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Lemon juice antioxidant activity against oxidative stress

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Abstract:

This study is conducted to evaluate the therapeutic and antioxidant effect of lemon juice on some hematological and biochemical parameters. Thirty female mice used in this study were exposed to oxidative stress through giving them hydrogen peroxide in drinking water for 30 days. Animals randomly distributed over 3 groups, each group contained 10 animals and treated as follows: T1 control group (drinking distilled water only), T2 (0.75% hydrogen peroxide in drinking water) and T3 (0.75% hydrogen peroxide in drinking water with daily drenching with 1 mL lemon juice). At the end of the experiment, blood samples were collected from animals for evaluating the following hematological and biochemical parameters: Haemoglobin concentration (Hb), red blood cells count (RBC), white blood cells count (WBC), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelet count (PLT), level of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and total protein. The results showed that T3 exhibited an enhancement in RBC count, Hb concentration, WBC, lymphocyte and total protein and reduction in the level of AST and ALT compared to T2. These findings clearly revealed the advance protective and antioxidant features of lemon juice on hematological and biochemical parameters of the oxidatively stressed female mice.

Key words: Antioxidants, Biochemical, Hematological, Lemon juice, Oxidative Stress.

Introduction:

Oxidative stress is an imbalance between oxidants and antioxidants levels in biological systems (1). Highly reactive atoms or molecules such as free radicals and reactive oxygen species (ROS) are the main cause of oxidative stress. These reactive species can be formed when oxygen interacts with certain molecules and when a cellular macromolecule accepting or losing a single electron therefore, behaving as oxidants or reductants and subsequently cause cellular damage (2-4). Modern lifestyle and eating habits are the most common oxidative stress inducers. Thus, researchers become more interested in rich antioxidants foods such as fruits and vegetables in particularly lemon juice that is showing highly protective features for human health against oxidative-stress related diseases (5, 6).

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Fruit extracts that are rich in antioxidants such as lemon juice were used as an effective agent in decreasing intracellular ROS concentration and protecting lipid, DNA and mitochondrial functionality from the damage induced by free radicals (7-9). Lemon fruit is the most important field crop as it can be used in a wide array of functions (10). For example, fresh consumption, producing juicing, decorating dishes, culinary products, preservatives to maintain food stability (11-14). Moreover, it is showing various health benefits, such as anticancer effect, antimicrobial effect, lipid-lowering effect, protective effect against cardiovascular diseases and antifungal activity (5, 15, 16). Furthermore, it is utilised for the treatment of stomach problem, constipation, teeth problems, memory loss, fever, bleeding, rheumatism, burns, breathing disorders, cholera, atherosclerosis, high blood pressure, treating liver ailments, promotes digestion and prevent urinary tract infections (17-21). In addition, there are much more applications such as cleaning agents and for hair and skin care (11, 16).

Lemon juice has several important chemical components with therapeutic features such as citric acid (Vitamin C, 2-hydroxy-1,2,3-propanetricarboxylic acid). Lemon juice also

contains high concentrations of polyphenols including: rutin, hesperidin, flavonoids, tannins, coumarins, quercitrin, quercetin, eriocitrin, narirutin, didymin, naringin, neohesperidin, chlorogenic acid, luteolin, kaempferol, monoterpene hydrocarbons, γ-terpinene, β-pinene, sabinene, α-pinene, and myrcene (10, 22-24). Micronutrients such as magnesium, potassium, folic acid, limonoids, xanthoxyletin, acids, volatile oils, carotenoids, and glycosides have also been reported in lemon juice. Moreover, a trace amount of waxes are also present in lemon juice (24, 25). These antioxidant are well-known in their elimination effect for free radicals and prevent diseases occurrence due to stress factors by alkalizing body through their acidic nature (15, 26-29).

Therapeutic features of lemon juice belongs to its component of citric acid. Around 5% of lemon juice is citric acid (Vitamin C), which gives lemon a sour taste (14, 30). Citric acid is classified as a weak organic acid formed naturally in many fruits, partially in citrus fruits, also found as a trivalent anion in animal fluids and tissues. Citrate salts have the ability to deliver minerals in biological systems (31). The largest amount of citric acid *in vivo* is related to ATP production in the citric acid cycle (32). Citric acid has a strong inhibitory effect and an efficient scavenger of reactive oxygen species because it has ability to diffuse between the membrane and exterior wall of the bacterial cell. As a result the acid will accumulate in the cell cytoplasm, and consequently acidification of the cytoplasm, disruption of the proton motive force, and inhibition of substrate transport (10, 26).

The present study is designed as a part of the therapeutic approach to evaluate the antioxidant effect of lemon juice *in vivo* on reduction of the deleterious effect of oxidative stress induced by hydrogen peroxide on some hematological and biochemical parameters in female mice. Results of this study could supply valuable information for our society that promotes lemon juice as an essential nutrient in the daily life.

Material and Methods:

Lemon juice preparation

Fresh Iraqi lemon fruits were brought from the local market in Al-Rifaee town, Thi-Qar province and washed with distilled water. Peels were manually separated and pulps deseeded. Juice was prepared by squashing the lemon pulps in a blender to extract the juice, and then the juice was filtered through a Whatman Filter Paper 42. Filtered juice was collected in a dark high-density PVC bottles (500 mL capacity) and kept overnight in the refrigerator before use at (4 ^{o}C).

Animals management

Thirty adult Swiss white female mice, aged 10-16 weeks old (initial weight 20-25 g) were brought from the animal house in College of Science-Thi-Qar University for the experiment. They were acclimatized for two weeks in plastic cages with sawdust bedding in a quiet room and given access to pelleted diet and water ad libitum. The room temperature was controlled (20 ± 2 C), and lights were on between 06:00 AM and 06:00 PM (12:12 h light: dark cycle). The mice were divided randomly into three groups; each group comprised of 10 animals. The study was conducted in the animal house in the College of Agriculture, University of Sumer and continued for 30 days from 20/3/2017 to 20/4/2017. The experimental protocol was approved by the Ethics Review Committee for Animal Experimentation of Sumer University.

Lemon juice and peroxide administration plan

The experiment was designed to have three treatments for 30 days.

T1: drinking water without any addition (Control group).

T2: 0.75% hydrogen peroxide in drinking water for 30 days.

T3: 0.75% hydrogen peroxide in drinking water and daily drenching with 1 mL lemon juice for 30 days from 20/3/2017 to 20/4/2017.

Blood samples collection

Sample collection and animal handling were approved in accordance with the CLSI guideline H21-A5 (33). At the end of the experiment (day 31 of experiment on Friday 21/04/2017), the mice were anesthetized with chloroform and the blood collected directly from the heart using syringes and needles. The collected blood samples (1 ml) were divided equally into two parts, the first was kept in well labelled (Tubes numbered 1-10 for group T1, 11-20 for group T2 and 21-30 for group T3) ethylenediamine tetra-acetic acid (EDTA) test tubes for hematological traits evaluation Hb, RBC, WBC, PCV, MCV, MCH, MCHC, PLT and the second part of blood was placed in normal labelled test tubes (Tubes numbered 31-40 for group T1, 41-50 for group T2 and 51-60 for group T3) for the separation of serum by using centrifuge machine at 1500 rpm for 15 minutes, while the blood cells were discarded. The serum was taken to the laboratory for the assessment of biochemical traits (level of AST, ALT and total protein).

Hematological investigation

The hematological parameters were measured by a fully automatic hemocytometer (Mythic^{TM}18 (Ringelsan CO. Turkey) in private hematological laboratory (Al-Noor for hematological and biochemical analysis). This hematological analyser performed complete blood count (CBC) on EDTA anticoagulated blood (34) for counting the cellular blood components, the Mythic^{TM}18 uses the impedance technique only. The analysis then was completed within a few minutes and the results printed directly from the machine. Haemoglobin measured spectrophotometrically (Cyanide-free method) by the formation of oxyhemoglobin at 555 nm. Hematocrit was measured by volume integration.

Biochemical analytical techniques

Liver function analyses were carried out to determine the serum concentrations of total protein, and the activities of liver enzymes AST and ALT. Spectrophotometric method was employed to determine these biochemical traits using a Helios gamma UV visible spectrophotometer, Thermo spectronic UK, by using diagnostic kits from (Quimica Clinica Applicada, S. A. Spain). Total protein was determined by the Biuret method (35). Alanine and aspartate aminotransferases were determined based on the colourimetric measurement of hydrazone formed with 2,4-dinitrophenyl hydrazine (36), alkaline phosphatase was determined according to the phenolphthalein monophosphate method (37) as manufacturer instructions.

Statistical analysis

The collected data are analysed according to statistical calculations of the mean value, standard deviations (SD), standard errors (SE) of the mean value and variability coefficient using SPSS software version 21. Student's *t*-test is employed to check the significant difference among results, and statistical significance is taken to be indicated by $P<0.05$ while, $P>0.05$ considered non-significant.

Results:

The results in **Table 1** show no significant differences between control group and T2 group in the following traits: RBCs (8.78±0.14 and 8.36±0.21 *vs.* 7.34±0.15 respectively), Hb (13.00±0.51 and 12.28±0.34 *vs.* 10.80±0.24 respectively), PCV% (41.74±0.58 and 41.10±0.38 *vs.* 37.50±0.32 respectively), while T1 group showed significant decrease ($P<0.05$) compared to control group and T2 group for the same parameters.

Table 1. Effect of hydrogen peroxide and lemon juice on some hematological parameters.

Parameters	Mean ± SD*		
	T1	T2	T3
RBCs x10^6/mL	8.78±0.14^a	7.34±0.15^b	8.36±0.21^a
Hb gm/Dl	13.00±0.50^a	10.80±0.24^b	12.28±0.34^a
PCV%	41.74±0.58^a	37.50±0.32^b	41.10±0.38^a

*Mean ± SD for 3 reduplications.
Mean values are preceded by different letters in same row indicate ($P<0.05$) probability as a significant difference.

T1 group exhibits significant increase ($P<0.05$) (Table 2) compared to control group in MCV (51.10±0.93 *vs.* 48.08±0.4), but no significant difference can be found when compared to T2 (49.20±0.89). However, no significant difference can be noticed among treatment in MCH (Table 2). T1 group has decreased significantly in MCHC compared to the control group (28.76 ± 0.48 *vs.* 31.06±0.95), but it shows non-significant difference compared to T2 (29.82±0.60) (Table 2). Platelet count in T1 shows significant increase ($P<0.05$) compared to control and T2 groups (650.20±25.25 *vs.* 428.20±10.30 and 515.60±8.07 respectively) (Table 2).

Table 2. Effect of hydrogen peroxide and lemon juice on some hematological parameters.

Parameters	Mean ± SD*		
	T1	T2	T3
MCV	48.08±0.40^b	51.10±0.93^a	49.20±0.89^{ab}
MCH	15.02±0.46^a	14.68±0.29^a	14.64±0.12^a
MCHC	31.06±0.95^a	28.76±0.48^b	29.82±0.60^{ab}
Platelet x10^3/mL	428.20±10.30^c	650.20±25.25^a	515.60±8.07^b

*Mean ± SD for 3 reduplications.
Mean values are preceded by different letters in same row indicate ($P<0.05$) probability as a significant difference.

Results in Table 3 reveal significant decrease in T1 group compared to control and T2 for WBCs (4.80±0.26 *vs.* 7.46±0.29 and 7.28±0.26 respectively) and lymphocyte (70.08±0.54*vs.* 76.80±0.92 and 74.98±0.45 respectively) while, granulocyte count shows significant increase ($P<0.05$) among T1 group compared to control and T2 (15.78±0.38 *vs.* 10.00±0.32 and 10.82±0.35 respectively). However, monocyte count did not affect in all treatment groups and no significant difference can be find among treatments (Table 3).

Table 3. Effect of hydrogen peroxide and lemon juice on WBCs and differential WBCs count.

Parameters	Mean ± SD*		
	T1	T2	T3
WBCs	7.46±0.29[a]	4.80±0.26[b]	7.28±0.26[a]
Lymphocyte	76.80±0.92[a]	70.08±0.54[b]	74.98±0.45[a]
Monocyte	13.20±0.70[a]	13.94±0.57[a]	13.80±0.34[a]
Granulocyte	10.00±0.32[b]	15.78±0.38[a]	10.82±0.35[b]

*Mean ± SD for 3 reduplications.
Mean values are preceded by different letters in same column indicate ($P<0.05$) probability as a significant difference.

The results in Table 4 reveal a significant increase ($P<0.05$) in AST and ALT levels of T1 compared to control and T2 (89.18±2.60 *vs.* 41.76±0.88 and 47.93±1.31 respectively) (106.10±2.23 *vs.* 51.60±0.99 and 56.81±1.32 respectively). While, total protein concentration for T1 decreased significantly compared to control and T2 (4.08±0.09 *vs.* 6.06±0.14 and 5.81±0.17 respectively).

Table 4. Effect of hydrogen peroxide and lemon juice on AST, ALT and total protein levels.

Parameters	Mean ± SD*		
	T1	T2	T3
AST U/L	41.76±0.88[c]	89.18±2.60[a]	47.93±1.31[b]
ALT U/L	51.60±0.99[c]	106.10±2.23[a]	56.81±1.32[b]
Total protein gm/DL	6.06±0.14[a]	4.08±0.09[b]	5.81±0.17[a]

*Mean ± SD for 3 reduplications.
Mean values are preceded by different letters in same row indicate ($P<0.05$) probability as a significant difference.

Discussion:

Effect of H_2O_2 and lemon juice on hematological traits

The results presented above have clearly showed treatment with hydrogen peroxide causes deterioration in hematological parameters. Because it causes oxidative stress due to liberation of hydroxyl ion which has harmful effect on the plasma membrane of RBCs causing aging and deformities and finally death of cells (38). In addition, free radicals combine with heme molecule leading to a reduction in its concentration in RBCs (39). Platelets count increment in mice treated with H_2O_2 alone may refer to the fact that free radicals cause enhancement in the count and activity of platelets (40), while lemon juice inhibits clotting and reduces activity of platelets (41). Addition of lemon juice with hydrogen peroxide led to reduce deterioration in blood parameters which approximately returned to the normal values. Improvement in hematological traits belongs to the high content of natural antioxidant in lemon juice such as Vit. C, flavonoids and alkaloid which scavenge free radicals and reduce their harmful effect on the body (42). Hemoglobin oxidation was inhibited easily by flavonoids through the direct binding to hemoglobin. In addition to flavonoids, lemon juice exhibits therapeutic features by improving erythrocytes levels (43). Phytochemical components of lemon juice have played rules to protect hemoglobin from oxidizing agents as a result hemoglobin levels increased in the current study. Increasing levels of hematocrit may be related to the decrease in the destruction of RBC. It is well known lemon juice has a high concentration of antioxidants such as vitamin C that play ruled in reducing destruction effect on RBC (44).

Effect of H_2O_2 and lemon juice on WBCs and differential count

Results in Table 3 show exposing to H_2O_2 led to inhibit immunity through reducing WBCs and lymphocyte count. This result is in agreement with finding of (45). This reduction in WBCs and lymphocyte count may be due to the damage of DNA and cell membrane of WBCs, in addition oxidative stress causes enhancement of cortisone secretion which is considered as immunosuppressive (46). This decrease in immunity cell count was reversed to the normal number by treatment with lemon juice due to its content of natural antioxidants such as citrate, Vit.

C, Vit. E and flavonoid (44). Functions of vitamin C related to its ability to donate electrons. It is a potent antioxidant and a cofactor for a family of biosynthetic and gene regulatory enzymes. Vitamin C contributes to immune defense by supporting various cellular functions of both the innate and adaptive immune system. Vitamin C supports epithelial barrier function against pathogens and promotes the oxidant scavenging activity. Vitamin C accumulates in phagocytic cells, such as neutrophils, and can enhance chemotaxis, phagocytosis. The role of vitamin C in lymphocytes has been shown to enhance differentiation and proliferation of cells likely due to its gene regulating effects (47).

Effect of H_2O_2 and lemon juice on AST, ALT and total protein

Our results exhibit significance increase in the level of AST and ALT and reduction in the level of total protein after H_2O_2 exposure which refers to the liver damage caused by free radicals (hydroxyl ion) and reduction in its function specially protein formation (48, 49). Lemon juice reduces this harmful effect of H_2O_2. Liver enzymes such as ALT, AST, and ALP are known marker enzymes for the assessment of the functional integrity of the liver cells (50). These enzymes are usually raised in acute hepatotoxicity or mild hepatocellular injury, vitamins C and E have hepatoprotective effect against hepatotoxicity due to the adverse effects of generated free radicals (51). Similar findings were reported by (52, 53) found that lemon juice has therapeutic property in case of liver damage due to alcohol drinking that is lemon juice improves liver function through scavenge of free radicals which lead to raise the level of total protein and reduction in the levels of AST and ALT in serum. In conclusion H_2O_2 has a deleterious effect on hematological and biochemical parameters in female mice while supplementation with lemon juice reduces these negative effects and improve liver function.

Conclusions:

Lemon juice is a rich antioxidants source that can be used as a safe, cheap and acceptable drink at different concentrations. It has shown significance protection effect for a wide range of hematological and biochemical traits covered in this study.

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Ethical approval:

Animals were used in this study according to institutional, national and international guidelines for the care and use of animals. Also, all ethical standards of Sumer University employed carefully in studies involved animals.

Conflicts of Interest: None.

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فعالية عصير اليمون تجاه فرط الاكسدة

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الخلاصة:

أجريت هذه الدراسة لتقييم تأثير عصير الليمون على بعض الصفات الدموية والكيموحيوية لإناث الفئران التي تعرضت للإجهاد التأكسدي من خلال إعطائهم بيروكسيد الهيدروجين في مياه الشرب لمدة 30 يومًا .استخدم في هذه الدراسة ثلاثين من اناث الفأر، وزعت عشوائيا في 3 مجموعات، كل مجموعة تضم 10حيوانات عوملت على النحو التالي: مجموعة السيطرة (مياه الشرب دون إضافة)، مجموعة المعاملة الاولى (T1 75٪ بيروكسيد الهيدروجين في مياه الشرب)، والمعاملة الثانية (T2 75٪ بيروكسيد الهيدروجين في مياه الشرب مع التجريع اليومي بعصير الليمون بمقدار 1مل). في نهاية التجربة ، تم قتل الحيوانات وجمع الدم لتقييم المعايير الدموية والكيموحيوية التالية: تركيزخضاب الدم، عدد كريات الدم الحمر، حجم الكريات المرصوص، متوسط حجم الكرية، متوسط خضاب الكرية، عدد خلايا الدم البيض، عدد الخلايا البيض التفريقي، عدد الصفائح الدموية ومستوى AST، ALTوالبروتين الكلي. أتضح من النتائج أن T2أظهرت تحسن في عدد كرات الدم الحمراء، تركيز Hb، WBC، الخلايا اللمفاوية والبروتين الكلي وانخفاض في مستوى ASTو ALTمقارنة بالمعاملة الاولى T1.وقد استنتج أن عصير الليمون له تأثير معززللصفات الدموية والكيموحيوية لأناث الفئران المعرضة للإجهاد التأكسدي.

الكلمات المفتاحية: عصير اليمون، فرط الاكسدة، مضادات الاكسدة.