

## Effect of crude polyphenol extracted from black olive fruit (*olea europae*) on some physiological and immunological parameters in Males Rats Treated with Hydrogen Peroxide

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Accepted on 24/1/2012

### Summary

The present study was designed to demonstrate the effect of crude alcoholic extract of polyphenol in olive (200 mg/kg) on some physiological and immunological parameters in male rats treated with 1% hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>). Twenty rats were divided randomly into four equal groups and treated for (30) days as follows: control group (c) which were given ordinary tap water, first treated group (G1) was given 1% H<sub>2</sub>O<sub>2</sub> in drinking water, the second treated group (G2) was given alcoholic extract of polyphenol in olive (200 mg/kg) by oral intubation, third treated group (G3) was given H<sub>2</sub>O<sub>2</sub> and polyphenol. Blood samples were collected after 30 days for measuring the following parameters: Total WBC count, the percentage of WBC differential count (%), platelet count, serum protein, albumin and globulin, Neutrophil /lymphocyte index, Albumin /Globulin and Phagocytic index. The results showed a significant increase in, total WBC count of polyphenol treated group (G2) and polyphenol compounds plus H<sub>2</sub>O<sub>2</sub>, Lymphocyte percentage in both polyphenol (G2) and polyphenol with H<sub>2</sub>O<sub>2</sub> (G3) treated groups, percentage of neutrophil in H<sub>2</sub>O<sub>2</sub> (G1) group and monocyte percentage in both H<sub>2</sub>O<sub>2</sub> (G1) and H<sub>2</sub>O<sub>2</sub> with polyphenol (G3) groups as compared with control group. Significant depression in platelet count in H<sub>2</sub>O<sub>2</sub> group (G1) as compared with other treated groups was also recorded. The immunological test showed a significant increase in phagocytic index in polyphenol and H<sub>2</sub>O<sub>2</sub> group (G3) as compared with other groups. The result also pointed a significant increase in the concentration of protein and globulin in polyphenol compounds with H<sub>2</sub>O<sub>2</sub> group (G3) and also a significant increase in albumin concentration of group G2, while there was a significant decrease in globulin concentration of H<sub>2</sub>O<sub>2</sub> (G1) group as compared with other groups. On conclusion it seems that treatment with alcoholic extract of polyphenol in olive at concentration of 200 mg/kg B.W. has the ability to correct the adverse effect of hydrogen peroxide in male rats.

**Keywords: Olive, polyphenol, H<sub>2</sub>O<sub>2</sub>, phagocytic index, total protein, albumin, globulin.**

### Introduction

The olive tree (*Oleo europaea*) produces the olive fruit. In the later part of the 20th century; olive fruit extract is believed to exert its biological benefits mainly via constituent antioxidants. Although the composition of olive fruit is complex, the major groups of compound thought to contribute to its observed health benefits include polyphenols, which has been found to inhibit oxidative stress (1).

Epidemiological evidence and numerous animal studies indicate that crude polyphenols of olive fruit may possess potent antioxidant activity. The chemoprevention ability of polyphenols occurs in highest level in this fruit (2). In vitro, the antioxidant activity of crude

polyphenols in olive fruit has been demonstrated, for example it was shown that polyphenols protects different cell types such as white blood cells, Platelets, erythrocytes and other type of cells from hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), which induced cytotoxicity (3).

Oxidative stress included by hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) is known to induce a broad range of physiological, biological and immunological dysfunctions in laboratory animals and humans. Furthermore, it causes cell death and trigger apoptosis due to DNA damage (4). Recent evidence indicates that WBC, lymphocytes, monocytes and platelets decreased when they exposed to high levels of reactive oxygen species (ROS) to which H<sub>2</sub>O<sub>2</sub>

is belonged because of mitochondrial depolarization which in turn leads to apoptosis of cells (5). In vitro treatment of platelet with free radicals causes proteolysis of glycoproteins in platelets membrane that play important role in its function, therefore, loss function and life span in circulation decreased (6). Because H<sub>2</sub>O<sub>2</sub> are known to be involved in the inflammatory process developed in many disease, phagocytic function increased as phagocytic index and efficiency after exposed to H<sub>2</sub>O<sub>2</sub> (7). Also, H<sub>2</sub>O<sub>2</sub> has important effect on blood proteins especially albumin. In general albumin represent the major and predominant physiological antioxidant, previous works have shown that more than 70% of free radical such as H<sub>2</sub>O<sub>2</sub> trapping activity of serum was due to serum albumin (8). As the immune system play an integral role in the body's defenses, it is crucial to maintain their repair against certain stress conditions. In the present study, it assessed the effect of extraction crude polyphenols in olive fruit on some immunological and physiological parameters of H<sub>2</sub>O<sub>2</sub> treated rats.

### Materials and Methods

Twenty mature (200 – 220 gm) adult males' rats were randomly divided into four equal groups (5/group) they had free access to food and water. In the first group, rats were received tap water and considered as control group. Animals of the second group received 1% H<sub>2</sub>O<sub>2</sub> in drinking water. Third group received 200 mg/kg B.W of crude polyphenols compounds which extracted from fruits of black olive by alcohol extraction method then using magnetic stirrer for 18 hrs and dried the extracts with incubator at 40 ± 2C°, quality determination of phenolic compounds showed positive results (9). The rats in the fourth group received 1% H<sub>2</sub>O<sub>2</sub> in drinking water with 200mg /kg B.W of crude polyphenols compounds. After 30days of the experiment blood samples were collected by heart puncture technique for measuring the following parameters. Total white blood cells (TWBC), the percentage of WBC differential count, platelets count as described by (10). Phagocytic activity (%) was measured as described by (11)

while neutrophil /lymphocyte (N/L) index was measured according to (12). These parameters were measured immediately after collection of blood samples. Besides, serum sample were used for measuring total protein and albumin enzymatically by using kit of linear chemicals. Also globulin concentration estimated indirectly by measuring of albumin in serum and then it was subtracted from the result of total protein (13). Differences between experimental groups were evaluated using one-way analysis of variance (ANOVA) specific group differences were determined using least significant differences (LSD) for all analysis, P<0.05 was considered to be significant, according to (14).

### Results and Discussion

The effects of crude polyphenols and 1% H<sub>2</sub>O<sub>2</sub> on cellular immunity of male rats was demonstrated in tables (1, 2, 3, 4, 5 and 6). Table,1 showed significant increase (P<0.05) in total leukocyte count following intubation of crude polyphenols compound comparing with H<sub>2</sub>O<sub>2</sub> group and other groups Antioxidant is substance that fights against ROS (reactive oxygen species) and protect the cells from their damaging effects. Production of ROS during cellular metabolism is balanced by their removal by antioxidant. Any conditions are leading to increase levels of ROS results in oxidative stress which promotes a large number of human diseases including cancer. Therefore, antioxidants may be regarded as potential anticarcinogenes, as they slow down or prevent development of cancer by reducing oxidative stress (15).

**Table, 1: Effect of crude polyphenols of olive fruit (*Olea europae*) at dose (200 mg /kg) and 1% H<sub>2</sub>O<sub>2</sub> on the total WBC count (x10<sup>9</sup> cell /L) in male rats.**

Groups	Total WBC count
Control©	B 5380.000 ± 160.60
H <sub>2</sub> O <sub>2</sub> (G1)	C 4720.000 ± 62.60
Crude polyphenol(G2)	A 1038.000 ± 120.30
H <sub>2</sub> O <sub>2</sub> with crude polyphenols(G3)	A 9740.000 ± 120.10

Values expressed as means ± SE. (n = 5/group)

Capital letters denote between groups differences (P<0.05)

Table,2 pointed to significant increase in percentage lymphocyte at the level of (P<0.05)

in both crude polyphenols group and H2O2 with crude polyphenolic compounds group, comparing to control and H2O2 groups. The results revealed that exposure of rats to H2O2 for 30 days showed significant increase at the level of (P<0.05) in neutrophil percentage comparing to control and other groups while exposure of animals to H2O2 and H2O2 with crude polyphenol compounds resulted in a significant at the level of (P<0.05) increase of monocyte percentage as compared to control and crude polyphenols compounds groups.

**Table, 2: Effect of crude polyphenols of black olive fruit (*Olea europae*) at dose (200 mg /kg) and 1% H2O2 on WBC differential count (%) in male rats.**

Groups	Lymphocyte	Neutrophil	Monocyte	Basophil	Eosinophil
Control©	B 63.4 ± 1.3	B30.06 ± 0.58	C 4.0 ± 0.83	0.04 ± 0.04	0.40 ± 0.40
H2O2(G1)	C25.00 ± 0.7	A38.80 ± 1.2	A 34.0 ± 0.55	0.40 ± 0.40	0.04 ± 0.04
Crude polyphenols (G2)	A70.80 ± 1.2	C 21.4 ± 0.51	B 8.2 ± 0.92	0.04 ± 0.04	0.8 ± 0.8
H2O2 with crude polyphenols(G3)	A72.60 ± 1.8	D 14.2 ± 1.46	B 11.2 ± 2.56	0.10 ± 0.10	0.1 ± 0.1

Values expressed as means ± SE. (n = 5/group)  
Capital letters denote between groups differences (P<0.05)

Table,3 clarified a significant increase (P<0.05) in phagocytic activity in a treatment groups as comparing with control, especially H2O2 with crude polyphenols compound group.

**Table, 3: Effect of crude polyphenols of olive fruit (*Olea europae*) at dose (200 mg /kg) and 1% H2O2 on percentage of phagocytic activity (%) in male rats**

Groups	Phagocytic activity %
Control©	D 67.60 ± 0.51
H2O2(G1)	B 75.60 ± 0.51
Crude polyphenols(G2)	C 72.80 ± 0.90
H2O2 + crude (G3)polyphenols	A 91.60 ± 1.10

Values expressed as means ± SE. (n = 5/group)  
Capital letters denote between groups differences (P<0.05)

H2O2 alone causes a significant depression in platelets count as shown in table (4) comparing to H2O2 with crude polyphenol group and other groups. Fruits and vegetables are rich source of antioxidants. Moreover, a number of phytochemicals present in medicinal plants are known to possess antioxidant activity such as the crude polyphenols

compounds which extracted from fruits of black olive that's known to exhibit pharmacological effect on the body (16).

**Table, 4: Effect of crude polyphenols of olive oil(*Olea europae*) at dose (200 mg /kg) and 1% H2O2 on platelets (x10<sup>6</sup>/L) in male rats.**

Groups	Platelets count
Control©	B 822.80 ± 2.30
H2O2(G1)	C 218.80 ± 16.90
Crude polyphenols(G2)	A 849.20 ± 1.70
H2O2 with crude polyphenols(G3)	AB 837.60 ± 9.70

Values expressed as means ± SE. (n = 5/group)  
Capital letters denote between groups differences (P<0.05)

The present study revealed that crude polyphenols compounds of olive fruits have an important role in protection the immune system from the harmful effect of 1% H2O2. For instance, olive polyphenols correct the adverse effect of H2O2 on the immunological parameters such WBC, differential leukocytes, platelets, total serum protein, globulines, albumin, phagocytic activity, and N/L index. Observation of increased total WBC count after exposure to 1% H2O2 and treatment with crude polyphenols compounds of olive agree with the results of (1), that polyphenols from olive had been shown a significantly increased white blood cells during inflammation by its protective properties. From present results, olive polyphenols compounds seem to modulate the oxidative stress on lymphocyte, monocyte percentage and platelet counts. Recent studies had shown that excess of reactive oxygen species (ROS) such as H2O2 had the ability to induce biochemical alteration in macromolecules like DNA, lipids and proteins through which these molecules mediate their effects on the inflammatory process and cell degradation (17). There is substantial evidence demonstrating that many genes and signal transduction pathways are influenced by H2O2 and antioxidants. Excess H2O2 are known to effect the expression of a number of genes and transcription factors such as NF-KB factor and activated it in monocyte and lymphocyte then cell death (18). For these reasons the chemoprevention ability of olive fruit has been ascribed to polyphenolic

compounds that possess a potent antioxidant activity which reduced DNA damage in peripheral blood lymphocytes and monocytes. H<sub>2</sub>O<sub>2</sub> causes DNA strand breaks by generation of the hydroxyl radical (OH<sup>·</sup>), via the Fenton reaction in the presence of Fe<sup>+2</sup>, therefore, olive polyphenols may act, in addition to their interference as free radical scavengers, as metal ion chelates (19). Polyphenols stimulates phagocytosis as response of the immune system during inflammation (20).

Table,5 indicated that crude polyphenols compounds in olive fruit with H<sub>2</sub>O<sub>2</sub> causes a significant increase in total protein and globulins as compared with H<sub>2</sub>O<sub>2</sub> and other groups. Meanwhile, albumin concentration increased significantly in crude polyphenol group and crude polyphenol with H<sub>2</sub>O<sub>2</sub> group as compared with other groups.

**Table, 5: Effect of crude polyphenols of olive fruit (*Olea europae*) at dose (200 mg /kg) and 1% H<sub>2</sub>O<sub>2</sub> on the total proteins, albumin, globulin and Albumin/ Globulin (A/G) index in male rats.**

Groups	Total proteins	Albumin	Globulin	A/G
Control©	B 6.50 ± 0.50	C 3.60 ± 0.13	A 2.90 ± 0.57	A 1.54 ± 0.40
H <sub>2</sub> O <sub>2</sub> (G1)	B 5.30 ± 0.20	C 3.60 ± 0.11	B 1.74 ± 0.14	A 1.54 ± 0.16
Crude polyphenols (G2)	A 7.20 ± 0.43	A 5.10 ± 0.12	B 2.10 ± 0.35	A 3.26 ± 0.90
H <sub>2</sub> O <sub>2</sub> with crude polyphenols (G3)	A 7.74 ± 0.40	B 4.50 ± 0.20	A 3.30 ± 0.31	A 3.82 ± 0.37

Values expressed as means ± SE. (n = 5/group)  
Capital letters denote between groups differences (P<0.05)

Data concerning the health benefits of crude polyphenols of olive have been reported that polyphenols diminish oxidant stress by stabilize the NF-kB system and prevent the activation of this system (21). Thus, hypothetically modulation of NF-kB activation could be a target to reduce inflammatory response and this may decrease the injury to the cells which may be one of the first events in the development of disease (22).

Table,6 demonstrated that Neutrophil/lymphocyte index show a significant increase at the level of (P<0.05) in H<sub>2</sub>O<sub>2</sub> group as compared with control and other groups. Therefore, the health effects of olive

polyphenols may be due to their antioxidant and anti-inflammatory action (23).

**Table, 6: Effect of crude polyphenols of olive fruit (*Olea europae*) at dose (200 mg/kg) and 1% H<sub>2</sub>O<sub>2</sub> on Neutrophil /Lymphocyte (N/L) index in male rats.**

Groups	N/L
Control©	B 0.470 ± 0.010
H <sub>2</sub> O <sub>2</sub> (G1)	A 1.600 ± 0.050
Crude polyphenols(G2)	C 0.300 ± 0.004
H <sub>2</sub> O <sub>2</sub> with crude polyphenols(G3)	D 0.198 ± 0.020

Values expressed as means ± SE. (n = 5/group)  
Capital letters denote between groups differences (P<0.05)

Oxidative stress by H<sub>2</sub>O<sub>2</sub> leading to production of several hormones of stress such as cortisol, epinephrine and nore-pinephrine which they causes increase in neutrophil percentage and N/L index in blood (24). Also exposure to H<sub>2</sub>O<sub>2</sub> leading to inflammation, and this reaction characterized by movement of neutrophils and monocytes from blood to the extravascular tissue. Furthermore, this reaction causes increase in many mediators such as monocyte- chemoattractant protein 1 (MCP1) and macrophage inflammatory molecule 1α (MIP1α). Therefore, monocytes and neutrophil increased in circulation and phagocytosis increased in tissue, to prevent extensive damage to the host (25).

Many studies strongly support the idea that polyphenols have the capacity to modulate the immune response and have potential anti-inflammatory activity. It has been observed that polyphenol compounds are able to decrease the expression of different pro-inflammatory cytokines /chemokines in many cell types, and then enhanced the resistance to oxidative stress (26). Therefore, neutrophil percentage decreased in polyphenol with H<sub>2</sub>O<sub>2</sub> treated group. Free radicals also can impart important changes in the platelets glycoproteins. The proteins become more susceptible to proteolysis following free radical injury, because oxidative stress can causes desialylation of platelet glycoprotein's, then the life span of platelets in blood is decreased (27), and that's appear in H<sub>2</sub>O<sub>2</sub> group. Meanwhile, crude polyphenols of olive fruit protect platelets from H<sub>2</sub>O<sub>2</sub> effect and keep it within normal range due to the protective properties of

polyphenols, occur mainly in H<sub>2</sub>O<sub>2</sub> with crude polyphenol group. In vivo, studies have added further evidence that free radicals increase the incidence of abnormal blood clots, while olive polyphenols help to prevent this abnormal blood clotting. In addition, polyphenols compounds prevent platelets from sticking together and reduce the risk of blood clots (28).

Another component of blood that is affected by oxidative stress is albumin. Albumin, known as a physiological antioxidant, is the most abundant protein in serum. Recent evidence indicates that albumin may provide antioxidant protection by functioning as a serum peroxidase in the presence of reduced glutathione, which is an intracellular antioxidant (13), so low serum albumin may also reflect a serum antioxidant deficit that contributes to the risk of oxidative stress (29). Moreover, oxidative stress induces oxidative degradation of protein in vitro.

Therefore, globulin is also modified and then decreased after attack by H<sub>2</sub>O<sub>2</sub> (30). Recent studies have described the affinities between olive polyphenols and plasma proteins (31), such as globulin, and protect it from oxidative stress, in which obviously occurs in crude polyphenol with H<sub>2</sub>O<sub>2</sub> group. Administration of crude polyphenols of olive showed restoration of serum albumin towards normal and these effects were more pronounced in animals exposed to H<sub>2</sub>O<sub>2</sub> with polyphenols. This infers that the therapeutic effect of polyphenols could be attributed to the biological activities of it, then strengthens the immune system and inhibits oxidative stress (32). Finally, all these benefits of crude polyphenols compounds of black olive fruit make it a greater fighter against oxidative stress and boost the immune system.

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induced hepato carcinogenesis in rats. The Free library by Farlex, PP: 1-8.

### تأثير المتعدد الفينولي الخام المستخلص من ثمار الزيتون الأسود *Olea europae* في بعض الصفات الفسلجية والمناعية لذكور الجرذان المعاملة بيروكسيد الهيدروجين

لمى وليد خليل و ليلى هاشم علول و انوار ابراهيم عبيد  
فرع الفسلجة والأدوية - كلية الطب البيطري - جامعة بغداد - العراق

#### الخلاصة

صممت هذه الدراسة لمعرفة تأثير متعدد الفينولي المستخلص الكحولي من الزيتون الأسود بتركيز 200 mg/kg في بعض المعايير الفسلجية والمناعية في ذكور الجرذان البالغة السليمة والمعاملة بيروكسيد الهيدروجين (1% استعملت عشرون جرذاً، اذ تم تقسيمها إلى اربعة مجاميع متساوية وعولمت لمدة (30) يوماً كالآتي: مجموعة السيطرة (C) أعطيت ماء الشرب العادي. مجموعة معاملة بيروكسيد الهيدروجين 1% (G1) وقد أعطيت ماء الشرب العادي المضاف اليه بيروكسيد الهيدروجين (H2O2). مجموعة معاملة بالمستخلص الكحولي لمتعدد الفينول بتركيز 200 mg/kg عن طريق الفم (G2) ومجموعة معاملة بالمستخلص الكحولي لمتعدد الفينول مع بيروكسيد الهيدروجين (H2O2) ورمز إليها (G3) وبنفس التراكيز السابقة. تم سحب الدم من الحيوانات بعد 30 يوم لغرض اجراء الفحوصات الآتية: العدد الكلي لكريات الدم البيضاء، العد التفرقي، حساب الاقراص الدموية، نسبة الخلايا المتغايرة / اللمفية، معامل البلعمة، قياس تركيز البروتين الكلي والاليومين والكلوبيولين في مصل الدم، حساب نسبة الألبومين / الكلوبيولين. لقد أظهرت النتائج حصول ارتفاع معنوي في العدد الكلي لكريات الدم البيض في المجموعتين (G2) و (G3) المعاملة بالفينول المتعدد فقط والمعاملة بالفينول المتعدد وبيروكسيد الهيدروجين بالإضافة إلى حصول ارتفاع معنوي في نسبة الخلايا اللمفاوية في المجموعتين (G2) و (G3) كذلك حصول ارتفاع معنوي في نسبة العدلات في المجموعة (G1) المعاملة ب H2O2، ولوحظ حصول انخفاض معنوي في عدد الأقراص في المجموعة المعاملة بيروكسيد الهيدروجين (H2O2) الدموية مقارنة مع بقية المجاميع، أما نتائج الفحوصات المناعية فقد أظهرت ارتفاع معنوي في معامل البلعمة للمجموعة (G3) المعاملة بالفينول المتعدد (polyphenol) وبيروكسيد الهيدروجين (H2O2) أما بالنسبة لتركيز البروتين الكلي والكلوبيولين وجد ان هناك ارتفاع معنوي في المجموعة (G3) المعاملة بالفينول المتعدد وبيروكسيد الهيدروجين مقارنة مع بقية المجاميع أضف إلى ذلك وجود ارتفاع معنوي في الألبومين في المجموعة (G2). ووجد هناك نقصان معنوي لتركيز الكلوبيولين في المجموعة المعاملة (G) مقارنة مع باقي المجموعات. نستنتج من هذه الدراسة ان متعدد الفينول المستخلص من الزيتون له القدرة في التغلب على التأثيرات الضارة لبيروكسيد الهيدروجين في الجرذان.

الكلمات المفتاحية: الزيتون، متعدد الفينول، بيروكسيد الهيدروجين، معامل البلعمة، البروتين الكلي، الألبومين، الكلوبيولين.