

Hypolipidemic effect of date palm pollen and isolated flavonoids in sera of adult male rabbits

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Summary:

This study presents the effects of date palm pollen grains-DPP (*Phoenixdactylifera*) and isolated flavonoids on the level of cholesterol, triglycerides-TG, high-density lipoprotein cholesterol HDL-C, low-density lipoprotein cholesterol LDL-C and very low-density lipoprotein VLDL in sera of 49 adult male rabbits divided randomly into seven groups (7 animals in each group), in which G₁, G₂ and G₃ treated with (30,60 and 90) mg/kg/day of DPP respectively, while the G₄, G₅ and G₆ were treated with (7.5,15 and 30) mg /kg/day of isolated flavonoids from DPP respectively compared with non-treated group C₁ as control. The results indicate that the level of cholesterol significantly decreased in sera of G₁, G₂ and G₃, and also in sera of rabbits in G₄ and G₅ as compared with C₁, while the level of TG show slightly reduced in sera of G₁,G₃ and significantly increased in G₆ as compared with C₁. In the other hand our results indicate that the important effect to improve the level of HDL-C may be due to the action of flavonoids at dose 15mg/Kg of body weight with no important effect of DPP to the serum level of HDL-C, and also reduced the levels of LDL-C in all groups treated with DPP and flavonoids, with no important effect to the level of VLDL-C in sera of all groups. Our results also indicate that DPP grains and flavonoids have anti atherosclerotic effects in high dose. From all the above results we can conclude that pollen grains of date palm and its isolated flavonoids have hypolipidemic and anti atherosclerotic effects.

التأثير الخافض لمستوى الدهون في الدم لطلع النخيل والفلافونيد المعزول في امصال دم الارانب البيض البالغة

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مفتاح البحث: طلع النخيل، الفلافونيد، دهون الدم

الخلاصة:

تم اجراء دراسة تاثير حبيبات طلع النخيل *Phoenix dactylifera* والفلافونيد المعزول على مستوى الكوليسترول، الكليسيريدات الثلاثية، الدهون عالية الكثافة، الدهون واطنة الكثافة والدهون واطنة الكثافة جدا في مصل دم 49 ارنب بالغ ، قسمت الحيوانات الى عشوائيا الى سبعة مجاميع (7 حيوان في كل مجموعة)، حيث جرعت المجاميع G_1 ، G_2 و G_3 بـ (30، 60 و 90 ملغم/كغم/يوم من طلع النخيل وعلى الترتب، في حين جرعت المجاميع G_4 ، G_5 و G_6 بـ (7.5، 15 و 30) ملغم/كغم/يوم من الفلافونيد المعزول من طلع النخيل وعلى الترتب، تم مقارنة النتائج مع مجموعة غير معاملة كمجموعة سيطرة C_1 . اظهرت النتائج ان مستوى الكوليسترول انخفض معنويا في امصال دم المجاميع G_1 ، G_2 و G_3 وايضا في امصال دم ارنب المجاميع G_4 و G_5 مقارنة بمجموعة السيطرة C_1 ، في حين اظهر مستوى الكليسيريد الثلاثي انخفاض طفيف في امصال دم G_1 و G_3 وارتفاع معنوي في امصال دم المجموعة G_6 مقارنة بمجموعة السيطرة C_1 من ناحية اخرى اظهرت النتائج ان التأثير المهم هو تحسين مستوى الدهون عالية الكثافة والذي قد يعزى الى الجرعة 15 ملغم/كغم/يوم من الفلافونيد ، في حين لم يكن لطلع النخيل دورا على مستوى الدهون عالية الكثافة، كما انخفض مستوى الدهون واطنة الكثافة في جميع المجاميع المعاملة بطلع النخيل وبالفلافونيد المعزول في حين لم يكن لهما تاثير على مستوى الدهون الواطنة جدا في امصال دم جميع الحيوانات قيد الدراسة. كما واطهرت دراساتنا الحالية ان لحبيبات طلع النخيل والفلافونيد المعزول تاثيرا ضد التصلب العصيدي عند الجرعات العالية. لهذا يمكن الاستنتاج ان لطلع النخيل والفلافونيد المعزول منه تاثير خافض لمستوى الدهون في الدم اضافة الى تاثيرهما ضد التصلب العصيدي.

Introduction:

Date palm (*Phoenix dactylifera*L., Palmae) is native to the Middle East region over centuries ago⁽¹⁾. In Folkloric practice, date represents an essential meal in some Arab area^(2,3). Extracts of fruits, pits and edible kernels showed improvement of vital activities and increased the hormonal concentration in rat and the pollen has been used by Egyptians to improve fertility in male⁽⁴⁻⁷⁾. Some reports on the previous phytochemical studies on the date palm pollen-DPP indicated the presence of sterols, flavonoids, , triterpenoidal, saponins and tannins [Egyptian cultivars]^(8,9). Abbas & Ateya (2011) also revealed the presence of estrone, estradiol, clionasterol, β -sitosterol and cholesterol besides five flavonoids compounds [rutin, luteolin -7-O- β -D - glucoside, apigenin, isorhamnetin-3-O- glucoside and naringin were isolated for the first time from the pollen]⁽¹⁰⁾.

The flavonoids are a category of natural substances belonging to the family of polyphenols⁽¹¹⁾. Which have been reported to exert wide range of biological activities, includes: anti-inflammatory, antibacterial, antiviral, anti-allergic^(12,13). In addition to inhibit lipid-peroxidation⁽¹⁴⁾, and reduced the activity of variety of enzymes like hydrolases, alkaline phosphatase, phosphodiesterase, lipase, α -glucosidase, lipoxygenase and aldose reductase⁽¹⁵⁻¹⁷⁾. The present study has been undertaken to establish the hypolipidemic effect of isolated flavonoids from DPP to the

levels of cholesterol, triglycerides-TG, high-density lipoprotein cholesterol HDL-C, low density lipoprotein cholesterol LDL-C and very low density lipoprotein VLDL.

Material and methods:

- Material: Plant material: Pollen grains of Iraqi date palm (*Phoenix dactylifera* L. variety El-GhanmiAhmar) were collected at the end of March to the end of April 2011. The pollens were separated from the kernels with a fine gauze sieve and left for 3 hours in an incubator at 35°C, and then kept in refrigerated (4°C) in closed container.

-Methods:

I-Extraction of flavonoids from DPP: Extraction of flavonoids was done according to (Chen *et al*, method) with some modification⁽¹⁸⁾, in which one hundred grams of DPP were extracted with 200ml diethyl ether using soxhlet apparatus for 3 hours to remove fatty contents. The defatted plant material was dried at 35 C° in an air oven, and then extracted twice with 250ml (70%) ethanol solution at 90°C for 2h. The solution was filtered and centrifugation at 3000 rpm for 15 min. The solvent was evaporated and the aqueous extract was condensed under reduced pressure. The extract was weight, labeled as Ex-F and stored at 4°C until used.

II-Animals: All animals used in this study were local male rabbits purchased from General Company for Drug Industries / Samarra. Male rabbit (1200-1550 g weight) were used at (3-3.5) month average age. Groups of rabbits were housed at room temperature with a lighting schedule of 12 hours light and 12 h dark. Animals had free access to a standard pellet diet and tap water as drinking solution. Different concentration of DPP grain and isolated flavonoids were prepared according to the table (1) and the extract orally administered in daily dose of 1ml /kg/day for 4 weeks:

Table(1):The dosage concentration of DPP and isolated flavonoids

Doses	Concentration mg/ml of dietary oil*		
	C ₁	C ₂	C ₃
DPP grain	30	60	90
Flavonoids	7.5	15	30

*sunflower oil.

The experimental study was divided the rabbits randomly into seven groups (7 animals in each group) described as follow:

Control-C₁: Orally 1ml /kg/day administered daily dose dietary oil only (1ml /kg/day).

Group-G₁: Orally administered daily dose 1 ml of C₁ of DPP grain.

Group-G₂: Orally administrated daily dose 1 ml of C₂ of DPP grain.

Group-G₃: Orally administrated daily dose 1 ml of C₃ of DPP grain.

Group-G₄: Orally administrated daily dose 1 ml of C₁ of flavonoids.

Group-G₅: Orally administrated daily dose 1 ml of C₂ of flavonoids.

Group-G₆: Orally administrated daily dose 1 ml of C₃ of Flavonoids.

III- Collection of blood samples: Before taking the blood samples animals were fasted for 12 hours. The blood serum was collected by centrifuge the blood at 2500 rpm for 15 minutes, then was divided into 3 parts in eppendorf tube and stored at -20°C until analyzed. Determination of plasma levels of total cholesterol⁽¹⁹⁾, TG⁽²⁰⁾ and HDL-C⁽²¹⁾ were performed by enzymatic and colorimetric methods, while LDL-C and very low density lipoprotein (VLDL) levels were calculated from the Friedewald formula^(22,23).

$$\text{LDL-C(mg/dl)} = \text{Total cholesterol} - \left(\text{HDL-C} + \frac{\text{TG}}{5} \right)$$

$$\left(\frac{\text{Triglycerides}}{5} \right) = \text{VLDL}$$

IV-Statistical analysis: Results were analyzed statistically using analysis of variance test-ANOVA by using the statistical program Minitab. Averages were compared to calculation of the characteristics of the application Duncan's Multiple Range Test by probability level $P \leq 0.05$.

Results and discussion:

Table(2) shows the mean \pm SD of lipid profile [cholesterol, TG, HDL-C, LDL-C and VLDL] in sera of rabbits treated with DPP, isolated flavonoids and not-treated as control group.

The mean \pm SD of cholesterol in sera of C₁ and three groups G₁, G₂, G₃ treated with DPP were (97.88 \pm 8.19)mg/dl, (86.06 \pm 3.71) mg/dl, (69.66 \pm 5.63)mg/dl and (72.17 \pm 4.59)mg/dl respectively. The results indicate that the levels of cholesterol were significantly decreased ($p \leq 0.05$) in sera of rabbits in all three groups G₁, G₂ and G₃ as compared with C₁, while the levels of cholesterol were significantly lower in G₂ and G₃ as compared with G₁, with no significant change between G₂ and G₃.

The mean \pm SD of cholesterol in sera of three groups G₄, G₅, G₆ were (78.78 \pm 7.73)mg/dl, (89.83 \pm 8.33)mg/dl and (99.42 \pm 5.90)mg/dl respectively. The results showed that the levels significantly decreased ($p \leq 0.05$) in G₄ and G₅ with no significant changes in G₆ as compared with C₁, but the level significantly decreased in G₄ as compared with G₅ and G₆, Fig.(1 Table(2): Mean \pm SD of lipid profile in sera of rabbits treated with palm pollen, flavonoids and not-treated as control group

Groups	Cholesterol mg/dl	TG mg/dl	HDL mg/dl	LDL mg/dl	VLDL mg/dl
C₁	97.88±8.19	48.548±6.362	33.197±4.920	54.978±4.599	9.710±1.272
Palm pollen grains					
G₁	86.06±3.71	41.681±5.391	31.874±6.809	44.053±10.862	8.336±1.078
p≤	0.05	0.05	NS	0.05	0.05
G₂	69.66±5.63	49.290±8.931	22.997±3.083	36.801±6.230	9.858±1.786
p≤	0.05	NS	0.05	0.05	NS
G₃	72.17±4.59	41.970±6.497	33.366±2.575	30.414±5.229	8.394±1.299
p≤	0.05	0.05	NS	0.05	0.05
Flavonoids					
G₄	78.78±7.73	45.622±9.007	25.481±6.074	44.172±10.162	9.124±1.801
p≤	0.05	NS	0.05	0.05	NS
G₅	89.83±8.33	52.260±5.218	38.685±11.356	40.700±8.802	10.452±1.043
p≤	0.05	NS	0.05	0.05	NS
G₆	99.42±5.90	55.083±8.938	55.138±5.314	33.262±6.003	11.017±1.788
p≤	NS	0.05	0.05	0.05	0.05

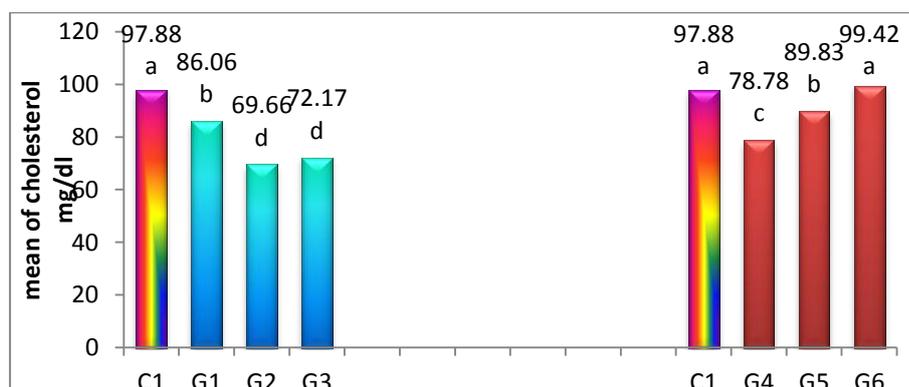


Fig.(1):Cholesterol levels (mg/dl) in sera of adult rabbits treated with DPP and flavonoids)

Blood lipid-lowering therapy had a great consideration in last few years due to the theories and clinical evidences that link elevated blood lipid, including cholesterol and triglycerides-TG with up growing incidence of many diseases include; cardiovascular diseases and diabetes mellitus⁽²⁴⁾. Our results indicate that DPP and isolated flavonoids reduced the level of cholesterol. These results were in agreement with the finding of Al-Shagrawi⁽²⁶⁾ who showed that the level of cholesterol significantly decreased in sera of rats consumed modified diets containing DPP, that's may be due to its contain of phytosterols⁽¹⁰⁾, which lowers the serum cholesterol by inhibiting intestinal uptake of the sterol, their ability to displace cholesterol from micelles in the small intestine underlies the

mechanism that inhibits cholesterol absorption, leading to a 10% reduction in total serum cholesterol. Numerous well designed studies have documented the beneficial actions of these phytosterols on serum cholesterol⁽²⁶⁻²⁷⁾. While the hypolipidemic effect of flavonoids was in agreement with finding of Demonty, et al⁽²⁸⁾, who showed that pure flavonoids don't lower serum cholesterol in moderately hypercholesterolemic men and women.

While the mean \pm SD of TG in sera of C₁ and three groups G₁, G₂, G₃ treated with DPP were (48.548 \pm 6.362)mg/dl, (41.681 \pm 5.391)mg/dl, (49.290 \pm 8.931)mg/dl and (41.970 \pm 6.497)mg/dl respectively. This results indicate that the level of TG significantly decreased ($p \leq 0.05$) in G₁ and G₃ as compared with C₁, with no significant differentiation between G₁ and G₃, but the levels in two groups significantly lower as compared G₂, Fig. (2).

The mean \pm SD of TG in sera of three groups G₇, G₈, G₉ treated with isolated flavonoids were (45.622 \pm 9.007) mg/dl, (52.260 \pm 5.218) mg/dl and (55.083 \pm 8.938) mg/dl respectively. The result indicate that the levels of TG significantly increased ($p \leq 0.05$) in G₉ with no significant change in G₇ and G₈ as compared with C₁, while between the three groups, the level of TG in G₇ was significantly lower than G₈ and G₉.

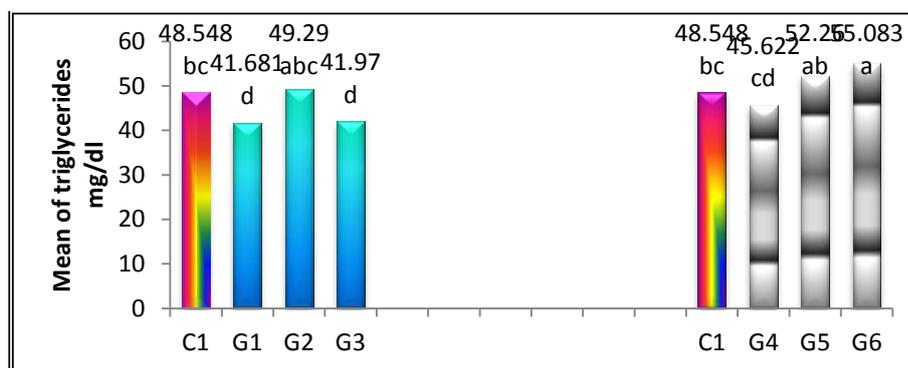


Fig.(2):TG levels (mg/dl) in sera of adult rabbits treated with DPP, and flavonoids

Our result suggest that's the effect of DPP and flavonoids didn't have high effect to the levels of triglycerides in sera of rabbits but we only show slightly reduced in the level of TG. Our results were in agreement with other finding, which found that DPP may slightly decreased the level of TG in sera of experimental rats fed on 2% date pollen grains powder⁽⁵⁾, with no significant effect to the level of TG in sera of infertile male treated with 500 mg DPP packed in capsule⁽²⁹⁾. But with high dose for DPP in the diet (4.0g/100 g DPP in the diet) the level of TG significantly high decreased⁽²⁵⁾. On the other hand many studies found that the flavonoids not effect to the serum concentration of triglycerides⁽³⁰⁾.

In another hand our results showed that the mean \pm SD of HDL-C in sera of C₁ and three groups G₁, G₂, G₃ treated with DPP were (33.197 \pm 4.920)mg/dl, (31.874 \pm 6.809) mg/dl,

(22.997±3.083)mg/dl and (33.366±2.575)mg/dl respectively. The results that the levels of HDL-C was significantly decreased ($p \leq 0.05$) in sera of rabbits in G_2 , with no significant change in G_1 and G_3 as compared with C_1 , while the levels were significantly lower in G_2 as compared with G_1 and G_3 , with no significant change between G_1 and G_3 .

The mean \pm SD of HDL-C in sera of three groups G_4 , G_5 , G_6 were (25.481±6.074) mg/dl, (38.685±11.356) mg/dl and (55.138±5.314) mg/dl respectively. The results showed that the levels significantly elevated ($p \leq 0.05$) in G_5 and G_6 with significant changes in G_4 as compared with C_1 , but the level significantly higher in G_6 as compared with G_4 and G_5 , Fig(3).

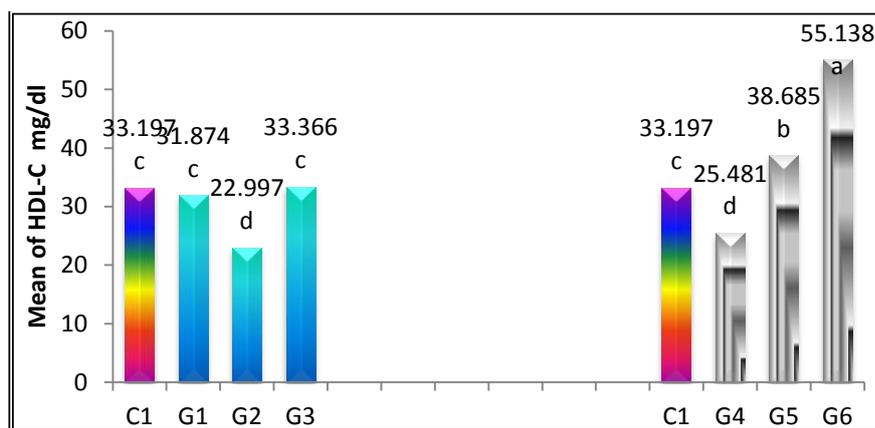
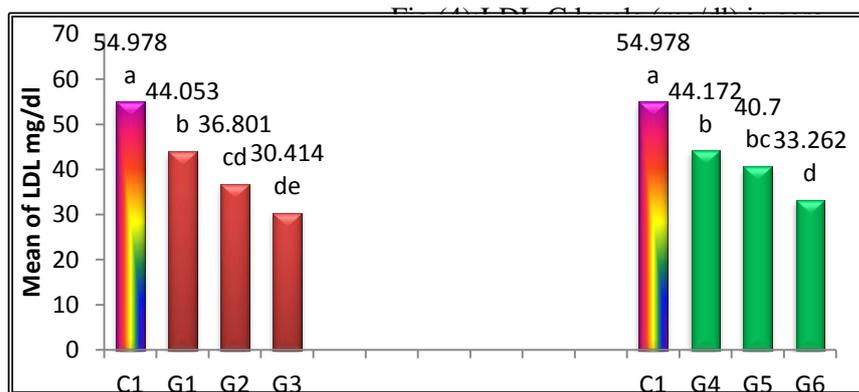


Fig.(3):HDL-C levels (mg/dl) in sera of adult rabbits treated with DPP, and flavonoids.

Our results indicate that the important effect to improve the level of HDL-C may be due to the action of flavonoids at dose 15mg/Kg of body weight with no important effect of DPP to the serum level of HDL-C. Our data about the effect of DPP to the level of HDL-C was disagreement with finding of Al-Shagrawi⁽²⁵⁾, which indicate that DPP may be elevated the level of HDL-C 19%.

Our results indicate that the mean \pm SD of LDL-C in sera of C_1 and three groups G_1 , G_2 , G_3 treated with DPP were (54.978±4.599)mg/dl, (44.053±10.862)mg/dl, (36.801±6.230)mg/dl and (30.414±5.229)mg/dl respectively. This results showed that the level of LDL-C significantly decreased ($p \leq 0.05$) in three groups G_1 , G_2 and G_3 as compared with C_1 , with no significant differentiation between G_2 and G_3 , and the level in G_3 significantly lower as compared G_1 .

The mean \pm SD of LDL-C in sera of three groups G_4 , G_5 , G_6 treated with isolated flavonoids were (44.172±10.162) mg/dl, (40.700±8.802) mg/dl and (33.262±6.003) mg/dl respectively. The result indicate that the levels of LDL-C significantly decreased ($p \leq 0.05$) in all three groups as compared with C_1 , while the level of LDL-C significantly lower in G_6 s compared with G_4 and G_5 , Fig(4).



of adult rabbits treated with

DPP and flavonoids

Our results suggest that the level of LDL-C significantly reduced by the effect of DPP and flavonoids, and the most effective dose for DPP was 90mg/Kg and 30mg/Kg for flavonoids. Our findings were in support with the studies conducted by Al-Shagrawi⁽²⁵⁾. The mechanism of antiatherosclerotic and hypolipidemic effects of pollen grains may be due to its known constituents, such as phytosterols⁽¹⁰⁾, polyunsaturated fatty acids⁽³¹⁾ (which reduced plasma total and LDL-C compared with saturated fatty acids). Also, fatty acids and sterols in pollen grain may interfere with intestinal absorption of cholesterol^(26,27).

VLDL levels were calculated in sera of all adult male rabbits groups [C₁, treated with DPP and flavonoids]. The mean ± SD of VLDL for these groups were shown in table (2) and Fig. (5). The mean ± SD of VLDL in sera of C₁ and three groups G₁, G₂, G₃ treated with DPP were (9.710±1.272) mg/dl, (8.336±1.078) mg/dl, (9.858±1.786) mg/dl and (8.394±1.299) mg/dl respectively. This results showed that the level of VLDL significantly decreased (p≤0.05) in G₁ and G₃ as compared with C₁, with no significant differentiation between G₁ and G₃.

The mean ± SD of VLDL in sera of three groups G₄, G₅, G₆ treated with isolated flavonoids were (9.124±1.801) mg/dl, (10.452±1.043) mg/dl and (11.017±1.788) mg/dl respectively. The result indicate that the levels of VLDL significantly increased (p≤0.05) in G₆ as compared with C₁.

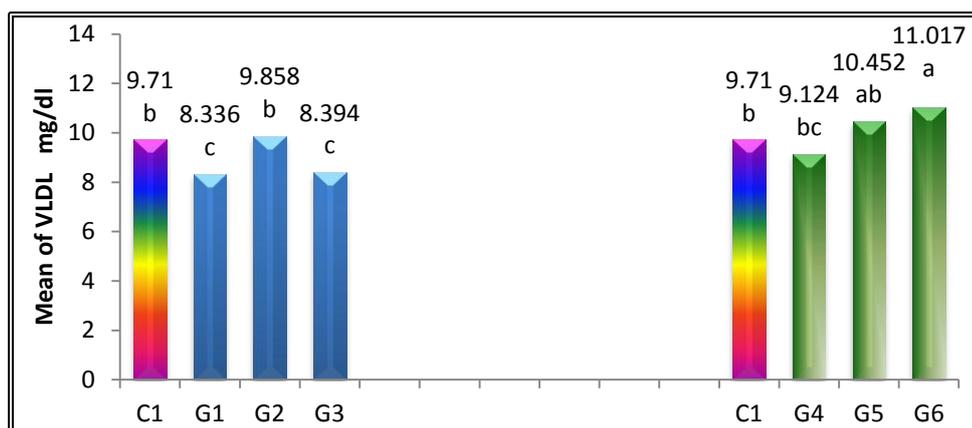


Fig.(5): VLDL levels (mg/dl) in sera of adult rabbits treated with DPP, flavonoids

Our results indicate that, no important effect for DPP and flavonoids to the level of VLDL, this finding was in agreement with other studies which found that DPP didn't effect to the level of VLDL in men⁽²⁹⁾.

Table(3): Mean \pm SD of atherogenic index in sera of rabbits treated with palm pollen, flavonoids and not-treated as control group

Groups	Cholesterol/ HDL-C	LDL-C/HDL-C
C₁	2.979 \pm 0.327	1.681 \pm 0.240
Palm pollen grains		
G₁	2.808 \pm 0.683	1.495 \pm 0.698
p \leq	0.05	0.05
G₂	3.069 \pm 0.429	1.709 \pm 0.560
p \leq	0.05	0.05
G₃	2.169 \pm 0.145	0.917 \pm 0.174
p \leq	0.05	0.05
Flavonoids		
G₄	3.152 \pm 0.665	1.838 \pm 0.624
p \leq	0.05	0.05
G₅	2.527 \pm 0.948	1.226 \pm 0.799
p \leq	0.05	0.05
G₆	1.813 \pm 0.173	0.613 \pm 0.161
p \leq	0.05	0.05

The mean \pm SD of atherogenic index (Cholesterol/ HDL-C) ratio in sera of C₁ and three groups G₁, G₂, G₃ treated with DPP were (2.979 \pm 0.327), (2.808 \pm 0.683), (3.069 \pm 0.429) and (2.169 \pm 0.145) respectively. The results that the levels of (Cholesterol/ HDL-C) ratio were significantly decreased (p \leq 0.05) in G₃, increased in G₂ with no significant change in G₁ as compared with C₁ group, while the

levels of ratio significantly lower in G₃ as compared with G₁ and G₂ with no significant change between G₁ and G₂. Fig.(6).

While the mean ± SD of (Cholesterol/ HDL-C) ratio in sera of G₄, G₅, G₆ were (3.152±0.665), (2.527±0.948) and (1.813±0.173) respectively. The results showed that the ratio significantly decreased (p≤0.05) in G₆ with no significant changes in G₄ and G₅ as compared with C₁, but the level significantly decreased in G₆ as compared with G₄ and G₅.

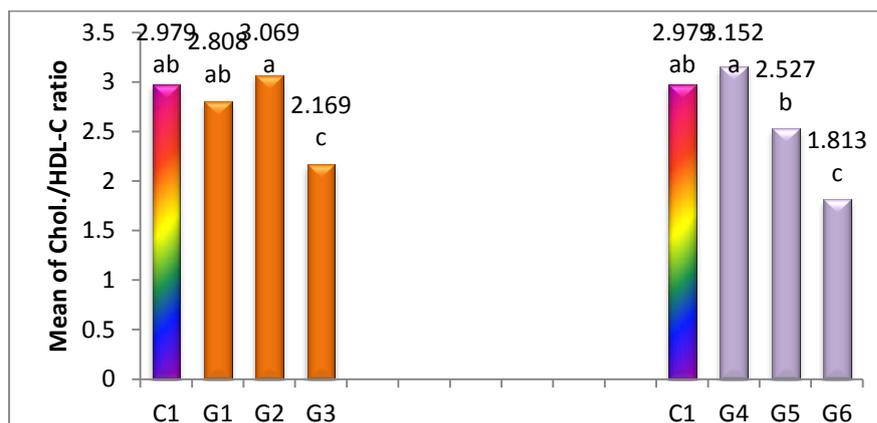


Fig.(6): Cholesterol/HDL ratio in sera of adult rabbits treated with DPP, isolated (sterols and flavonoids)

The mean ± SD of atherogenic index (LDL-C/ HDL-C) ratio in sera of C₁ and three groups G₁, G₂, G₃ treated with DPP were (1.681±0.240), (1.495±0.698), (1.709±0.560) and (0.917±0.174) respectively. The results that the levels of (LDL-C / HDL-C) ratio were significantly decreased (p≤0.05) in G₃, with no significant change in G₁ and G₂ as compared with C₁ group, while the levels of ratio significantly lower in G₃ as compared with G₁ and G₂ with no significant change between G₁ and G₂. Fig.(7).

While the mean ± SD of (LDL-C/ HDL-C) ratio in sera of G₄, G₅, G₆ were (1.838±0.624), (1.226±0.799) and (0.613±0.161) respectively. The results showed that the ratio significantly decreased (p≤0.05) in G₅ and G₆ with no significant changes in G₄ as compared with C₁, but the level significantly decreased in G₆ as compared with G₄ and G₅.

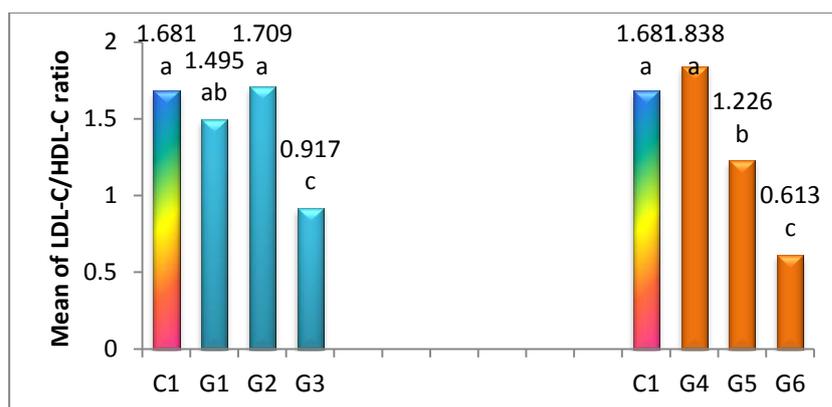


Fig.(7): LDL-C/HDL-C ratio in sera of adult rabbits under oxidative stress treated with DPP, isolated (sterols and flavonoids)

Our results indicate that DPP grains and flavonoids have anti atherosclerotic effects in high dose. The mechanism of antiatherosclerotic effects of pollen grains may be due to its known constituents, such as polyunsaturated fatty acids, fatty acids and sterols in pollen grain interfere with intestinal absorption of cholesterol⁽²⁵⁾. While flavonoids seem to suppress LDL oxidation and inflammatory progression in the artery wall. A Japanese study reported an inverse correlation between flavonoid intake and total plasma cholesterol concentrations, other clinical studies, as mentioned earlier, stated that flavonoid intakes protect against coronary heart disease^(32,33). This anti-atherosclerotic effect of flavonoids may be derived from their antioxidant properties, but that relationship remains unclear⁽³⁴⁾.

Conclusion:

From all the above results we can conclude that pollen grains of date palm and its isolated flavonoids have hypolipidemic and anti atherosclerotic effects

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