

The Protective Role of Date Palm Pollen (*Phoenix dactylifera* L.) on Liver Function in Adult Male Rats Treated with Carbon Tetrachloride

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Summary

The present study was carried out to investigate the protective role of date palm pollen aqueous suspension against the toxic effects of carbon tetrachloride on liver function in adult male rats by studying the following parameters, estimation of ALP, AST, ALT enzymes activity, total serum bilirubin, and histological study of liver. Forty adult male rats aged 12-14 weeks and weighed 275-325 gm were randomly divided into four equal groups (10 rats/group) and were treated for 42 days as follows: rats of the first group were received 1 ml tap water orally once a day and olive oil 0.5 ml /kg B.W. intraperitoneally twice a week which considered as group C, rats of the second group were received date palm pollen suspension orally (150 mg/kg B.W) once a day and olive oil 0.5 ml /kg B.W. intraperitoneally twice a week (group T1), rats of the third group were treated intraperitoneally with 500mg / kg B.W. of CCL4 mixed with equal volume of olive oil twice a week (group T2), while rats of the fourth group were received date palm pollen suspension (150 mg/kg B.W) once a day orally and treated intraperitoneally with 500 mg / kg B.W. of CCL4 mixing with equal volume of olive oil twice a week (group T3). The blood samples were collected at (zero, 14, 28, 42) days for biochemical parameters. After that, six rats from each group were sacrificed, and then samples of liver were taken for histological study. The results revealed no significant differences ($P > 0.05$) in Liver Enzymes activity (ALT, AST, ALP) as well as serum Bilirubin (TSB) in T1 group treated with date palm pollen comparing with control group while a significant elevation ($P \leq 0.05$) in liver Enzymes (ALT, AST, ALP) activity and total serum bilirubin (TSB) in group T2 which exposed to carbon tetrachloride. The protective role of date palm pollen against carbon tetrachloride was recorded in group T3 which manifested by significant differences ($P > 0.05$) in liver enzymes activity (ALT, AST, ALP) and serum bilirubin (TSB) as compared to the control. The histological study of liver of date palm pollen treated rats (group T1) indicated proliferation of kupffer cells and no nuclear lesion, while group T2 showed severe vacuolation in the hepatocytes, while group T3 showed moderate vacuolation in hepatocytes. From the result obtained, it was concluded that it seems likely that dosage of rats with (150 mg/kg B.W) of date palm pollen for 42 days of treatment caused improvement and enhancement of liver function against carbon tetrachloride harmful effects.

الدور الوقائي لغبار طلع النخيل (*Phoenix dactylifera* L.) على وظائف الكبد في ذكور

الجرذان البالغة المعاملة برباعي كلوريد الكربون

جواد كاظم عراق وميساء علاء عبدالحسين

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الخلاصة

صممت هذه الدراسة لمعرفة الدور الوقائي للمعلق المائي لغبار طلع النخيل ضد التأثيرات السمية لرباعي كلوريد الكربون على وظائف الكبد للجرذان البالغة وذلك بدراسة المعايير التالية: قياس نشاط انزيمات الكبد, ALT, AST, ALP, مستوى الصبغة الصفراء والدراسة النسيجية للكبد. تم استخدام (40) من ذكور الجرذان البالغة يتراوح معدل أعمارها 12-14 أسبوع ومعدل أوزانها 275-325 غم، قسمت عشوائياً إلى أربعة مجاميع وبواقع عشرة حيوانات/مجموعة وعوملت كالتالي لمدة 42 يوم: جرعت فمويًا المجموعة الأولى الماء العادي يوميًا وحقنت بزيت الزيتون 0,5 مل/كغم من وزن الجسم مرتين في الأسبوع داخل غشاء الخلب وهدت كمجموعة سيطرة (Group C)، في حين جرعت فمويًا حيوانات المجموعة الثانية المعلق المائي لغبار طلع النخيل 150 ملغم / كغم وزيت الزيتون 0,5 مل/كغم من وزن الجسم داخل غشاء الخلب مرتين في الأسبوع (group T1)، في حين أعطيت حيوانات المجموعة الثالثة رباعي كلوريد الكربون

500ملغم/كغم من وزن الجسم ومزج مع نفس الحجم من زيت الزيتون داخل غشاء الخلب مرتين في الأسبوع (group T2)، أما حيوانات المجموعة الرابعة فجرعت فمويًا المعلق المائي لغبار طلع النخيل 150 ملغم / كغم من وزن الجسم ورباعي كلوريد الكاربون 500ملغم/كغم من وزن الجسم ومزج مع نفس الحجم من زيت الزيتون داخل غشاء الخلب مرتين في الأسبوع (group T3). تم سحب عينات الدم في الأيام (0, 14, 28, 42) لإجراء الفحوصات الكيميائية، وفي نهاية التجربة تم قتل (6) حيوانات من كل مجموعته وتم أخذ عينات من الكبد لأجراء الفحوصات النسيجية. أظهرت النتائج عدم وجود فرق معنوي في المجموعة (T1) التي عولجت بغبار طلع النخيل في انزيمات الكبد , ALT , AST , ALP ومستوى الصبغة الصفراء. مقارنة بمجموعة السيطرة. كما لوحظ وجود ارتفاعاً معنوياً ($P < 0.05$) في نشاط انزيمات الكبد ALP , AST , ALT , مستوى الصبغة الصفراء في الدم , مستوى الصبغة الصفراء في الدم المقارنة بمجموعة السيطرة. أظهر الفحص النسيجي لمقاطع الكبد في المجموعه التي عولجت بغبار طلع النخيل زيادة اعداد خلايا كوفر (kupffer cells) بينما اظهرت المجموعة (T2) وجود تحوصل شديد في خلايا الكبد، أما المجموعه (T3) التي عولجت بغبار طلع النخيل ورباعي كلوريد الكاربون شوهد قلّه في نشوء التحوصل في خلايا الكبد يستدل من نتائج هذه التجربة أن معاملة ذكور الجرذان البالغة لمدة 42 يوم بجرعة (150 ملغم/كغم من وزن الجسم) من المعلق المائي لغبار طلع النخيل أدى إلى تحسن وتعزيز وظيفة الكبد لذكور الجرذان ضد التأثيرات المؤذية لرباعي كلوريد الكاربون.

Introduction

Phoenix dactylifera L (Date palm) belonging to family Arecaceae, called 'Nakhla' and the 'Tree of Life' by the Arabs. It is a member of the monocotyledon family Arecaceae(1). The palm family is a symbol of prosperity and love to Muslims. In fact, Muslims believe that "who eats seven dates every morning will not be affected by poison or magic on the day he eats them" (2).

DPP applications in the rites, and its uses in traditional and herbal medicine, have been recorded throughout history. A variety of pollen containing food products, such as candy and chocolate bars, are commercially available in health food stores in the Western world (3). They contain concentrations of phytochemicals and nutrients and are rich in carotenoids flavonoids and phytosterols(4). Moreover, they are good source of protein, amino acids, vitamins, dietary fiber, fatty acids, enzymes, hormones and minerals (5).

Carbon tetrachloride, also known as tetrachloromethane, is a colourless, nonflammable, heavy liquid with a sweet, aromatic, non-irritating odour (6,7). It is used as an agricultural fumigant and as a solvent in the production of semiconductors, in the processing of fats, oils and rubber and in laboratory applications (8,9). Exposure to high concentrations of carbon tetrachloride (including vapor) can affect the central nervous system, degenerate the liver and kidneys(10; 11). Chronic exposure to carbon tetrachloride can cause liver (12,13) and kidney damage and could result in cancer(14).

Within the body, CCl₄ breaks down to highly toxic trichloromethyl (CCl₃) and trichloromethylperoxyl (CCl₃O₂) free radicals by cytochrome P450 enzyme and causes damage to hepatocytes(15,16). Hepatotoxicity action of CCl₄ and leakage of liver enzymes into blood were recorded by several investigators (17, 18).

Several data suggests that CCl₄ induced liver damage and renal injuries in rats can be reversed by treatment with date palm pollen grains. There are little information about the protective role of DPP on liver and kidney function in animal after exposure to ccl₄. Therefore, this experiment designed to demonstrate this role by study the following parameters which are related to liver function: 1.Serum aspartate aminotransferase (AST), Alanine transaminase (ALT) and alkaline phosphatase (ALP) activities.2.Total serum bilirubin concentration.3.Histologic study of liver

Materials and Methods

A total number of (40) Albino Wistar male rats averages weight (275- 325 gm) was used in this investigation. Their ages ranged (2.5 – 3) months. Animal were left for fifteen (15) days for adaptation with the experimental condition and kept under a suitable environmental condition of (21-25 °C) in an air conditioned room and photoperiod of 12-hours light/dark

cycle the animals were housed in plastic cage of diameters (70×50×15) cm. The cages were cleaned twice a week and animals had free access to water and standard pellets diet along the experimental period. Care was taken to avoid any unnecessary stress and the cages were cleaned once a week. Rats were apparently healthy and they were identified by color tail marks.

Forty male rats were divided randomly into four equal groups (10 animals per group) for six weeks :Control Group: Ten rats treated orally with distilled water daily via gavage needle and olive oil 0.5ml/kg B.W. intraperitonally twice a week; Group T1: Ten rats treated orally with (2ml)150 mg/kg B.W. of DPP suspension daily via gavage needle and olive oil 0.5ml/kg B.W. intraperitonally twice a week ; T 2: Ten rats treated intraperitonally with (0.5 ml)500 mg / kg B.W. of CCL4 and mixed with an equal volume of olive oil 0.5 ml/kg B.W. twice a week (19);Group T 3:Ten rats treated orally with 150 mg/kg B.W. from DPP suspension via Gavage needle and intraperitonally with 500 mg/kg B.W. of CCL4 mixed with an equal volume of olive oil 0.5 ml/kg B.W. twice a week . At zero, 14 ,28, 42 days of the experiment, animals were anesthetized by intramuscular injection of (ketamine 90mg/Kg B.W and Xylazine 40mg/Kg B.W), blood samples were obtained via cardiac puncture technique from each anesthetized animal using disposable syringe needles 3cm .Samples were centrifuged at 2500 (rpm) for 15 minute , and then serum was stored in a freezer at -18 C till use,for measuring the following Biochemical parameters:Serum Alkaline Phosphatase (ALP) Activity (IU/L) Colorimetricly (20);Colorimetric method was adapted for the detection the activity of ALT and AST by using ALT and AST kit in serum(21);Total serum bilirubin concentration was determined according to (22,23). For histological studies, rats were anesthetized, sacrificed by withdrawal of blood from heart. Immediately, after scarification, liver and kidney were excised blotted, opened longitudinally and preserved in 10% neutral formalin buffer solution till the preparation of histological sections. Several tissue sections were prepared according to (24).Results are expressed as mean± SE. Two- way analysis of variance (ANOVA II) .Group differences were determined using least significant difference (LSD) test at P<0.05 (25).

Results

Alkaline Phosphatase (ALP) (IU/L)

There were no significant differences in the values of DPP treated group (T₁) at the day 14, 28 and 42 of treatment (124.18±12.8), (126.20±2.8)and (133.30±4.78), comparing to control with mean value of (138.54±4.8), (138.20±7.6), (141.00±9.8) respectively and DPP+CCL4 treated group (T₃) with mean value of (140.8±5.20), (139.80±2.26) and (146.20±5.23) respectively. It seems to be that there was significant (P<0.05) increase in ALP of (T₂) compared with control group and other treated groups (T₁,T₃). There were no significant differences in ALP of (T₃) treated group compared with control group.(table 1).

Table (1) Effect of DPP, CCL4 and DPP plus CCL4 on ALP activity (IU/L) of adult male rats.

Group Time	Control group	T1 group (DPP)	T2 group (CCL4)	T3 group (DPP + CCL4)
Zero time	137.60± 7.76 A a	141.30±7.42 A a	140.80±8.36 A c	143.80±10.45 A a
Day 14	138.54±4.8 B a	124.18±12.8 B a	165.70±8.23 A b	140.80±5.20 B a
Day 28	138.20±7.6 B a	126.20±2.8 B a	186.20±8.8 A a	139.80±2.26 B a
Day 42	141.00±9.8 B a	133.30±4.78 B a	190.40±8.26 A a	146.20±5.23 B a

L.S.D = 18.6

- Values are expressed as mean ± SE, n = 10 each group.

- Capital letters denote differences between groups, P<0.05 vs. control.

-Small letters denote significant differences within group (P<0.05).

Serum aspartate aminotransferase (AST) (IU/L).

There were no significant differences (P>0.05) in the values of DPP treated group (T₁) at the day 14, 28 and 42 of treatment (124.78±2.99), (134.98±13.8), (130.00±7.02), comparing to control group with mean value of (135.14±22.6), (137.32±12.52) and (142.80±3.70) respectively and DPP+CCL4 (T₃) with mean value of (151.20±1.82), (139.90±7.58) and (148.00±5.35) respectively. It seems that there was a significant (P<0.05) increase in AST of (T₂) group compared with (T₁) group and (T₃) group and control group. There was no significant difference in AST of (T₃) treated group compared with control group during all the period (table 2).

Within group, there were no significant differences of T₁, T₃ treated groups at all the period of treatment except within group T₂ treated group, thus there was a significant (P<0.05) increment in AST activity at last period of treatment compared with 14 and 28 days.

Table (2): Effect of DPP, CCL4 and DPP plus CCL4 on AST activity (IU/L) of adult male rats.

Group Time	Control group	T1 group (DPP)	T2 group (CCL4)	T3 group (DPP + CCL4)
Zero time	135.80± 3.36 A a	133.24±5.98 A a	132.20±5.84 A c	132.40±4.069 A a
Day 14	135.14±22.6 B a	124.78±2.99 B a	158.92±23.71 A b	151.20±1.827 B a
Day 28	137.32±12.52 B a	134.98±13.8 B a	178.70±3.40 A b	139.90±7.58 B a
Day 42	142.80±3.70 B a	130.00±7.02 B a	195.30±5.19 A a	148.00±5.35 B a

L.S.D = 22.7

- Values are expressed as mean ± SE, n = 10 each group.

- Capital letters denote differences between groups, P<0.05 vs. control.

-Small letters denote significant differences within group (P<0.05).

Alanine aminotransferase (ALT) (IU/L)

There were no significant differences (P>0.05) in the values of DPP treated group (T₁) at the day 14, 28 and 42 of treatment respectively, comparing to control and DPP+CCL4 treated group (T₃). There was a significant (P<0.05) increase in serum activity of ALT of CCL4 treated group (T₂) compared with T₁, T₃ and control group. There was no significant differences in ALT of (T₃) treated group compared with control group.

Within time, DPP treated group (T₁) and (T₃) showed no significant differences in the value of ALT continuously at all treated period while CCL4 treated group (T₂) showed significant increase in the value of ALT at days (28,42) compared with day (14).

Table (3): Effect of DPP, CCL4 and DPP plus CCL4 on ALT activity (IU/L) of adult male rats.

Group Time	Control group	T1 group (DPP)	T2 group (CCL4)	T3 group (DPP + CCL4)
Zero time	47.40 ±4.4 A a	45.50±3.79 Aa	41.80±3.78 A c	40.80±2.63 A a
Day 14	45.62±6.61 B a	46.44±2.92 B a	64.24±3.14 A b	47.60±1.13 B a
Day 28	45.02±3.41 B a	42.60±2.61 B a	89.81±4.39 A a	49.14±1.18 B a
Day 42	47.62±7.44 B a	48.22±3.40 B a	92.60±5.74 A a	48.00±4.81 B a

LSD =10.81

- Values are expressed as mean ± SE, n = 10 each group.

- Capital letters denote differences between groups, P<0.05 vs. control.

-Small letters denote significant differences within group (P<0.05).

Total Serum Bilirubin (TSB) (mg/dl)

there was no significant differences (P>0.05) in the level of TSB in DPP treated group (T₁) at the day 14, 28 and 42 of treatment comparing to control, and DPP+CCL4 (T₃) group

At the same periods significant (P<0.05) increase in the value of TSB in CCL4 treated group (T₂) as compared with control group and other two treated groups (T₁, T₃). There was no significant difference in TSB of (T₃) treated group compared with control group.

Within time, DPP treated group (T₁) and (T₃) showed no significant difference in the value of TSB continuously at all treated period while CCL4 treated group (T₂) showed significant increase in the value of TSB at all period of experiment.

Table (4) Effect of DPP, CCL4 and DPP plus CCL4 on total serum Bilirubin (mg/dl) of adult male rats.

L.S.D = 0.04

Group Time	Control group	T1 group (DPP)	T2 group (CCL4)	T3 group (DPP + CCL4)
Zero time	0.47± 0.011 A a	0.46±0.010 A a	0.48±0.012 A d	0.49±0.015 A a
Day 14	0.44±0.012 B a	0.45±0.07 B a	0.60±0.02 A c	0.46±0.013 B a
Day 28	0.45±0.024 B a	0.43±0.01 B a	0.82±0.166 A b	0.47±0.086 B a
Day 42	0.46±0.005 B a	0.42±0.06 B a	1.11±0.19 A a	0.48±0.022 B a

- Values are expressed as mean ± SE, n = 10 each group.

- Capital letters denote differences between groups, P<0.05 vs. control.

The histological changes in liver and kidney:

1.Liver

Light microscopic examination of liver samples of rats received orally 150 mg/kg B.W of DPP suspension daily orally for 42 days (T₁ group) clarified proliferation of kupffer cells and no nuclear lesion (Figure2) compared with control group which showed normal liver pranchyma (Figure 1). Figure (3) pointed to histological section of liver in animals treated with 1ml/kg B.W CCL4 with olive oil, showed severe vacuolation in the hepatocytes while histological section in the liver of rat received DPP suspension + CCL4 with olive oil

for 42 days , showed moderate vacuolation in hepatocytes (figure 4) , compared with control group.

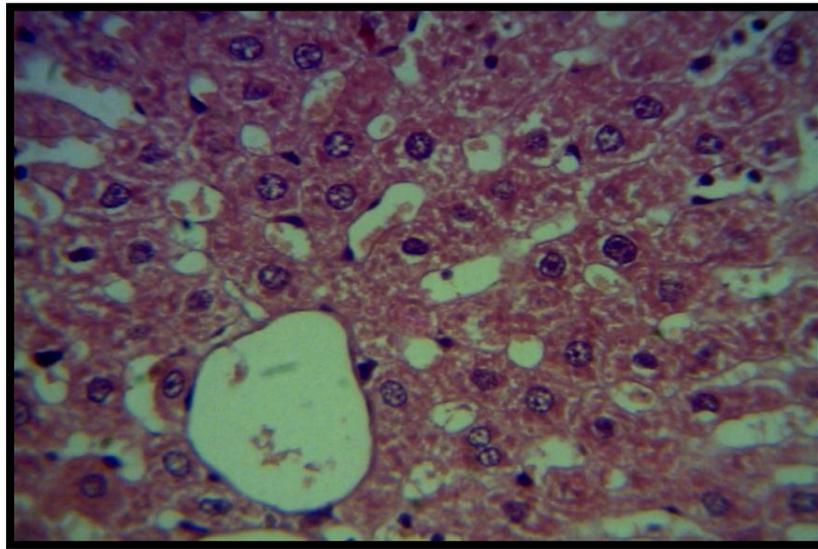


Figure (1):- Histological section in the liver of control group shows normal liver parenchyma (H and E, X 40).

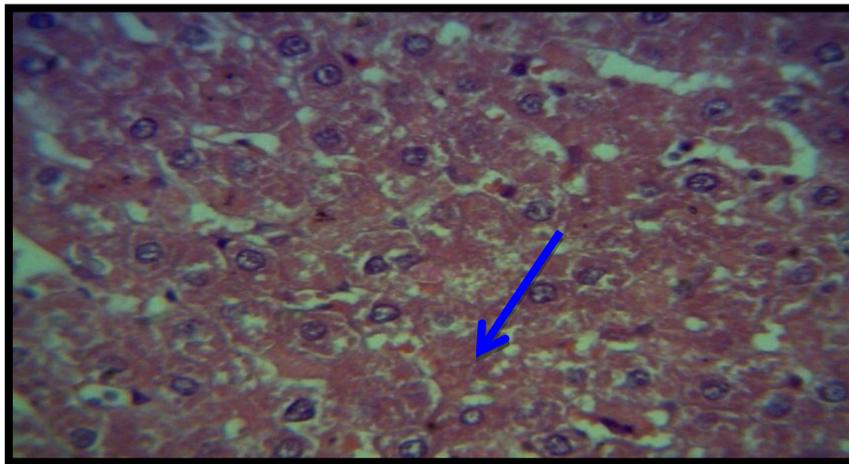


Figure (2):- Histological section in the liver of rat received 150 mg/kg B.W. of DPP suspension for 42 days, shows proliferation of kupffer cell () and no nuclear lesion (H and E, x 40).

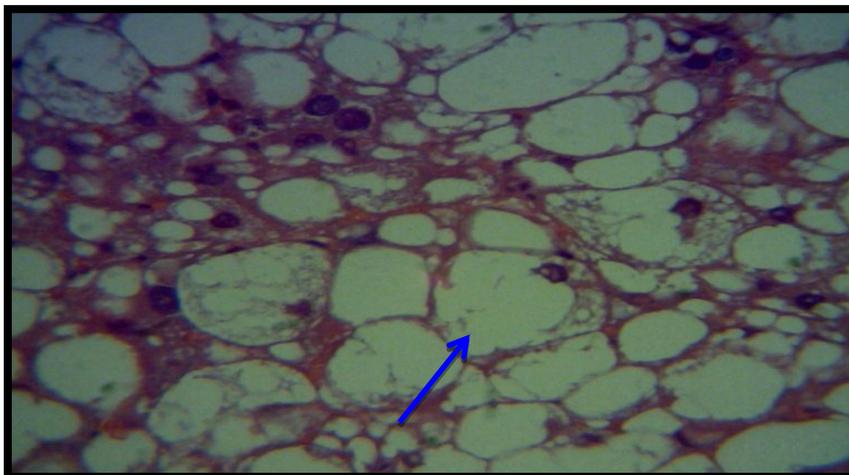


Figure (3):- Histological section in the liver of rat received 1 ml / kg B.W. of CCL4 with olive oil, shows severe vacuolation in the hepatocyte  (H and E, x 40).

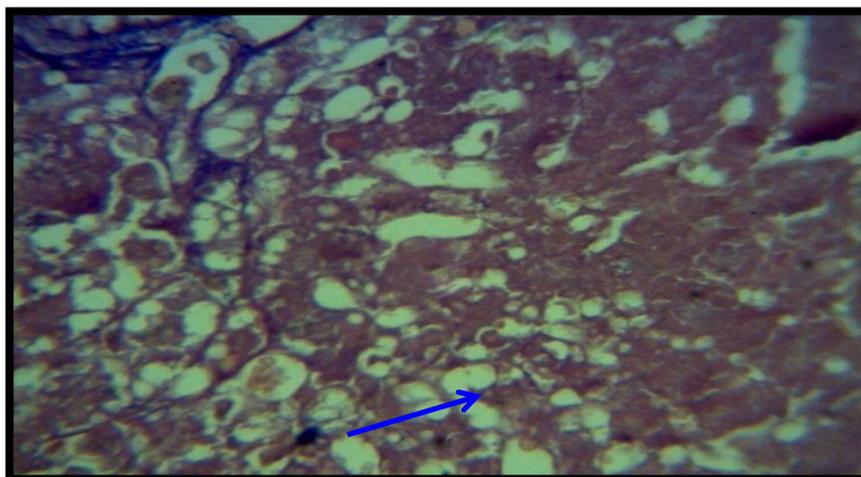


Figure (4):- Histological section in the liver of rat received 150 mg/kg B.W. of DPP suspension +1ml/kg B.W. of CCL4 with olive oil for 42 days , shows moderate vacuolation in hepatocyte → (H and E, x 40).

Discussion

The results of this study showed that DPP play a significant role in modulating liver enzyme activity in treated animal that confirming the phytochemical antioxidant role of DPP and other component like vitamins A,E or minerals like zinc in maintaing and improving liver function (26).

Vitamin E and zinc regulate many enzymes and gene transcription systems that are essential for metabolism , growth and production.

a significant elevation in liver enzyme activities (indicate hepatic dysfunction) in CCL4 treated group during the experiment may attributed to the effect of CCL4 as oxidative factor decreasing cellular based metabolic rate and destruction change of liver , also it causes liver injury and cirrhosis (26 and 27). Increase in serum levels of AST, ALT, ALP activities because they are entracellular enzyme released from the damage tissues into the blood stream (28, 29).

ALP activities on the other hand are related to functioning of hepatocytes, its increase in serum is due to increased synthesis in the presence of increased biliary pressure (30, 31). On the other hand, activities of ALT, AST and ALP decreased in rats treated with protective dose of pollen grains (group T3) compared with CCL4 treated group, the decrement of these hepatic enzymes may be attributed to the antioxidant properties of pollen grains, it is reported that phenolic compounds can act by scavenging free radicals against oxidative damage (32), important factor in the hepatoprotective activity of any drug is the ability of its constituents to inhibit the aramatose activity of cytochrome p-450, by their favoring liver regeneration. On those bases, it is suggested that flavonoids in phoenix dactylifera L. could be contributing as a factor to its ability for hepatoprotection through inhibition of cytochrome p-450 aramatase (33, 34) Flavonoids (quercetin) also protects antioxidative defense mechanism by increasing the absorption of vitamin C (35).

Non-significant decrease in total serum bilirubin concentration in DPP treated rats compared with control group, indicated that total palm pollen grains have ability to maintain liver function due to its components such as vitamins C,E (36) the flavinoids which protects cell membrane form oxidation and directly influence cellular response to oxidative stress through modulation of signal transduction pathways (37).

Administration of CCL4 induced hepatic damage in rats was evidenced by a significant increase of bilirubin in the blood. Elevation of bilirubin levels may be due to interfering of CCL4 with bilirubin synthesis and discharge of bile acids , cholesterol and bilirubin (38). CCL4 may affects the integrity of liver cells , by the same manner it effect on the structure and function of erythrocyte membrane and increase the erythrocyte fragility , this

in turn leads to erythrocyte hemolysis and hemoglobin breakdown (39). On other hand, increase of bilirubin in CCL4 treated rats may be attributed to hepatic damage which ends up in cirrhosis associated to oxidative stress where oxidation of fatty acids in hepatocytes membrane and the formation of a thick extracellular matrix are the main causes of the loss of the homeostatic equilibrium in the liver (40). Rats received DPP with CCL4 showed decreasing in serum bilirubin level due to protection effects and antioxidants scavenge ring activities of DPP component .vitamin C and E protection has been observed bilirubin against extrahepatic biliary obstruction (41) and Kupffer cell activation (42). Furthermore DPP have some flavonoids , such as quercetin and rutin , which play important role as antioxidant agents(34).

Animal received DPP suspension showed normal hepatic lobular architectures with central veins and radiating hepatic cords (43,44). the pathological changes of liver after CCL4 received showed disarrangement of normal hepatic cells with centrilobular necrosis, vacuolization of cytoplasm , damage of parenchymal cells and diffuse fat globules deposits indicating diffuse hepatic steatosis(fatty hepatic degeneration).The reactive radicals of CCL4 interact with polyunsaturated fatty acids of membrane lipids, which initiate lipid peroxidation and cause functional and morphological changes in the cell membrane (45).

Animals administered DPP plus (CCL4 with olive oil) showed few changes might because of antioxidant components of DPP like vitamin E which was found to significantly reduced the elevated levels of enzyme, lipid peroxide in liver, , these results indicate that DPP received orally can circumvent the CCL4 toxicity (41).

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