doi.org/10.52866/esj.2021.03.17.16

Estimation of ascorbic acid in some medical plants and their effect on Superoxide dismutase (SOD) enzyme and some hematological parameters in male rats

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Abstract

The experiment was conducted to evaluate the effect of aqueous extract of *Spinacia oleracea, Zea mays* and *Capsicum annuum* plants on SOD enzyme levels in rats. Ascorbic acid was estimated in these plants by using High-Performance Liquid Chromatography (HPLC). Twenty-five male albino rats in age (8-12) weeks and weight (120-200 g) were divided randomly into five groups; each group was kept in a separate polypropylene rat cage by using CRD complete random design with five replicates for each treatment. In addition, histological study was conducted to liver after treatment of rats with the plant aqueous extracts. The result showed that *S.oleracea* and *Z.mays* increased the level of SOD 16.32 U/ml. While the result of *C. annuum* administration increased Hb and RBC levels 13.40 g/dl, 6.9010^12/L. Also ascorbic acid results were 3.376mg/ml, 0.645mg/ml and 0.579mg/ml, for *S. oleraceay, C. annuum*, and *Z. mays*, respectively The evidence of the study showed that *Z.mays* is an excellent antioxidant in rats. Also ascorbic acid significantly increased RBC and Hb in rats. The Histological study result inducted that the use of these plants in current diets has no pathophysiological implications.

Keywords: Ascorbic acid , medicinal plants, SOD, Antioxidant, corn, red chili pepper.

تقدير حامض الاسكوربك في بعض النباتات الطبية وتأثيره على انزيم الاكسدة Superoxide dismutase (SOD) وبعض معلمات الدم في ذكور الجرذان

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الخلاصة:

اجريت الدراسة لتقييم تأثير المستخلص المائي في نباتات السبانخ، الذرة والفلفل الأحمر الحار على مستويات انزيم الأكسدة في الفئران. في هذه الدراسة تم تقدير نسبة حامض الاسكوربك في هذه النباتات باستخدام تقنية كروماتوغرافيا السائل عالية الدقة. خمسة وعشرون جرذ ذكر البينو بعمر (8–12) اسابيع و وزن (120–200غم) قسمت عشوائياً الى خمسة مجاميع: كل مجموعة حفظت في قفص بوليبروبلين للجرذان باستخدام التصميم الكامل العشوائي. بالإضافة، تم اجراء دراسة نسيجية على الكبد بعد معاملة الجرذان بالمستخلصات النباتية المائية. اظهرت النتائج ان الذرة و السبانخ رفعت من مستوى انزيم الأكسدة 16.32 وحدة انزيم/ مل. وكانت نتيجة التجريع بالفلفل الاحمر الحار هي رفع نسبة الهيموغلوبين و كريات الدم الحمر 13.40غم/ ديسيلترإ، 6.90 (10^12/ لتر). في حين كانت نتائج حامض الاسكوربك 3.376 ملغم/ مل، 0،645 ملغم/ مل و 0،579 ملغم/ مل للسبانخ و الفلفل الاحمر الحار و الذرة على التوالي. لقد بينت النتائج هذه الدراسة ان الذرة هي مضاد اكسدة ممتاز في الجرذان. كذلك حامض الاسكوربك قد رفع من نسبة الهيموغلوبين و كريات الدم الحمر في الجرذان. و أخيراً، فقد اظهرت النتائج النسيجية ان استخدام هذه النباتات ضمن الحمية الغذائية ليس لديه اي تأثير مرضي على الخلايا النسيجية.

1.Introduction

The cells have a sufficient number of antioxidants to guard against or restore ROS damage and to control redox-sensitive signals (1). Three of the key antioxidant enzymes that are thought to be essential in all cells that metabolize Oxygen (2) include superoxides are dismutase (SOD), catalases and substrate specific peroxidases, glutathione peroxidase(GPx) in mammalian cells. The SODs turn hydrogen peroxide and molecular oxygen (O2) into superoxide dramatically, while the catalase and peroxidases translate hydrogen peroxide into water and in the case of catalase into oxygen and water. The end effect is the conversion to the water of two potentially toxic species, superoxide and hydrogen peroxide(3).

In the pathogenesis of many infectious diseases, oxidative stress is a basic denominator. Antioxidants are also typically used for protecting and reverse oxidative damage to cells and tissues. The complex interplay between oxidative stress and antioxidants in many physiological processes is well known to be the foundation of iron metabolism. Iron deficits and iron overloads can affect redox conditions, and iron supplementation and iron chelation respectively can return these conditions to physiological conditions. Similarly, antioxidants are introduced as a potential therapeutic solution to the attenuation of oxidative stress-induced tissue harm. In particular, several bioactive plant-derived compounds, likely through interactive mechanisms, have been shown to influence both iron metabolism and redox state (4). A natural antioxidant(NAO) in the spinach has been identified, which includes flavonols, polyphenols, and vitamins (A, B1, B2, B3, B6, C, E, K, and folic acid) hence, it can rise the SOD level (5,6). As calculated by radical-trapping antioxidant agents, the pepper (RAA) was one of the top 10 antioxidant agent suppliers, in most regions of the world, prevents macromolecule from harmful free radicals, i.e. reactive Oxygen species (ROS), Chili and red pepper (7). Several experiments have shown that maize has antioxidant properties that can theoretically be used as a ready-to-use and effective bioactive source of natural antioxidants (8,9). The results showed that maize should up-regulate the function of antioxidant enzymes to defend against oxidatives and increase the level of SOD(10).

Most local spices of herbal plants have been reported to contain rich medicinal properties (11). According to this study, traditional medicine has developed from environmental capital, which the people of the community have adapted to the desperation of disease survival and the preservation of good health. *Capsicum annumm* is a flowering plant species in the Solanacae family of the night shade. Its species are endemic to the Americas where it has been grown for thousands of years (12). *Capsicum annuum*, is a rich source of antioxidant compounds, including capsaicinoids and phenolic compounds, especially flavonoids (13). Capsicum have anti-inflammatory, antioxidant, anti-platelet, anti-hypertensive, hypoglycemic and hypocholesterolemic properties in both in vitro and in vivo models (14). Red pepper is commonly used as a condiment and has a variety of pharmacological and physiological characteristics(15,16). In Latin and Central America, Asia, and Africa, it is popular for red pepper to be used to increase the amount of spice in food(17), however, it is well known to be problematic when eaten in excess of(18,19). Red chili pep-

per has numerous pharmacological and chemical properties, such as drug groups, which are capable of causing tissue deterioration([20). Over the last few decades, it has been experimentally established that a variety of traditional spices will exhibit beneficial physiological activities(21). The active compounds present in spices have important roles in promoting physiological effects, including antioxidant and hypolipidemic activity(22,23,24). Capsaicin (8-methyl-N-vanillyl-6-nonenamide), an active pepper compound responsible for its spicy properties, has captivated the attention of researchers for more than a century who have speculated that it could have pharmacological and physiological effects(20). The purpose of this research is to evaluate the effect of aqueous extract of *Spinacia oleracea, Zea mays* and *Capsicum annuum* on SOD levels and hematological properties in rats.

2.Material and Methods:

2.1 Collection of the plants

The plants samples included the leaves of *Spinacia oleracea* (spinach), the fruits of *Capsicum annuum*(red chili pepper), and the kernels of *Zea mays* (corn) were obtained from the local markets in Baghdad, Iraq. The samples were kept in an oven at 40 °C for one week, until they were hard and then grinded by a grinder to give a small size pieces 2 mm. Then all the samples stored in glass container at room temperature in a dry dark place until use.

2.2 Preparation of aqueous plant extract

An aqueous extract was prepared for each plant by water extraction method without boiling. Different concentration of each plant was prepared by dissolving certain weight of each plant powder according to the needed concentration in normal saline (25).

2.3.4 Ascorbic acid estimation by HPLC

The determination of ascorbic acid was carded by using HPLC technique (26).

2.4 Laboratory animals

Thirty male albino rats in age (8-12) weeks and weight (120-200 g) were purchased from Al-Nahrain University Biotechnology Research Centery, and were housed with room temperature and the experimental protocol design according to (27).

2.5 Experimental design

Rats were divided it to five groups each group contained five rats

Group one(G1) involved the administration of distilled water(control).

Group two(G2) involved the administration of only spinach extract.

Group three(G3) involved the administration of spinach and red chili pepper extract.

Group four(G4) involved the administration of spinach and corn extract.

Group five(G5) involved the administration of all plant extract.

,and they received 1ml of the aqueous extract orally (mg/kg) (27).

2.6 Collection of blood samples and organs

Five milliliters of blood were collected from the heart of the animals directly by cardiac puncture using a medical syringe and each blood samples were placed in EDTA tube that contain anticoagulant for complete blood count(CBC) tests including red blood cells (RBCs) and hemoglobin (Hb) (28) and kept in refrigerator in sloping way. After dosing period (1 month), the rats were dissected and liver was excised from the animals, and placed in formalin 10% solution for histological examination(30).

2.7 Estimation of superoxide dismutase (SOD) enzyme

Anion free radical superoxide (O^{2-}) can

be formed by reaction system of xanthin and xanthan oxidase; hydroxylamine can be oxidized as Nitrit; it becomes purple by developers' reaction. SOD can inhibit the free radical anion superoxide(O^{2-}) when samples that contain SOD are measured. SOD's inhibitory effect will decrease the nitrite formation, and the sample tube absorbance value is less than the tube control (29).

2.8 Statistical Analysis:

The program that used to detect the effect of difference factors in study parameters was Statistical Analysis System-SAS (2012) by using CRD complete random design with five replicates for each treatment. Least significant difference -LSD test (Analysis of Variation-ANOVA) was used to significant compare between means. Evaluation of Regression coefficient between variables (Standard curve) in this study(31).

3.Results and discussion:

3.1 Hematological parameters

3.1.1 The effect of ascorbic acid on Hemoglobin (Hb) and Red blood cells (RBC) level:

The result in table (1) showed that the highest level of ascorbic acid was 3.376 mg/ml for *S.oleracea*, while the lowest level was 0.579 mg/ml in *Z. mays*.

Table 1: The concentration of ascorbic acid in the plants of present study

Plants	Ascorbic acid con. (mg/ml)
Spinach (*Spinacia oleracea*)	3.376
Red chili pepper (*Capsicum annuum*)	0.645
Corn (*Zea mays*)	0.579

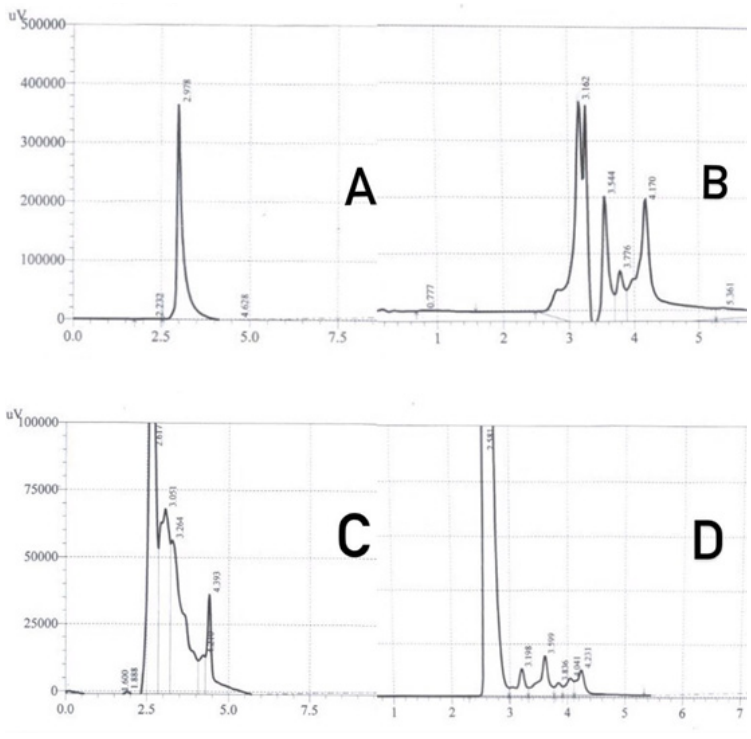

Figure1: Ascorbic acid estimation by HPLC (A; standard, B; *C.annuum* C; *S.oleracea*, D; *Z.mays*).

The result shown in (table 2) revealed that the administration of *C. annuum* and *S.oleracea* in group 3 raised the number of erythrocytes cells (6.90 10^12/L) and the concentration of hemoglobin (13.40g/dl), however group five that included the administration of all the plants extract showed declining in concentration of hemoglobin was (6.02 10^12/L) and red blood cells were(11.80g/dl).

Table2: The levels of red blood cells and hemoglobin in the groups of present study:

Groups	Mean ± SE	
	RBC (10□12/L)	Hb (g/dl)
G1(control)	6.06 ±0.19 b	12.76 ±0.53 ab
G2	6.32 ±0.17 b	12.26 ±0.29 ab
G3	6.90 ±0.09 a	13.40 ±0.31 a
G4	6.19 ±0.18 b	12.20 ±0.56 ab
G5	6.02 ±0.20 b	11.80 ±0.54 b
LSD value	0.547 *	1.526 *
Means having with the different letters in same column differed significantly * (P≤0.05).		

G1: Control (D.W), G2: Spinach, G3: Spinach + Red chili pepper, G4: Spinach + Corn, G5: Spinach + Zea mays + Red chili pepper.

The aquatic extract administration of *S. oleracea* in group two faintly increased RBC level and slightly decreased the Hb levels. In general the leaves of spinach increase the levels of RBC and Hb in the body owing to the phytochemical compound in it, such as flavonoids, saponins, terpens, and glycosides(32). It has been documented that saponins and alkaloids have anti-anemic potential(33). Since saponins are membrane active agents that lyse red blood cells or other cell walls, it is likely that this plant initially lysed red blood cells, by developing glycosidic enzymes that cleave some of the terminal sugars of the saponin, the cells overcome this inhibition and detoxify it(34). Thus, this detoxification of saponins strengthened the correct use of iron found in the *Spinacia oleracea* aqueous extract to synthesize heme / hemoglobin for fresh red blood cells. This contributes to enhanced RBC and Hb.

C. annuum increase the iron level in the blood due to it is high amount of ascorbic acid (35), and when the bioavailability of iron rises the red blood cells and hemoglobin increase (36). Other studies suggested that the capsaicin component in *C. annuum* can increase the number of micronucleate normochromic red blood cells in bone marrow cells and the peripheral blood and this due to dihydrocapsaicin compound in it (37,38).Some studies indicate that the capsaicin in *C. annuum* can effect on erythropoiesis and that diminish RBC count, and when this happens it will inhibit the heme biosynthesis that correlated erythropoiesis and make Hb concentration decrease (39).

In group four, the effect of *Z. mays* was slightly low. Because of the phytic content

in this plant that is known for it is ability to lower the ferritin levels, thus lowering Hb and RBC, and make the iron levels low(40). Group 5 that included the taken of all the plant extract mix together, it showed a significant decrease in RBC and Hb level. This decrease may be influenced by antagonistic effect of all the compounds in the chosen plants, specially the phytic component that effect directly on Hb levels (41).

3.2 Super Oxide Dismutase (SOD) enzyme

In (table3-3), group four was significantly high in SOD enzyme level 16.32 U/ml in comparing with the control group which received oral administration 1 ml of only D.W.

Table 3: The SOD level in the groups of present study

Groups	Means ± SE
	Level of SOD (U/ml)
G1(control)	5.10 ±1.77 b
G2	7.59 ±2.56 b
G3	12.66 ±3.84 ab
G4	16.32 ±10.32 a
G5	11.21 ±8.20 ab
LSD Value	8.179 *
Means having with the different letters in same column differed significantly * (P≤0.05).	

G1: Control (D.W), G2: Spinach, G3: Spinach + Red chili pepper, G4: Spinach + Corn, G4: Spinach + Zea mays + Red chili pepper.

Group two (G2) showed insignificant increased level of SOD. The water extraction of *S. oleracea* plant showed high antioxidant effect. (42) reported that spinach is a rich source of nutrient and carotenoids as well as polyphenols. Polyphenoles is now commonly recognized as physiological antioxidants with an essential potential to protect against the many degenerative diseases associated with free radical tissue harm(43).

The *C. annuum* administration in group (treated with spinach and red chili pepper) raised the level of SOD. Red chili pepper is rich with antioxidant, such as capsaicinoids, tocopherols (Vitamin E), and carotenoids(44). Tocopherols are among the most significant lipid-soluble antioxidants in food and human and animal tissues (45). Vitamin E is widely recognized as one of the most active antioxidants in existence. The biological function of vitamin E is not restricted to antioxidant properties(46). Thus the level of SOD increased.

The process of oxidation the unsaturated fatty acid is called lipid peroxidation that is responsible for long time injury to cell (47,48), the inhibition of this process is important to avoid free radicals that may cause several diseases (49). In group four (treated with spinach and corn), plant extract administration of *Z. Mays* inhibited lipid peroxidation products and enhanced

the function of antioxidant enzymes such as SOD, which are considered the first line of the antioxidant defense mechanism to minimize oxygen-free radicals (50). This beneficial effect is due to the phytochemical compounds, flavonoids, fatty acid, vitamins, fiber, phenolic compound and the fructose component in corn plants(51,52).

The taken of all the plant extract in group five made the level of SOD enzyme to some extent increase. This may be because of an antagonistic effect between the component in the plants. Findings of some studies showed that the reaction between phenols , sugars (glucose, fructose, etc.), and organic acid is antagonistic(53). Other studies showed that the interaction of flavonoids and ascorbic acid is also antagonistic(54).

3.3 Histological study of liver

3.3.1 Control group

In the control group, histology of the liver of the rats showed hexagonal lobules revealed in the central vein hepatocytes are arranged in cords around a central vein figure 3-2(A1+A2).

3.3.2 Group of *Spinacia oleracea*

In compare with sections of control group the sections of the liver in group two showed mild marked disarrangement of hepatic cords of the lobules and central vein . The magnified sections of the hepatic lobule have revealed mild micro and macro fatty degeneration within the cytoplasm of the hepatocyte and hyper cellularity of kupffer cells figure2(B).

3.3.3 Group of *Spinacia oleracea and C. annuum*

The sections of liver were showed mild in congestion sinusoidal and central vein figure 2 (C).

3.3.4 Group of *Spinacia oleracea* and *Z. maize*

The section of liver in group four was similar to the control group figure 2(D).

3.3.5 Group of All the plants extract

The sections of liver in group five was also similar to the control group figure 2 (E).

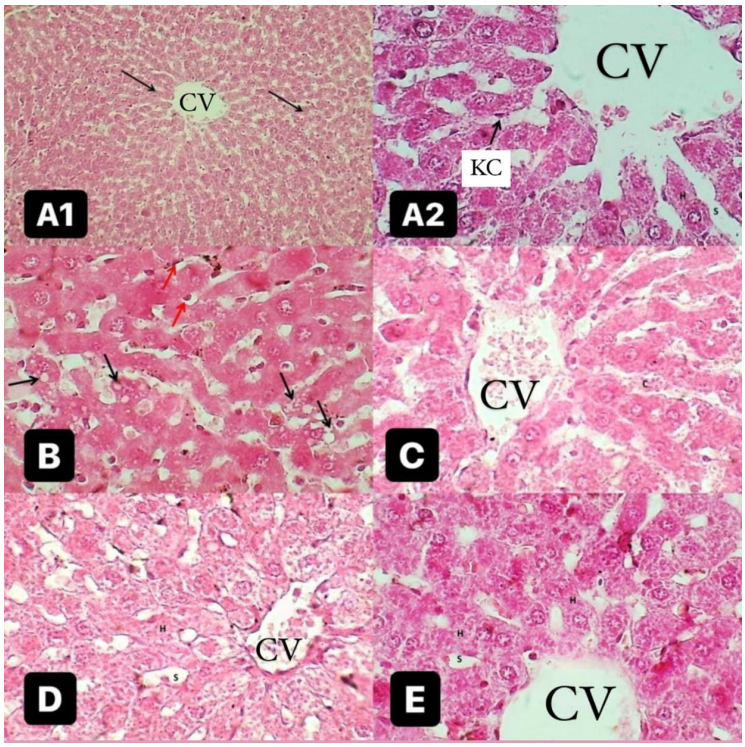

Figure 2:Section through rat liver lobule shows Central vein (CV), hepatocyte (H), sinusoid (S) & kupffer cells(KC). H&E stain.400x(A1+A2: control, B: S.oleracea, C: C.annuum+ S.oleracea, D: Z.maize+ S.oleracea, E: all plant extract).

Conclusion:

The oral administration of ascorbic acid proved to be an effective way to increase RBC and Hb and can lead to iron increasing in the blood. Also, *Z.maize* had positive effect on SOD enzyme levels. Further studies are warranted to examine if these in *vitro* effects translate into (patho)physiologically relevant changes in animal and human.

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