Assessment the Antioxidant and Hypolipidmic Effect of Black Cumin (*Nigella sativa L.*) Flavonoids in Induced Oxidative Stressed Male Rabbits

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Summary

Qualitative and quantitative assessment of flavonoids, sugar moiety and antioxidant scavenging activity (*in vitro and vivo*) of black cumin seed (*Nigella sativa* L.) as well as serum lipid profile inhydrogen peroxide (H_2O_2) induced oxidative stress and flavonoids treated adult male rabbits were the main aim of the current study. The results showed that black cumin seeds contained 4.50gmcrude flavonoids per kg crushed seeds and the high performance liquid chromatography indicated the existence of 22 flavonoids, only seven of which were identified flavonoids represented nearly 2.751gm/kg of the total flavonoids. The concentration of kaempferol was the highest whereas quercetin the lowest. Glucose, fructose and sucrose were the sugar moiety of flavonoids with an average concentration of 3.22, 2.21 and 3.31 g/kg crushed seeds respectively. Assessment the antioxidant scavenging activity of the extracted flavonoids and its effect on lipid profile in H2O2 treated rabbits were carried out as follows; eighteen adult male rabbits were randomly divided into three equal groups. Rabbits in the first group were received tap water and served as control (C), whereas animals in 2nd group received 0.5% H2O2 in drinking tap water (T1), animals in the 3rd group were received 0.5% H2O2 in drinking water with 27.5mg/kg B.W. of black cuminseeds flavonoids.Fasting blood samples were collected at 0, 4, 8 and 12 weeks of the experiment. The results clarified that 0.5% H_2O_2 in drinking water caused significant elevation in serum malondialdehyde (MDA) and reduction in glutathione (GSH) concentration of treated animals respectively.The incidence of antioxidant scavenging activity in *vitro* was more pronounced than that recorded in *vivo* particularly at the early stages of administration and extracted flavonoids ameliorate to a great extent the incidence of hydrogen peroxide induced oxidative stress in male rabbits. The results revealed that administration of 0.5% H_2O_2 in drinking water for 12 weeks to male rabbits caused significant $p<0.05$ elevation in TC, TAG, LDL-C and VLDL-C concentration, with significant $p<0.05$ reduction in serum HDL-C concentration as compared with the control where as *Black Cumin* seed flavonoids caused significant reduction in the serum concentration of TC, TAG, LDL-C, and VLDL-C and significantly $p<0.05$ elevation in HDL-C concentration comparing to H_2O_2 treated group.

Conclusion: This study clarified the significant role of black cumin flavonoids in modulation of glutathion (GSH) content and malondialdehyde(MDA) equivalent and exhibited highly hypolipidmic effect.

Keywords: antioxidant, hypolipidmic,*Nigella sativa L,* Flavonoids, Oxidative Stressed Male Rabbits.

تقييم دور فلافونويدات الحبة السوداء *Nigella Sativa* كمواد مانعة للأكسدة وخافضة للدهون في ذكور الأرانب المعرضة للكرب التأكسدي

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الخلاصة

تقدير فلافونويدات بذور الحبة السوداء الكمي والنوعي , ومحتواها السكري ونشاطها المانع للأكسدة (في الزجاج والكائن الحي) وكذلك تأثيرها في الصورة الدهنية في ذكور الأرانب البالغة المعرضة للكرب التأكسدي المستحدث بواسطة بيروكسيد الهيدروجين كان الهدف الرئيسي للدراسة الحالية .أظهرت النتائج أن بذور الحبة السوداء تحتوي على 4.5 غم

فلافونويدات/كغم من مسحوق بذور الحبة السوداء , وأظهرت نتائج كروماتوغرافيا السائل عالي الأداء (HPLC) وجود 22 مركب فلافونويدي , تم تشخيص سبعة منها فقط شكلت 2.75 غم من كمية الفلافونويدات الكلية , وتبين أن الكامبافيرول هو الأعلى تركيزا والكوارستين الأقل تركيزا , ووجد أن الكلوكوز , الفركتوز والسكروز تشكل الشطر السكري في الفلافونويدات وبمعدل 3.22 , 2.21 و 3.31 غم/كغم من مسحوق البذور على التوالي. تم تقسيم ثمانية عشر من ذكور الأرانب البالغة الى ثلاثة مجاميع متساوية , تركت المجموعة الأولى بدون أي معاملة واعتبرت مجموعة سيطرة (C) , عوملت حيوانات المجموعة الثانية أعطيت بيروكسيد الهيدروجين وبتركيز 0.5% في ماء الشرب (T1) . عوملت المجموعة الثالثة 0.5% بيروكسيد الهيدروجين في ماء الشرب أضافة الى الفلافونويدات المستخلصة وبمقدار 27.5 غم/كغم من وزن الجسم (T2) .سحبت عينات دم من الحيوانات خلال الفترات 0 , 4 , 8 و 12 أسبوع من التجربة وأظهرت النتائج أن 0.5% من بيروكسيد الهيدروجين في ماء الشرب سبب زيادة معنوية في المالونالديهايد في مصل دم الأرانب وأنخفاضا معنويا في الكلوتاثايون في الأرانب المعاملة . ومن جانب آخر فأن كفاءة فلافونويدات بذور الحبة السوداء كمواد مانعة للأكسدة في الزجاج (In vitro) كان أكثر وضوحا عما هو عليه في الكائن الحي (الأرانب) وخاصة في المراحل الأولى من التجريع وأن الفلافونويدات لعبت دورا مهما في تقليل مضار الكرب التأكسدي في ذكور الأرانب . كما أظهرت النتائج أن اعطاء 0.5% بيروكسيد الهيدروجين في ماء الشرب لذكور الأرانب ولمدة 12 اسبوعا سببت زيادة معنوية في تركيز الكولسترول الكلي والكليسرول ثلاثي الأسيل والكولسترول ذو البروتينات الواطئة الكثافة والواطئة الكثافة جدا , وانخفاضا معنويا في تركيز الكولسترول ذو البروتينات عالية الكثافة في مصل دم الأرانب المعاملة مقارنة بمجموعة السيطرة , في حين سببت فلافونويدات بذور الحبة السوداء في حصول انخفاض معنوي $p<0.05$ في تركيز كل من الكولسترول والكليسرول ثلاثي الأسيل والكولسترول ذو البروتينات الواطئة الكثافة والواطئة الكثافة جدا وزيادة معنوية $p<0.05$ في تركيز الكولسترول ذو البروتينات عالية الكثافة في مصل دم الأرانب مقارنة بمجموعة السيطرة .

الأستنتاج من نتائج هذه الدراسة أن لفلافونويدات بذور الحبة السوداء دورا معنويا في تحوير المعايير التي لها علاقة بالكرب التأكسدي وخفض الدهون .

الكلمات المفتاحية: مانعة للأكسدة ،خافضة للدهون، ذكور الأرانب، فلافونويدات، الحبة السوداء ،الكرب التأكسدي

Introduction

Flavonoids and their conjugates are very large diverse group of low- molecular mass natural polyphenolic substances. They are found as glycosides with sugar moieties include D-glucose and L-rhamnose in many plants tissues where they are present (1 and 2). Flavonoids can be divided according to their substituent's into eight different classes ,which all share the common phenylchromromance structure consisting of two aromatic rings (A and B) and oxygenated heterocyclic ring C (3 and 4).The flavonoids have been long recognized to possess anti-inflammatory, anti-oxidant, estrogenic effects, anti-allergic, hepatoprotective, anti-thrombotic, anti-viral, and anti-carcinogenic activities (5 and6). Although the antioxidant efficiency of flavonoids and its interference with different radical producing system as well as enrichments of antioxidant defense mechanisms of the body which continuously threated by damage caused by free radicals and reactive oxygen species have been documented (7), its function *in vivo* is uncertain. Therefore, this study was conducted to evaluate qualitative and quantitative constituents of black cumin seed flavonoids and to elucidate the role of crude flavonoids (an exogenous non-enzymatic antioxidant) in ameliorating the incidence of oxidative stress induced by H2O2 (major forms of ROS, since there is no enzymatic defense system against reactive hydroxyl radical OH´) in male rabbits as well as to assess the effect of black cumin seed flavonoids on serum lipid profile.

Materials and Methods

Black cumin seeds were purchased from the local market (Baghdad) and the vouchers specimen of the seeds were deposited to be identified and authenticated at the National Herbarium of Iraq Botany Directorate, under scientific name *Nigellia sativa L.* belongs to the family Ranun culacear. The method of Harborne (8) modified by AL-Kawary (9) was used for the extraction of flavonoids.

High performance liquid chromatography was used for the identification of black cumin seed flavonoids according to (10), using Shim-pack C_{18} (ODS) column and water: acetic acid: methanol at the ratio 42:8:50 V/V at flow rate 1ml/min as mobile phase, UV detection at wave length 254. Standard flavonoids obtained from Fluak Company.

The method of Lamblin *et al.,* (11) were used for the qualitative and quantitative determination of sugar moiety of black cumin seed flavonoids under the following experimental condition, HPLC with Shim-Pack CLC-NH_2 column, H_2O: acetonitrile 3:1 V/V at a flow rate 1 ml/min as mobile phase and refractive index detector.

Eighteen adult male rabbits were randomly divided into three equal groups and treated daily for twelve weeks as follows: Animals in first group were received regular standard diet, tap water and served as control (C), rabbits in the 2nd group were received a daily normal diet and 0.5% H_2O_2 in drinking water (T1), while animals in the 3rd group were received a daily normal diet and 0.5% H_2O_2 in drinking water with 27.5 mg/kg B.W. of black cumin flavonoids (12). Fasting blood samples were collected at 0, 4, 8 and 12 weeks of the experiment. Blood was drawn by cardiac puncture technique from anesthetized rabbits and kept in tube not more than 4hours followed by centrifugation for 15 minutes at 3000 rpm. Serum was isolated and frozen at -20 C° until analysis.

1,1-Diphenyl-2- Picryl Hydrazyl (DPPH) was used for the determination of antioxidant scavenging activity of black cumin seed flavonoids (0.02%) as compared with standard butylated hydroxy toluene (BHT) (13).Determination of serum reduced glutathione(GSH) was carried out using 5,5-Dithiobis (2-nitrobenzoic acid) (14). While thiobarbituric acid assay (15) was used for the determination of serum malondialdehyde concentration in H2O2 and flavonoid treated animals.

Serum total cholesterol (TC) concentration was measured according to (16) using Randox assay kit.Enzymatic estimation of serum triacylglycerol (TAG) concentration was carried out using Biomerieux kit (17). While serum high-density lipoprotein cholesterol HDL-C was measured enzymatically using Linear enzymatic kit (Linear chemicals, Barcelona, Spain). Serum low density lipoprotein cholesterol (LDL-C) and very low density lipoprotein-cholesterol (VLDL-C) concentrations were calculated according to (18).

Statistical analysis of data was performed on the basis of Two-Way Analysis of Variance (ANOVA) using a significant level of ($P<0.01$ and 0.05). Specific group differences were determined using least significant differences (LSD) as described by (19).

Results and Discussion

The result of this study showed that black cumin seeds contained 4.50 gm flavonoids/kg crushed seeds, and the high liquid chromatography indicated the existence of 22 flavonoids, only seven of identify flavonoids were represented 2.751 g/kg. The concentration of kaempferol was the highest while quercetin the lowest (Table 1 and Figure 1 and 2).The results indicated that *Black Cumin* seeds contained relatively high amount of flavonoids as compared to other resources such as apples 5.95- 13.2mg/100 gm of fruit (20), and for 115 edible plant in Taiwan the flavonoids contents as glycones were ranged from zero to 254mg/100gm fresh mass(21), with a wide spectrum of flavonoid aglycones as compared to other plant recourses.

Glucose, fructose and sucrose with an average values 0.322,0.221 and 0.331 gm/100 gm crushed seeds respectively were represented the major sugar fraction of the glycosides after acid hydrolysis (Table 2).

Table (1): High performance liquid chromatography analysis (qualitative and quantative) of *Nigla sativa L.* seeds.

Flavonoids	Retention time of standard flavonoids/ min	Retention time of Nigella sativa L. flavonoids/ min	Flavonoids amount g/kg seeds
Kaempferol	3.1	3.1	1.15
Catechin	2.4	2.4	1.00
Myricitin	2.6	2.6	0.57
Hesperdin	6.02	6.02	0.02
Slopetin	3.5	3.6	0.004
Rutin	3.9	3.8	0.004
Quercetin	8.3	8.3	0.003

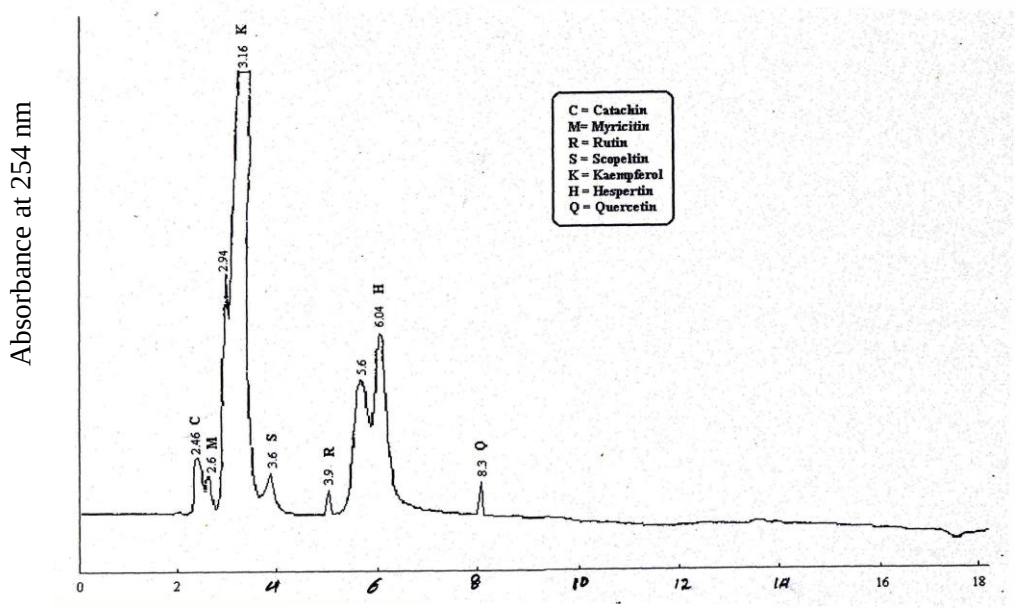

Figure1: High performance liquid chromatography standard of *Black Cumin* flavonoids.

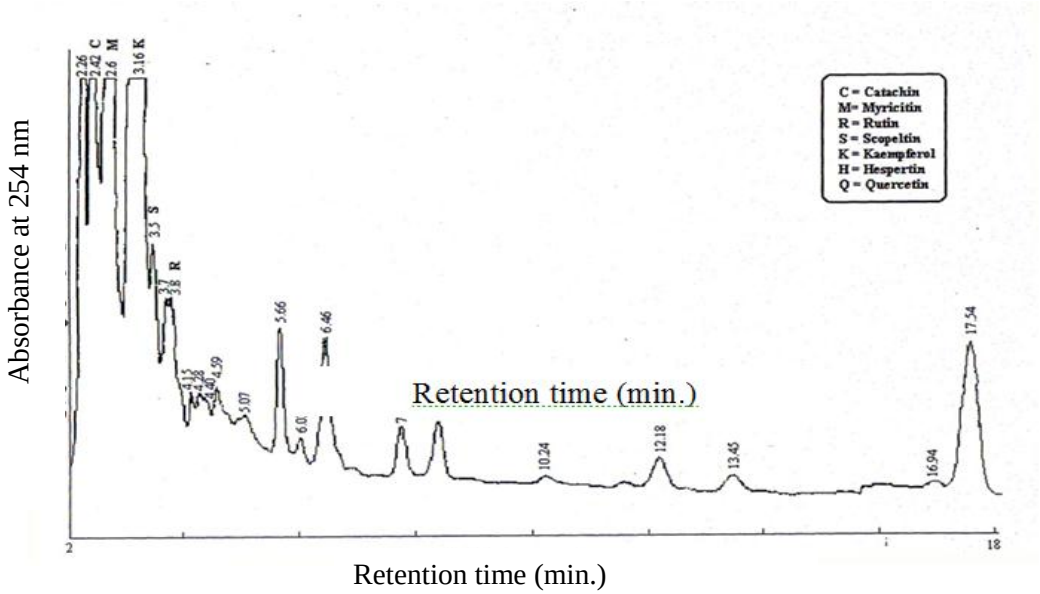

Figure 2: High performance liquid chromatography of *Black Cumin* flavonoids.

Table (2): High performance liquid chromatography analysis of the sugar fractions of the glycosides (flavonoids) of *Nigella sativa L.* on Shim-Pack CLC- NH2 column, using water: acertonitrile 3: 1 V/V at a how rate 1ml/min. as mobile phase and refractive index detector.

Sugar	Retention time of standard sugar/ min	Retention time of *Nigella sativa* L. glycoside sugar/ min	Sugar quantity g/ kg seed
Glucose	4.63	4.69	3.22
Fructose	4.09	4.12	2.21
Sucrose	6.36	6.34	3.31

The antioxidant scavenging activity of *Black cumin* seeds flavonoids and BHT (DPPH scavenging assay) in *vitro*, were 37.18 and 42.12 µg/ mole respectively (Table 3). This higher percent (88.27%) of antioxidant scavenging capability in comparison to standard BHT at the same concentration and under similar experimental conditions clarified the potent antioxidant effect of *Black cumin seed* flavonoids.

Table 3: Antioxidant scavenging activity of 0.02% black cumin flavonoids as compound to Butalated hydroxyl toluene (BHT) by DPPH scavenging assay in vitro.

Antioxidants	Antioxidant scavenging activity µg/mole
Black cumin flavonoids	37.18
BHT	42.12

2. Antioxidant effect of *Black cumin seed* flavonoids *in vivo*:

a. Serum reduced glutathione (GSH) concentration (mmol/l).

The mean values of serum GSH concentration in the control and treated groups are depicted in Table (4). There was a significant decrease (P<0.01) in GSH in T1 (H_2O_2 treated group) as compared with control, during the experimental period. Flavonoids plus H2O2 treated group (T2) also showed a significant (P<0.01) reduction in GSH concentration (13.16 ± 0.12) mmol/l up to twelve weeks of treatment as compared with the control 15.22 mmol/l, whereas no significant differences in the mean values of GSH 14.96 and 14.99 mmol/l after 8 and 12 weeks of treatment respectively as compared to the control was recorded.

Table (4): Effect of flavonoids extracted from black cumin seeds for twelve weeks on serum glutathione (GSH) concentration (µmol) in control and H_2O_2 treated adult male rabbit.

Weeks	Control	T1animals eceived 0.5% H_2O_2	T2animals eceived 0.5% H_2O_2+ 27.5 mg flavonoids
Zero	15.11 ± 0.14 A a	15.44 ± 0.12 A a	15.22 ± 0.09 A a
4	15.30 ± 0.09 A a	12.70 ± 0.09 B b	13.16 ± 0.12 B b
8	15.27 ± 0.08 A a	10.79 ± 0.11 C c	14.96 ± 0.03 A a
12	14.96 ± 0.13 A a	9.34 ± 0.09 C c	14.99 ± 0.12 A a

- Values are expressed as mean ± SE, - n=6 rabbit per group. -Small letter within group differences, P<0.01 vs. pretreatment values. -Capital letter between group differences, P<0.01 vs. control group.

b. Serum malondialdehyde (MDA) concentration (mmol/l): The concentration of serum MDA in H_2O_2 and flavonoids plus H2O2 treated rabbits was clarified in table (5). The results

showed that the mean values of MDA in H_2O_2 treated group were significantly (P<0.01) increased during the experimental period as compared with the control. In H_2O_2 plus flavonoid treated group a significant elevation (P<0.01) in MDA mean value (0.63 ± 0.02), observed after four weeks of treatments as compared with control (0.48 ± 0.02), whereas no significant differences was recorded after 8 and 12 weeks of treatments.

Table (5): Effect of flavonoids extracted from black cumin seed on serum malondiadehyde (MDA) concentration (mmol/ l) in control, H_2O_2 treated adult male rabbit.

Weeks	Control	T1animals received 0.5% H_2O_2	T2animals received 0.5% H_2O_2+ 27.5 mg flavonoids
Zero	0.48 ± 0.04 A a	0.49 ± 0.03 A a	0.48 ± 0.02 A a
4	0.49 ± 0.03 A a	0.76 ± 0.39	0.63 ± 0.02 C b
8	0.50 ± 0.05 A a	0.83 ± 0.16 B b	0.52 ± 0.05 A a
12	0.47 ± 0.02 A a	0.97 ± 0.22 B d	0.50 ± 0.03 A a

- **Values are expressed as mean ± SE, n=6 rabbit per group -Small letter within group differences, P<0.01 vs. pretreatment values. - Capital letter between group differences, P<0.01 vs. control group.**

The results of the present study showed that oral administration of 0.5% H_2O_2 to male rabbits caused a significant elevation and reduction in serum MDA and GSH concentration respectively. Significant depletion of GSH level in H_2O_2 treated group may possibly attributed to the role of H_2O_2 in increasing O_2 production in the stomach followed by state of tissue hyperoxia which in turn lead to excessive formation of free radicals which lead to deterioration of biological molecules (22 and 23). Available evidence indicated that mechanisms of oxidative stress mediated by H_2O_2 injury may also be involved. The induction of gene expression which is regulated by nuclear transcription factor-XB (NF-XP) an oxidative stress esponsive transcription factors, these genes are found to be activated by H_2O_2 leading to higher depletion of intracellular GSH and a marked increase in oxidative stress (24 and 25). While depletion of cellular GSH could be attributed to the direct interaction of oxidants (H_2O_2) with GSH as well as differential expression of the GSH-metabilizing enzymes glutathione peroxidase (26).An elevation in free radicals generation caused a gradual cell injury and liberating lopoxygenase enzyme which oxidized unsaturated fatty acids, and subsequent production of MDA, overwhelming endogenous scavenging system including GSH resulting in oxidative stress (27 and 28).Flavonoids regarded as excellent free radicals scavenging agent (29) and potent inhibitors of LPO (30), prevent oxidative damage by utilizing free radicals and suppress its formation. Flavonoids contribute to the overall antioxidant activities of plant mainly due to their redox properties and nucleophilic thiol group participate in substances detoxification either by conjugation catalyzed by glutathione-S-transferase (GST) or chemical reaction with a reactive metabolite to form conjugate and donation of proton or hydrogen atom to reactive metabolites or free radicals. Reactive intermediates can react with GSH either by direct chemical reaction or by a GST-mediated reaction preventing possible cell death (31) Generally, the mechanism of phenolic compounds (flavonoids) for antioxidant activity are neutralizing lipid free radicals and preventing decomposition of hydrogen peroxide into free radicals (32 and 33).

Table (6) demonstrated a significant P<0.01 elevation in TC mean value in H_2O_2 treated group (T1) during the experiment period as compared to the control and T2 group were recorded, while flavonoid plus H2O2 treated group (T2) showed a significant P<0.01

decrease in serum TC after 8 and 12 weeks of the treatment comparing with the control rabbits .Statistical analysis indicated that the mean values of serum HDL-C concentration in H2O2 treated group T1 (Table 7) were tended to decrease during the experiment period after four, eight and twelve weeks of the treatment as compared with control , significant P<0.01 elevation in HDL-C concentration in T2 group was recorded during the experiment period as compared with the control.

A Significant P<0.01 elevation in serum LDL-C concentration was detected in hydrogen peroxide treated group (Table 8) as compared with control group at three period intervals, while serum LDL-C concentration in flavonoid plus H_2O_2 treated group showed a significant P<0.01 elevation after 4 weeks of the treatment as compared with the control and pretreated period .On the other hand, in the eight and 12 weeks of the experiment, a significant P<0.01 decrease in serum LDL-C concentration in T2 group was observed.

Table (6): Effect of flavonoids extracted from black cumin seeds for twelve weeks on serum total cholesterol (TC) concentration (mg/dl) in control H_2O_2 and H2O2 + flavonoids treated adult male rabbit.

Weeks	Control	T1animals received0.5% $_2O_2$	T2animals eceived 0.5% H_2O_2+ 27.5 mg flavonoids
Zero	155.91 ± 2.09 A a	157.42 ± 1.11 A a	159.11 ± 2.03 A a
4	157.80 ± 1.86 A a	253.0 ± 3.31 B b	169.32 ± 2.32 C b
8	160.0 ± 1.23 A a	291.0 ± 2.68 B c	160.71 ± 2.67 A a
12	160.30 ± 1.20 A a	306.51 ± 2.97 B d	159.33 ± 1.97 A a

-Values are expressed as mean ± SE. - n=6 rabbit per group. -Small letter within group differences, P<0.01 vs. pretreatment values. -Capital letter between group differences, P<0.01 vs. control group.

Table (7): Effect of flavonoids extracted from black seeds (Nigella sativa L.) for twelve weeks on serum high density lipoprptein (HDL-C) concentration (mg/dl) in control and H_2O_2 plus flavonoids treated adult male rabbit.

Weeks	Control	T1animals received 0.5% H_2O_2	T2 animals received 0.5% H_2O_2+ 27.5 mg flavonoids
Zero	54.08 ± 1.09 A a	52.40 ± 1.20 A a	52.90 ± 1.01 A a
4	53.04 ± 1.18 A a	49.10 ± 1.00 B ab	50.20 ± 0.92 AB a
8	52.80 ± 1.67 A a	45.81 ± 1.39 B b	50.0 ± 1.06 A a
12	53.20 ± 0.86 A a	47.20 ± 1.18 B c	51.97 ± 0.68 A a

- Values are expressed as mean ± SE. - n=6 rabbit per group. -Small letter within group differences, P<0.01 vs. pretreatment values. -Capital letter between group differences, P<0.01 vs. control group.

Table (8): Effect of flavonoids extracted from black cumin seeds for twelve weeks on serum low density lipoprotein (LDL-C) concentration (mg/dl) in control and H_2O_2 treated adult male rabbit.

Weeks	Control	T1 animals received 0.5% H_2O_2	T2 animals received 0.5% H_2O_2+ 27.5 mg flavonoids
Zero	76.77 ± 1.81 A a	79.22 ± 1.24 A a	80.90 ± 1.53 A a
4	78.41 ± 1.94 A a	170.0 ± 2.33 B b	89.98 ± 1.75 C b
8	81.0 ± 1.67 A a	208.48 ± 1.85 B c	85.23 ± 2.02 A ab
12	81.83 ± 1.53 A a	222.47 ± 2.84 B c	79.16 ± 1.93 A a

-Values are expressed as mean ± SE. - n=6 rabbit per group.
-Small letter within group differences, P<0.01 vs. pretreatment values.
-Capital letter between group differences, P<0.01 vs. control group.

Table (9) illustrated the mean value of VLDL-C concentration in the control and treated groups along the experimental period.The results showed that the mean values of VLDL-C in treated groups (T1) were significantly P<0.01increased as compared with the control and pretreated period. No significant P<0.01 differences in serum VLDL-C concentration in T2 group after four and eight weeks.While after twelve weeks of treatment significant P<0.01 elevation in VLDL-C in T2 group was recorded.

Table (9): Effect of flavonoids extracted from black cumin seeds for twelve weeks on serum very low density lipoprptein (VLDL-C) concentration (mg/dl) in control and H_2O_2 treated adult male rabbit.

Weeks	Control	T1 animals received 0.5% H_2O_2	T2 animals received 0.5% H_2O_2+ 27.5 mg flavonoids
Zero	25.06 ± 0.54 A a	25.88 ± 0.48 A a	25.30 ± 0.60 A a
4	25.75 ± 0.40 A a	33.6 ± 0.75 B b	26.14 ± 0.53 A a
8	26.20 ± 1.67 A a	37.82 ± 0.54 B c	25.48 ± 0.60 A a
12	25.27 ± 0.84 A a	36.93 ± 0.96 B c	28.5 ± 0.50 C b

- Values are expressed as mean ± SE. - n=6 rabbit per group.
-Small letter within group differences, P<0.01 vs. pretreatment values.
-Capital letter between group differences, P<0.01 vs. control group.

Table (10) demonstrated the mean values of serum TAG concentration (mmol/l) in untreated and treated groups along the experimental period.The table showed a general trend for TAG concentration to increase P<0.01 significantly after 4, 8 and 12 weeks of treatment in group T1 comparing with control and pretreated period. Whereas no significant difference inTAGconcentration in flavonoid plus H2O2 treated group along the experimental period was recorded.

Table (10): Effect of flavonoids extracted from black cumin seeds for twelve weeks on serum triacylglycerol (TAG) concentration (mg/dl) in control and H_2O_2 treated adult male rabbit.

Weeks	Control	T1 animals received 0.5% H_2O_2	T2 animals received 0.5% H_2O_2+ 27.5 mg flavonoids
Zero	125.30 ± 2.58 A a	129.40 ± 1.34 A a	126.50 ± 2.14 A a
4	128.76 ± 1.81 A a	168.0 ± 2.43 B b	130.70 ± 1.46 A a
8	131.0 ± 1.75 A a	189.10 ± 2.68 B c	127.40 ± 1.43 A a
12	126.35 ± 1.64 A a	184.67 ± 1.96 B c	131.50 ± 1.80 A a

- **Values are expressed as mean ± SE. - n=6 rabbit per group.**
- **Small letter within group differences, P<0.01 vs. pretreatment values.**
- **Capital letter between group differences, P<0.01 vs. control group.**

The result of the present study showed that administration of H2O2 in drinking water to rabbits caused a case of hypercholesterolemia and hypertriglyceredemia manifested by significant elevation in serum TC,LDL-C,VLDL-C and TAG and reduction in HDL-C .The changes in serum lipid profile could reflect the role of H2O2 in suppression of lipid metabolism, due to changes in lipoprotein metabolism (34). Moreover, elevationof LDL-C and VLDL-C may be due to changes in the hepatic expression of genes such as lipoprotein receptors, apolipo protein B and the microsomal TAG transfere protein (35 and 36).

Oral administration of flavonoids to male rabbits for 12 weeks exerted hypocholesterolemic effect (depression of serum TC, LDL-C) and elevation of HDL-C concentration and hypotriacylglyceridemic effect.

Recently it has been documented that flavonoids has the ability to directly scavenge off , protect HDL-C from oxidation and reduce serum cholesterol level (37) and improving the lipoprotein profile through decreasing the LDL-C concentration (38 and 39).

Flavonoids lower blood cholesterol level initially by enhancement of HMG-coA reductase phosphorylation indirectly thus diminish endogenous cholesterol production and secondly through probable flavonoid binding to cytoplasmic steroid receptor due to hydrophobicity of their aglycones portion and this complex is likely to interact with steroid regulatory elements. Alternatively, the flavonoids may inter calate it between the bases of DNA segments, leading to transcription of gene involved lowering blood cholesterol level (40).

Generally, the mechanisms of phenolic compounds for antioxidant activity are neutralizing lipid free radicals and preventing decomposition of hydroperoxides into free radicals (41).

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