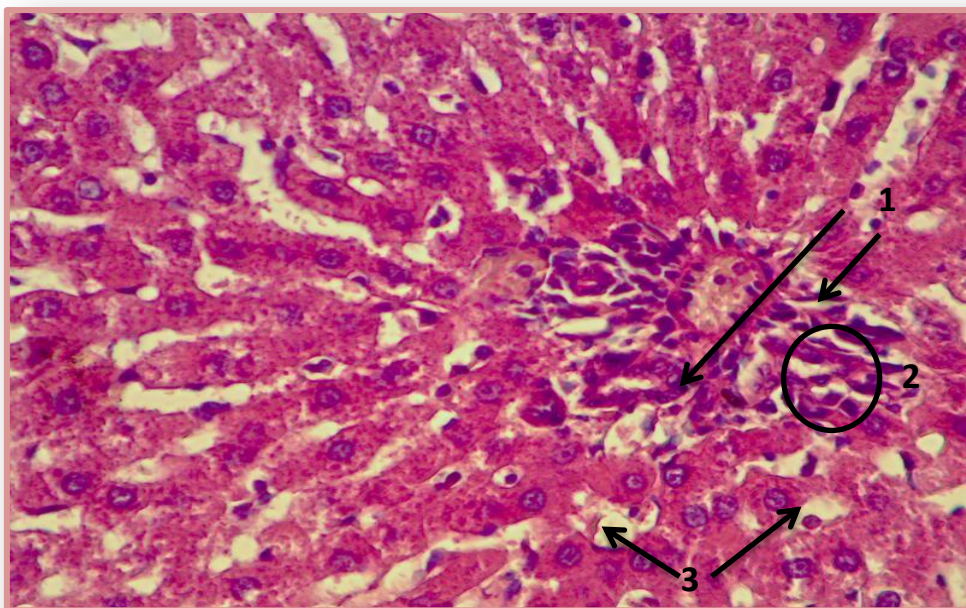


Figure (8) Section of liver from rat groups treated with 100g/kg of bw fennel for 10 days, showing: 1. Dispersed focal area of necrosis, 2. Hepatocyte cells with mild inflammatory cells infiltration, 3. Dilatation of sinusoids . (400x) H&E.

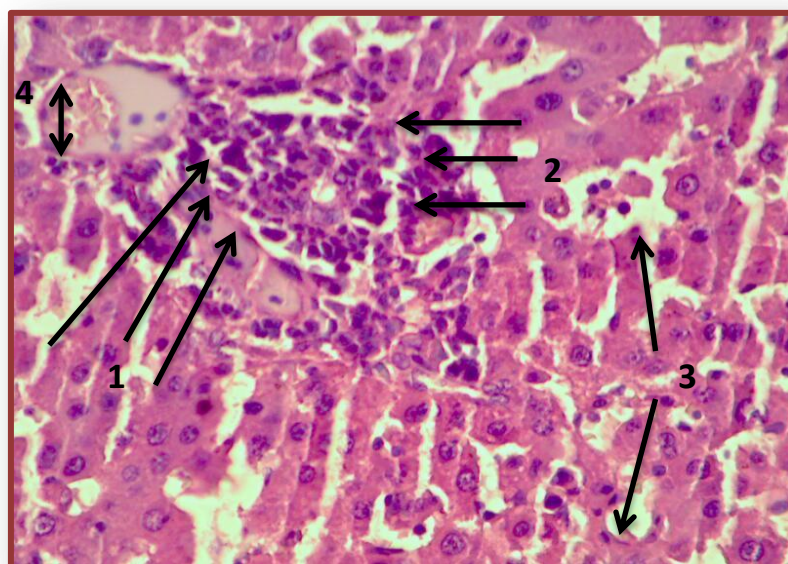


Figure (9) Section of liver from rat groups treated with 100g/kg of bw fennel for 10 days, showing : 1. More prominent appearance of inflammatory cells infiltration, 2. Necrosis, 3. Sinusoidal dilatation, 4. Congestion. (400x) H&E.



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Histological sections of liver from treated groups with 200mg/kg of bw fennel consumption for 10 days demonstrated focal area of necrosis and inflammatory cells infiltration and congestion of blood vessels (Figure 10), a beginning of congestion at mid doses(20 days) of fennel and more prominent appearance of inflammatory cells with necrosis and sinusoidal dilatation, but it seems to show worse effects in experimental groups of 30 days fennel consumption, as sections of liver showing wide area of necrosis and inflammatory cells infiltration in addition to sinusoidal dilatation with congestion of blood vessels (Figure 11). compared with the histological sections of liver from control groups of rats(Figure 5).

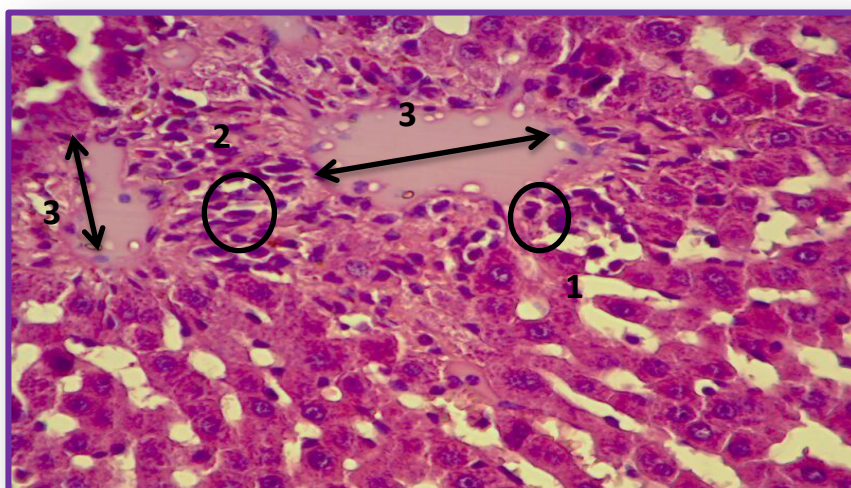


Figure (10) Section of liver from rat groups treated with 200g/kg of bw fennel for 10 days, showing: 1. Focal area of necrosis, 2. Inflammatory cell infiltration, 3. Congestion of blood vessels. (400x) H&E

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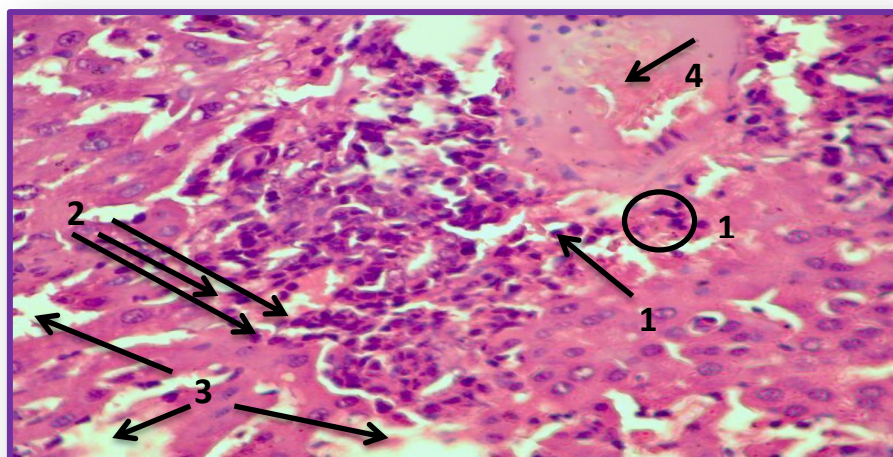


Figure (11) Section of liver from rat groups treated with 200g/kg of bw fennel for 30 days, showing: 1. Majority area of necrosis, 2. Wide area of inflammatory cells infiltration, 3. Sinusoidal dilatation, 4. Congestion of blood vessels (400x) H&E.

Liver is a vital organ in the body that is responsible for detoxification of toxic chemicals and drugs, thus it is a target organ to all toxic chemicals (Rabeh and Aboraya, 2014). Apiaceae plants produce coumarin compounds, either concentrated or isolated form of coumarins, they will stay toxic due to internal hemorrhage and liver toxicity (Stansbury 2016). The present study results agree with Al-Kassi, (2010) whom studied the effect of cumin (a member of Apiaceae family) feeding (0.5, 1, 1.5)% with diet on broiler chicks for 42 days, results showed significant increase in liver weights with increase in body weight gain but high concentration of cumin consumption in group 4 showed depression in growth due to the damage in intestine, liver and kidney. It is related to the mechanism due to components of the plant may cause damage to body tissues (Ibrahim *et al.*, 2007). Adverse effects of flavonoids may overbalance their beneficial effects, they also can inhibit the number of enzymes leading to altering normal body functions in addition to interfering with mineral absorption in our bodies (Fuhrman, 2007). Oral administration of D-limonene will be absorbed almost completely in the gastrointestinal tract of both humans and animals then rapidly distributes to various tissues in the body including liver, kidney, lungs, blood serum and in high concentrations found in adipose tissue (Crowell *et al.*, 1992). It has been demonstrated that mice treated 1000 mg/kg/day d-limonene for 28 weeks exhibited an increase in the incidence of multinucleated hepatocytes (National Toxicology Program, 2007).

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Estragole undoubtedly was found to induce liver DNA damage when cultured in F344 rat hepatocytes (Chan and Caldwell, 1992). Minimal to mild hepatocyte necrosis occurred in estragole-treated rat livers, with a dose-dependent increasing (Ding *et al.*, 2011). In a study by Liu *et al.*, (1999) they injected rats with safrole (0, 250, 500 and 1000)mg/kg, liver cell damage demonstrated by the increase of serum GOT and GPT levels, results show that safrole has the potential to induce oxidative damage *in vivo*, this type of damage may be involved in hepatocarcinogenic effects of safrole. In male and female rats exposed to safrole for 8 weeks, light microscopy of liver sections showed presence of individual cell necrosis (Gray *et al.*, 1972). Estragole occurs naturally in a variety of plants including fennel, tarragon and sweet basil, several studies with oral, inter peritoneal or subcutaneous administration to mice or rats shown the carcinogenicity of estragole (De Vincenzi *et al.*, 2000; Scientific opinion, 2001; Emea, 2004; Iten and Saller, 2004 and National Toxicology Program, 2011). Many plants synthesize hepatotoxic compounds, a study have been performed on three women to ingest *Centella asiatica*, results showed hepatic injury by promoting apoptosis and altering cell membranes, furthermore biopsies of the patients showed marked eosinophilic degeneration and cellular necrosis, Marked necrosis of hepatocytes and apoptosis may be accompanied by an inflammatory infiltrate surrounding injured cells with an increase in GPT and GOT with respect to ALP (Jorge and Jorge, 2005). Safrole might raise a potential concern for human health and would be of high priority for risk management (van den Berg *et al.*, 2011).

Congestion of blood vessels refers to advanced damage to liver tissue that could also effect liver functions, these outcomes had occurred after exposure to safrole and estragole which are potent components in fennel. The liver is a main site of biotransformation, as it converts xenobiotics to be water-soluble metabolites that can be effectively excreted. Liver cells and kidneys contain several enzymes that oxidize xenobiotics forming metabolites, enzymes conjugate to these metabolites forming a larger molecule and more polar to facilitate excretion and prevent accumulation of harmful substances in the body, however biotransformation also may induce toxicity when metabolites are not conjugated to enzymes, and so they bind to and damage cellular structures (binding to DNA induces mutation causing genetic toxicology), overloaded biotransformation causes massive destruction of essential proteins or lipid membranes and cause cell death.

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Different doses, animal species diversity, the specific part of herb used, routes of administration and duration of study are some of the effective factors that would cause varied of results of the studies mentioned above.

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## دراسة التأثيرات الكيموحيوية والنسجية لبذور الحبة

### الحلوة ( الشمار ) *Foeniculum vulgare* على

#### الكبد لذكور الجرذان

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#### المستخلص

ان تناول الاغذية الغنية بالاعشاب والمنتجات الطبية العشبية هي ذات استخدام واسع، الشعبية الراهنة تجعل تقييم سلامة هذه العشبة في الاستخدام ضرورة ملحة يجب ان تحتل مكانا بارزا في مسوحات الاغذية باعتبارها عوامل محتملة في حدوث السرطانات وبعض الامراض المختلفة الاخرى لأحتوائهم على مواد كيميائية ذات سمية عالية ومعادن ثقيلة اضافة الى سموم عضوية طبيعية، مما جعلت الدراسة الحالية تهدف الى التحقق من تأثير بذور نبات الشمر على اوزان الجرذان وبعض المعايير الوظيفية الاخرى والتغيرات النسجية للكبد.

لهذا الغرض تم استخدام 60 جرذ ابيض ذكر. تم تجريعهم فمويا يوميا ببذور الشمر الممزوجة مع الغذاء المخصص للجرذان بثلاث جرع مختلفة (50, 100, 200)غم/كغم لوزن الجسم ولفترات زمنية ثلاث مختلفة (10, 20, 30) يوم. قسمت الجرذان البالغة الى 12 مجموعة ، في كل مجموعة 5 جرذان كما التالي: (مجموعة 1, 2, 3) هي مجاميع سيطرة تغذت فقط على العليقة الخاصة بالجرذان لفترات زمنية ثلاث مختلفة (10, 20, 30) يوم على التوالي، مجموعة (4, 5, 6) هي مجاميع جرذان عوملت

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على التوالي ببذور الشمر الممزوجة بالعليقة بجرع ثلاث مختلفة (50, 100, 200) غم/كغم لوزن الجسم لمدة 10 ايام، (مجموعة 7, 8, 9) هي مجاميع جرذان عوملت على التوالي ببذور الشمر الممزوجة بالعليقة بجرع ثلاث مختلفة (50, 100, 200) غم/كغم لوزن الجسم لمدة 20 يوما، اما (المجموعة 10, 11, 12) هي مجاميع جرذان عوملت على التوالي ببذور الشمر الممزوجة بالعليقة بجرع ثلاث مختلفة (50, 100, 200) غم/كغم لوزن الجسم لمدة 30 يوما.

بعد انتهاء فترة التجريع وزنت الحيوانات المختبرية وجمعت عينات الدم لكل جرد عن طريق طعنة القلب وفصلت مباشرة وحفظت عند درجة -80 م<sup>0</sup> لأجراء التحليل الكيمائية الحياتية وبعض المعايير النسجية، تم تشريح الحيوانات وعزل الكبد وتم حفظه في 10% فورمالين للاعداد النسيجي.

بعد تجريع الجرذان بتراكيز عالية من الشمر ولفترات زمنية طويلة اظهرت النتائج المعطيات التالية:.

❖ حصول انخفاض معنوي عالي ( $p < 0.01$ ) في اوزان الكبد لمجاميع المعاملة (7, 8, 9, 10, 11, 12) مقارنة مع مجاميع السيطرة.

❖ حصول ارتفاع معنوي عالي ( $p < 0.01$ ) في مستويات انزيمات الكبد ( GOT, GPT) في مصل الدم لمجاميع المعاملة (6, 7, 8, 9, 10, 11, 12) ،في حين مستويات انزيم الكبد ALP اظهرت ارتفاع معنوي ( $p < 0.05$ ) في مجاميع المعاملة (10, 11, 12) مقارنة مع مجاميع السيطرة

❖ الفحص النسيجي لنسيج الكبد اظهر توسع الجيوب وتتكس وتخلل الخلايا الالتهابية مع احتقان الاوعية الدموية وتخر.

الكلمات المفتاحية : ببذور الشمر، الكبد، جرع، تنخر.