

Effect of Essential Oil Extracted from Dill Seed, Parsley Seed and its Mixture on Rats with Liver Disorder

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Abstract:

The main objective of the research is to study the effect of some plants essential oils , which include (dill seed essential oil , parsley seed essential oil and their mixture) to treat liver disorder using experimental animals. Mature male albino rats weighing 200 – 250 g were obtained from the Laboratory of Animal Colony, Institute Research Alrmdi , Giza , Egypt . The animals were divided into 8 equal groups; one group was kept as a (Control - ve), while the other 7 groups were injected by carbon tetrachloride (CCL4) in paraffin oil 50 % V / V (2ml / kg B. Wt.) twice a week for two weeks to induce chronic liver damage . Rats with liver intoxication were disported into 7 equal groups (n = 6 rats) using two doses (200 and 400 mg /kg of dill seed oil , parsley seed oil and mixture of them). At the end of experimental period (28 days) , blood samples were collected for serum separation to determine serum liver enzymes (GOT , GPT) total protein , albumin , globulin , albumin / globulin (A / G) ratio and random blood sugar . Results of the present study revealed that all tested plants essential oils showed a significant enhancement in liver intoxication and all tested parameters . According to these results , dill seed essential oil , parsley seed essential oil and their mixture could be used for impaired liver function

Key words : Anethum graveolens L (Dill). - Petroselinum crispum (Parsley) - Liver disorders.

Introduction:

Liver disorders :(Hepatitis , Cirrhosis) , it is an inflammation of the liver that may be caused by viral and bacterial infections , chemical toxicity as (CCL₄) Cirrhosis refers to scarring of the liver . Scar tissue form because of injury or long-term disease . It replaces healthy tissue. Scar tissue cannot do what healthy liver tissue does make protein, help fight infections , clean the blood , help digest food , asnd store energy for when you need it . Scar tissue also blocks the normal flow of blood through the liver . Too much scar tissue means that your liver cannot work properly . To live , you need a liver that works.(Tawheeda 2010). Meanwhile, Mahmood, et.,al (2014) Indicated that,parsley (*Petroselinum crispum*) is an important culinary herb originated from the Mediterranean region. It possesses small and dark seeds with volatile oil content. *Petroselinum crispum* is now planted throughout the world due to its usage in food industry, perfume manufacturing, soaps, and creams. Its main constituents subsume coumarins, furanocoumarins (bergapten, imperatori), ascorbic acid, carotenoids, flavonoids, apiole, various terpenoid compounds, phenyl propanoids, phthalides, and tocopherol. Due to these constituents, it has been announced to possess a number of possible medicinal emblematics including, antimicrobial, antianemic, menorrhagic, anticoagulant, antihyperlipidemic, antihepatotoxic, antihypertensive, diuretic effects, hypoglycaemic, hypouricemic, anti oxidative and estrogenic activities. In Morocco, Parsley is mostly used as an elixir to treat arterial hypertension, diabetes, cardiac and renal diseases. Antioxidant and antibacterial activities of parsley, made it propitious in food systems. Its ELI17 gene has been corroborated as a particularly fast-responding gene. There is a requisite for extensive research to avail the maximal benefits of this significant medicinal plant. Parsley has been used as carminative, gastro tonic, diuretic, antiseptic of urinary tract, anti-urolithiasis, anti-dote and anti-inflammatory and for the treatment of amenorrhea, dysmenorrhea, gastrointestinal disorder, hypertension, cardiac disease, urinary disease, otitis, snuffle, diabetes and also various dermal disease in traditional and folklore medicines. Phenolic compounds and flavonoids particularly apigenin, apiin and 6"-Acetylapiin; essential oil mainly myristicin and apiol; and also coumarins are the active compounds identified in *Petroselinum crispum*. Wide range of pharmacological activity including antioxidant,

hepatoprotective, brain protective, anti-diabetic, analgesic, spasmolytic, immunosuppressant, anti-platelet, gastroprotective, cytoprotective, laxative, estrogenic, diuretic, hypotensive, antibacterial and antifungal activities have been exhibited for this plant in modern medicine. Farzaei et., al (2013). Dill (*Anethum graveolens* L.) is an aromatic spice plant from the genus *Anethum* of the family *Apiaceae Umbelliferae* (Leung and Foster , 2003) . This plant is an important condiment crop with a characteristic aroma and odour (Pino et al., 1995) . It is well known as a medicinal herb with antimicrobial , hypotensive , antihyper-lipidemic , diuretic , antiemetic , laxative and spasmolytic effects (Tucakov , 1997, Hosseinnzadeh et al., 2002 and Koppula and Choi , 2011) . The medicinal parts of the plant are its seeds , fresh or dried leaves and the upper stem (Faber et al ., 1997 & Leung and foster ., 2003) . Various plant parts of dill have different odours (Faber et al ., 1997) . Dill seeds contain the highest concentration of medicinal and aromatic compounds , but an appreciable amount is also present in the leaves and flowers (Koppula and Choi , 2011) . The essential oils from dill seeds , leaves and herb were used as a flavouring agent in the food industry , especially for their characteristic aroma and odour (Jirovetz et al ., 2003) . Dill seeds are considered as a valuable source of essential oil (Ortan et al., 2009) . the main compounds of the herb essential oil are α -phellandrene and dill ether which are responsible for the typical herb odour . Besides α -phellandrene and dill ether , limonene and carvone is mainly responsible for the typical caraway note of the oils . This monoterpene ketone is the main component of the seeds essential oil (Faber et al., 1997) . With 2-5% of the essential oil dill seeds are deemed to be rich in the essential oil (Leung and Foster , 2003) . Carvon was reported to be the major constituent (20 – 60 %) in a number of instances (Leung and Foster , 2003 : Callan et al ., 2007 : Radulescu et al ., 2010 : Delaquis et al., 2002) . Limonene , apiole , dill apiole , α -phellandrene , α -pinene , α -trpinene , 1,8 -cineole ,dihydro carvone and p-cymene are also present in the oil (Leung and Foster , 2003 : Pino et al ., 1995) . Dill leaves and herb contain significantly less essential oil when compared to the seed (0.5 – 1.5 %) (Faber et al., 1997 : Leung and Foster , 2003)

The present study was designed to specifically investigate the antioxidant efficacy of dill seed oil , parsley seed oil and its mixed on mice with liver disorder .

Materials and Methods:

Expermental animales

Forty eight (48) Sprague Dawley strain were obtained from the Laboratory of Animal Colony , Institute research Alrmdi , Giza , Egypt . weighting (200-250)g , were used in this study . All rats were fed on basal diet for one week . Each rat was housed in an individual stainless steel cage under controlled condition , Diets were introduced to rats in aspecial non-scattering feeding cup to avoid loss of food and contamination . Tap water was provided to rats by mean of glass tubes projecting through wire cages from inverted bottles supported to one side of the cage .

Investigated samples :

Investigated samples consisted of some plants seeds. All Food items have been bought from local market and prepared for chemical analysis , which are suggested for treatment of liver disordered but in new blends , these plants seeds used after knowing their active constituents and their biological activities .

This investigation is used three (3)plants seeds , which are used as groups for treatment of liver disorder as the the following : (dill , parsley and their mixture) .

Preparation of essential oils :

Plant material

The seeds of dill and parsley were bought from Local market from Minouf city, Menofiya Government.

Extreaction of the essential oil

The air-dried plant material (100 g) was subjected to hydro distillation for 3 h using a clevenger type apparatus. The essential oil from leaves and stems samples were dried over anhydrous sodium sulphate and preserved in a sealed vial at 4 °C until further analysis.

Biochemical Analysis :

At the end of the experiment , rats were fasted (12)hours and anesthetized with di-ethyl ether . blood samples were collected in clean dry centrifuge tubes for 10 minutes at 3000 rpm from hepatic portal vein . All blood samples were collected in ethylene diamine tetra acetic acid (EDTA) as anticoagulant . Also blood samples for determination of liver enzymes (GOT , GPT) **Jacobs. *eT al.*,(2001)**

Histopathological examination :

Specimens from liver and kidney were collected after kept in formalin then embedded in paraffin 4-6 thick sections were prepared and stained with hemetoxlin and eosin according to **Carleton, (1978)**

Statistical analysis :

Statistical analysis were performed by using computer program Statistical Package for Social Science (SPSS) , and compared with each other using the suitable tests . All obtained results were analyzed by one way ANOVA test using duncans multiple range test and $p < 0.05$ was used to indicate significance between different groups (Snedecor and Corchran, 1967) .

Results and Discussion**Liver functions (GOT , GPT) :**

It is clear from Table (1) that rats in toxicities with CCL_4 , the serum level of GOT enzyme was $(114 \pm 4.24 \text{ IU/l})$ in (control -ve) group as compared to (control +ve) group which was $(144 \pm 2.82 \text{ IU/l})$ these results showed that there were significant increase in (control +ve) groups compared to (control-ve) group . All rats poisoned by CCL_4 and orally fed on (Dill seed oil 200 mg/kg) group ,(Parsley seed oil 200 mg/kg) group , (Parsley seed oil 400 mg/kg) group and (Mix . of two oils 200 mg/kg) group , showed a significant decrease and the best treatment in GOT when compared with control +ve which were $100 \pm 5.65 \text{ IU/l}$, $109 \pm 1.41 \text{ IU/l}$, $125.5 \pm 3.53 \text{ IU/l}$, $126.50 \pm 2.12 \text{ IU/l}$ and $144 \pm 2.82 \text{ IU/l}$ respectively . In group of mix. of two oils 400 mg/kg , showed liver returned to normal situation compared to all groups which was $113 \pm 8.48 \text{ IU/l}$. On the other hand the mean value of GPT for control-ve caused a significant decrease when compared with control +ve which were $47.5 \pm 3.53 \text{ IU/l}$ and $52.5 \pm 0.70 \text{ IU/l}$

respectively. while the mean value of GPT IU/L for groups fed on basal diet +Dill seed oil 200 mg/kg, Dill seed oil 400 mg/kg and Parsley seed oil 200 mg/kg were significant decrease when compared with control +ve, which were 35.5 ± 3.53 IU/L, 26.0 ± 4.24 IU/L, 44.5 ± 0.71 IU/L and 52.5 ± 0.70 IU/L respectively. In group fed with mixed of two oils 200 mg/kg showed liver returned to normal situation compared to all groups which was 46 ± 2.82 IU/L. Manal M. *et.al* (2013) : revealed that the *A. graveolens* extract has anti-hepatotoxic properties that may minimize the deleterious effects generated by hepatotoxin paracetamol. Thus, it could be used as a potent anti-hepatotoxic agent. Also, the extract should be encouraged in diets as it could be used as a functional food to prevent liver and kidneys damage because of its antioxidant properties. Moreover, dill could be recommended for clinical trial as hepatic support.

Table (1) : Effect of oral administration with dill seed oil, parsley seed oil, and its mixture on liver function (GOT and GPT) in rats intoxicated with CCL₄.

<i>Parameters</i> <i>Groups</i>	<i>GOT</i> <i>IU/L</i>	<i>GPT</i> <i>IU/L</i>
Control (-)	114± 4.24	47.5 ± 3.53
Control (+)	144± 2.82	52.5 ± 0.70
Dill seed oil 200 mg/kg	100 ± 5.65	35.5 ± 3.53
Dill seed oil 400 mg/kg	135.50 ± 13.43	26.0 ± 4.24
Parsley seed oil 200 mg/kg	109 ± 1.41	44.5 ± 0.71
Parsley seed oil 400 mg/kg	125.5 ± 3.53	54 ± 4.24
Mix. of two oils 200 mg/kg	126.50 ± 2.12	46 ± 2.82
Mix. of two oils 400 mg/kg	113 ± 8.48	50 ± 4.24
L.S.D	16.50	8.00

Values denote arithmetic means ± standard deviation of the means.

Serum Proteins (T.P , Alb , Glob , A/G) :

Table (2) showed the effect of dill seed oil, parsley seed oil and its mixture on serum proteins S.T.P, Alb, Glob, A/G (mg/dl) of control positive and different groups of rats which were induced by CCL₄. The mean value of S.T.P of control positive was lower than control negative which were (5.40 ± 0.14 g/dl and 5.65 ± 0.07 g/dl) respectively. Also, data indicated that, S.T.P for all groups were the highest values improvement when compared with control positive.

As regards the S. Alb , the mean values of control positive was lower than control negative which were $(3.25 \pm 0.07 \text{ g/dl}$ and $3.40 \pm 0.14 \text{ g/dl}$, respectively , also it can be noticed that groups fed with dill seed oil 400 mg/kg , parsley seed oil 200 mg/kg , parsley seed oil 400 mg/kg , mixed of two oils 200 mg/kg and mixed of two oils 400 mg/kg were the lowest values improvement when compared with control positive, it were being $2.95 \pm 0.35 \text{ g/dl}$, $3.20 \pm 0.28 \text{ g/dl}$, $3.20 \pm 0.14 \text{ g/dl}$, $3.15 \pm 0.07 \text{ g/dl}$, $3.10 \pm 0.14 \text{ g/dl}$ and $3.25 \pm 0.07 \text{ g/dl}$, respectively. Also , results noticed that S.Glob for control positive was lower than control negative which were $2.15 \pm 0.21 \text{ mg/dl}$ and 2.25 ± 0.07 respectively . Also all groups showed improvement in S.Glob in serum and the mean values were higher than control positive expects Dill seed oil 200 mg/kg group which was lower than control positive .

As for S. A/G ratio , data showed that the mean value of control positive as the same value of control negative which were 1.50 ± 0.28 and 1.50 ± 0.14 respectively ,also the highest value improvement for Dill seed oil 200 mg/kg , it was being 2.30 ± 0.28 , and the lowest value 1.05 ± 0.07 for Dill seed oil 400 mg/kg when compared with control positive .

Table (2) : Effect of dill seed oil , parsley seed oil and thiere mixture on serum proteins T.P , Alb , Glob and A/G mg/dl for control positive and different groups of rats which were induced by CCL4.

Parameters Groups	T.P (g/dl)	Alb (g/dl)	Glob (mg/dl)	A/G
Control (-)	5.65 ± 0.07	3.40 ± 0.14	2.25 ± 0.07	1.50 ± 0.14
Control (+)	5.40 ± 0.14	3.25 ± 0.07	2.15 ± 0.21	1.50 ± 0.28
Dill seed oil 200 mg/kg	4.95 ± 0.21	3.40 ± 0.14	1.55 ± 0.35	2.30 ± 0.28
Dill seed oil 400 mg/kg	5.85 ± 0.35	2.95 ± 0.35	2.90 ± 0.14	1.05 ± 0.07
Parsley seed oil 200 mg/kg	5.55 ± 0.35	3.20 ± 0.28	2.35 ± 0.07	1.35 ± 0.07
Parsley seed oil 400 mg/kg	5.70 ± 0.28	3.20 ± 0.14	2.50 ± 0.14	1.30 ± 0.00
Mix . of two oils 200 mg/kg	5.90 ± 0.14	3.15 ± 0.07	2.75 ± 0.21	1.15 ± 0.07
Mix . of two oils 400 mg/kg	5.55 ± 0.21	3.10 ± 0.14	2.45 ± 0.07	1.30 ± 0.00
L.S.D	0.60	0.450	0.450	0.450

Values denote arithmetic means \pm standard deviation of the means .

Random Blood Sugar :-

Table (3) showed the effect of dill seed oil < parsley seed oil and its mixed on random blood sugar (mg / dl) for control positive and different groups of rats which were induced by CCL₄. Its clear that random blood sugar for control positive was higher than control negative being 152.50 ± 7.77 and 137 ± 2.82 mg / dl , respectively . Also data noticed that the highest value for Parsley seed oil 200 mg / kg group . It was recorded 142.50 ± 3.53 mg / dl when compared with control negative . Also data noticed that the lowest value for Dill seed oil 400 mg / kg group being 128 ± 2.82 mg / dl as compared with control negative . Also data noticed that all groups were lowest than control positive .

Table (3): effect of dill seed oil , parsley seed oil , and its mixed on serum glucose (mg/dl) level of rats intoxicated with CCL₄

Parameters	Random blood suger (mg/dl)
Control (-)	137±2.82
Control (+)	152.50 ± 7.77
Dill seed oil 200 mg/kg	138 ±8.48
Dill seed oil 400 mg/kg	128 ± 2.82
Parsley seed oil 200 mg/kg	142.50 ±3.53
Parsley seed oil 400 mg/kg	133 ± 5.65
Mix . of two oils 200 mg/kg	132.50 ± 2.12
Mix . of two oils 400 mg/kg	130.50 ± 3.53
L.S.D	14.50

Values denote arithmetic means ± standard deviation of the means .

Histopathological Results :**. Liver**

.Microscopically, Liver of rat from group 1(which feeding on Standard diet + 200 mg dill oil /kg of rats) showing no histopathological changes which (Fig. 1) while , Liver of rat from group 2 (which feeding on Standard diet + 400 mg dill oil /kg of rats)

showing slight hydropic degeneration of hepatocytes which (Fig 2) , Examined Liver of rat from group 3 (which feeding on Standard diet + 200 mg dill oil and parsley oil /kg of rats) showing dilatation and congestion of central vein which (Fig. 3), as well as(Fig. 4) showed that Liver of rat from group 3(which feeding on Standard diet + 200 mg dill oil and parsley oil /kg of rats) showing slight hydropic degeneration of , moreover , Liver of rat from group 4 (which feeding on Standard diet + 200 mg parsley oil /kg of rats) showing slight vacuolar degeneration of hepatocytes which (Fig. 5) , vacuolar degeneration of hepatocytes were noticed in Liver of rat from group 5(which feeding on Standard diet + 400 mg parsley oil /kg of rats) which (Fig. 6) , as well as ,(Fig. 7) showed that Liver of rat from group 5 (which feeding on Standard diet + 400 mg parsley oil /kg of rats)showing slight congestion of central vein ,How ever, Liver of rat from group 6 (which feeding on Standard diet + 400 mg dill oil and Parsely oil /kg of rats)showing slight hydropic degeneration of hepatocytes which (Fig. 8) , Liver of rat from group 7(control +) showing vacuolar degeneration of hepatocytes which (Fig. 9) , Liver of rat from group 7 (control +) showing vacuolar degeneration of hepatocytes and portal infiltration with inflammatory cells which (Fig. 10) , Moreover, Liver of rat from group 8 (control -) showing the normal histological structure of hepatic lobule (Fig. 11) , As well as , Fig. (12) Liver of rat from group 8 (control -) showing the normal histological structure of hepatic lobule .

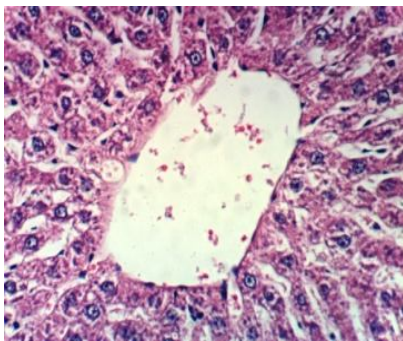


Fig. (1): Liver of rat from group 1 (which feeding on Standard diet + 200 mg dill oil /kg of rats) showing no histopathological changes (H & E X 400).

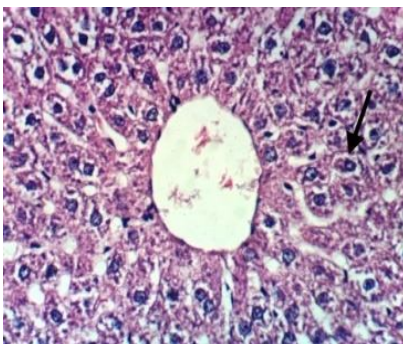


Fig. (2): Liver of rat from group 2 (which feeding on Standard diet + 400 mg dill oil /kg of rats) showing slight hydropic degeneration of hepatocytes (H & E X 400).

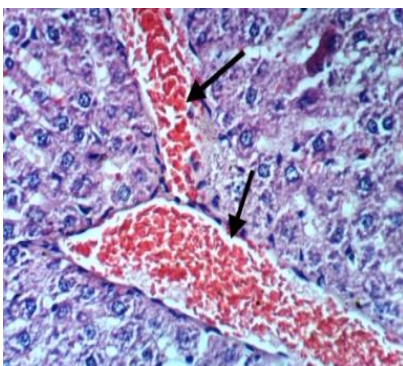


Fig. (3): Liver of rat from group 3 (which feeding on Standard diet + 200 mg dill oil and parsley oil /kg of rats) showing dilatation and congestion of central vein (H & E X 400).

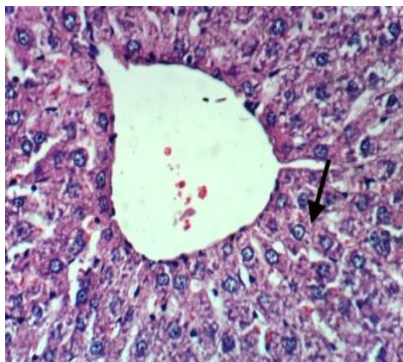


Fig. (4): Liver of rat from group 3(which feeding on Standard diet + 200 mg dill oil and Parsely oil /kg of rats) showing slight hydropic degeneration of hepatocytes (H & E X 400).

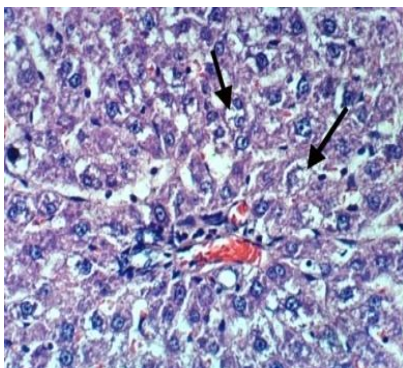


Fig. (5): Liver of rat from group 4 (which feeding on Standard diet + 200 mg Parsely oil /kg of rats) showing slight vacuolar degeneration of hepatocytes (H & E X 400).

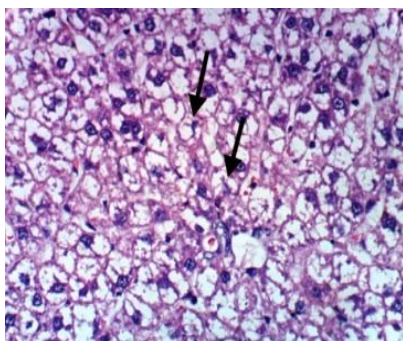


Fig. (6): Liver of rat from group 5(which feeding on Standard diet + 400 mg Parsely oil /kg of rats) showing vacuolar degeneration of hepatocytes (H & E X 400).

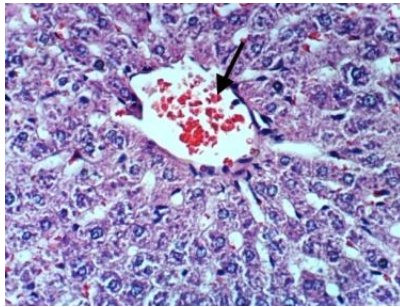


Fig. (7): Liver of rat from group 5 (which feeding on Standard diet + 400 mg Parsely oil /kg of rats)showing slight congestion of central vein (H & E X 400).

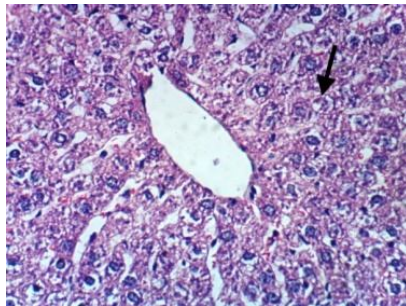


Fig. (8): Liver of rat from group 6 (which feeding on Standard diet + 400 mg dill oil and parsley oil /kg of rats)showing slight hydropic degeneration of hepatocytes (H & E X 400).

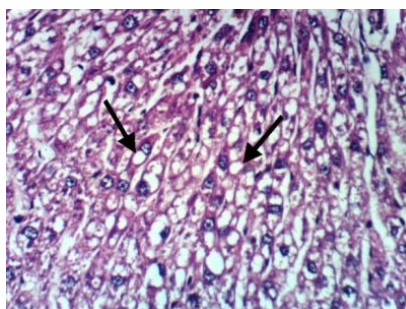


Fig. (9): Liver of rat from group 7(control +) showing vacuolar degeneration of hepatocytes (H & E X 400).

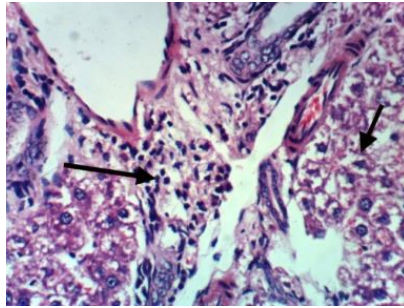


Fig. (10): Liver of rat from group 7 (control +) showing vacuolar degeneration of hepatocytes and portal infiltration with inflammatory cells (H & E X 400).

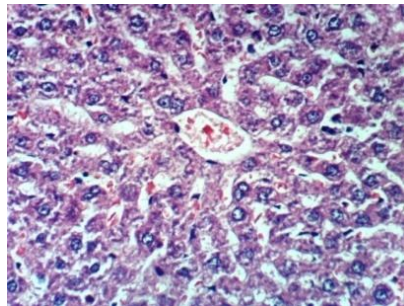


Fig. (11): Liver of rat from group 8 (control -) showing the normal histological structure of hepatic lobule (H & E X 400).

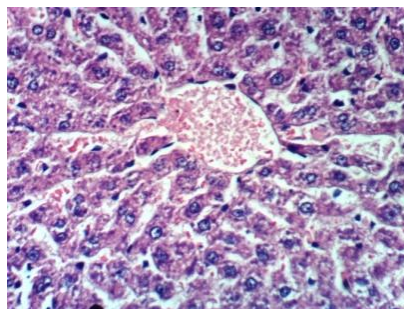


Fig. (12): Liver of rat from group 8 (control -) showing the normal histological structure of hepatic lobule (H & E X 400).

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تأثير الزيوت العطرية لبذور الشبث والبقدونس ومخلوط منهم في علاج الفئران المصابه بخلل فسيولوجي في الكبد

الملخص العربي :

يهدف البحث إلى دراسة تأثير بعض الزيوت العطرية والمشتملة على الزيت العطري للشبث والزيت العطري للبقدونس لعلاج بعض الأمراض الخاصة بالكبد وذلك من خلال استخدام فئران التجارب . تم استخدام ٤٨ فأر أبيض ذات أوزان ٢٠٠ - ٢٥٠ جرام وتم تقسيم الفئران إلى ٨ مجموعات متساوية ، وقد تم ترك المجموعة الأولى لكي تمثل المجموعة الضابطة السالبة بينما السبع مجموعات الأخرى فتم حقنها في الغشاء البروتوني تحت الجلد باستخدام رابع كلوريد الكربون والمخلوط مع زيت البرافين ٥٠ % بالحجم بنسبه ٢ مللى / كجم من وزن الجسم وذلك مرتين أسبوعيا لمدة أسبوعين لإحداث تسمم كبدي . والفئران المصابة بتسمم كبدي تم تقسيمهم إلى سبع مجموعات . تم ترك احد هذه المجموعات لكي تصبح المجموعة الضابطة الموجبة أما باقي المجموعات فتم معالجتهم بالزيوت العطرية المختبرة بجرعتين ٢٠٠ و ٤٠٠ ملليجرام / كجم لكل منهم وكذلك الأمر بالنسبة للمخلوط من كل الزيوت العطرية . وفى نهاية التجربة تم تجميع عينات الدم وفصل السيرم وتقدير أنزيمات الكبد والبروتين الكلى والالبومين والجلوبولين ومستوى السكر في الدم والفحص الهستوباثولوجى لعضو الكبد . وقد أظهرت النتائج أن جميع الزيوت العطرية للنباتات الطبية موضع الاختبار أحدثت تحسنا معنويا في حاله التسمم الكبدي في كل التحاليل المختبرة وعلى ذلك فقد أوضحت النتائج انه يمكن استخدام الزيوت العطرية للنباتات الطبية موضع الاختبار وهى زيت العطري لبذور الشبث والزيت العطري لبذور البقدونس في تحسين وظائف الكبد لدى المرضى المصابين بالخلل الفسيولوجي في الكبد .