Ameliorative effect of aqueous black current extract or vitamin E against oxidative stress induced alterations in hematological parameters and iron level in male rats

Amer Hassan Abed Al- Khadim
-College of Nursing-Thi-Qar University– Department of Adult Nursing

Abstract:

This study **was designed to evaluate some hematological parameters (Red blood cell count (RBCs), hemoglobin** estimation (Hb), Packed cell volume (PCV), blood indices (Mean corpuscular volume, Mean corpuscular hemoglobin, Mean corpuscular hemoglobin concentration) (MCV, MCH, MCHC) and the level of iron in rats exposed to oxidative stress by 0.5% hydrogen peroxide (H_2O_2) and then treated by aqueous of black currant **(*Vitis vinferia.L)*** extract and comparing to the vitamin E.

Twenty-four adult male rats were divided randomly into four equal groups (six rat/ group) and treated as follows for 42 days. The Rats in first group (GI) were received normal water with oral intubation of sun flower oil (1ml /rat) and consider as control group. Animals of the second group (GП) were received 0.5% H_2O_2 in drinking water, while rats of the third group (GШ) were received 0.5% H_2O_2 in drinking water with oral intubation of vit. E (400 I.U/Kg/B.W/day) diluting in sun flower oil.The animals in fourth group (GIV) were intubated daily (60 mg/kg/B.W/day) of aqueous extract of black currant plus 0.5% H_2O_2 in drinking water. Fasting blood samples were collected at 0, 21; 42 days of experiments. Blood hematological parameter and serum Iron were estimated. The results of the present study demonstrated that administration of 0.5% of H_2O_2 in drinking water caused a significant decreased in RBCs count, Hb content, PCV, MCV and Iron concentration in serum while there was no change in MCH and MCHC in group (GIV) compared to control and other group. In addition, there are a significant elevation in (RBCs, Hb, PCV, MCV, and MCH) in group treated with aqueous extract of black currant plus 0.5% H_2O_2in drinking water compared to control and H_2O_2 group, with no change in concentration of iron in (G Ш and G IV) at end of experiment. It was concluded at the end of experiment that the aqueous extract of black currant exerts protective effect against oxidative stress and it's more active than Vit.E. It was recommended to

evaluation the hematological parameter in some chronic diseases have relationship to biomarker of oxidative stress.

Introduction

Anemia is a decrease in normal value of red blood cells (RBCs) or less than normal quality in the blood **(1, 2),** in world having over 20 million people with anemia by unhealthy diet and nutritional deficiency with protein and fat intake **(3)**. Many reports showed that anemia is caused by iron deficiency in blood stream **(4)**, but in the reality anemia characterized by deficiency in hemoglobin of RBCs diminishing the ability of their blood to transport oxygen to our cell and remove CO_2**(4).** However, anemia is the most common disorder of blood there are several kinds of anemia produced in variety of underlying causes, based on morphology of RBCs **(5).** Iron is an essential element in all living cells. Approximately 75% of total body iron is associated with hemoglobin, which is responsible for oxygen transport **(6)**. Iron deficiency in the body limits the synthesis of heme, a prosthetic group of hemoglobin that in turn limits the synthesis of hemoglobin and decreases the production of RBCs in the bone marrow resulting in anemia **(7).** Since cellular energy metabolism is dependent on oxygen, anemia has a wide range of clinical consequences. One of the consequences of severe iron deficiency is a decrease in life span of RBCs in circulation **(8).** More recently it has been demonstrated that iron deficiency accelerates the suicidal death of RBCs (eryptosis), **(9).**Recent studies showed the oxidative stress play a significant role in pathophysiology of anemia **(10).** Oxidative stress (OS) is the term referring to a shift towards the pro-oxidant in the prooxidant/ antioxidant balance that can occur as a result of an increase in oxidative metabolism **(11).** It results in massive cell damage inducing cellular mutations, tissue breakdown and immune compromise **(12).** Endogenous antioxidant defenses are inadequate to completely minimize ongoing oxidative damage to the DNA, lipids, proteins and other biological molecules. Hence diet-derived antioxidants may be particularly important in protecting against diseases resulting from cellular damages **(13).** The organism is well equipped with antioxidant defense systems with compounds like glutathione peroxidase **(14),** superoxide dismutase **(15),** catalase and glutathione reductase **(16, 17).** The non-enzymatic line of defense include substances such as reduced glutathione (GSH), vitamin A, C,

E, selenium and zinc, that can efficiently neutralize the damaging effects of free radicals and protect the body **(18).** Also Fruits and vegetables have important roles in the prevention of cellular oxidative damage **(19).** Today, health care professionals use standardized extract of grape seed (fresh and skin) to treat arrange of health problem related to free radical damage, including age related disease, DNA damage, cancer, blood sugar regulating problems and heart disease (**20)**.Grapes poanthocyanidin (PCOS) have been shown potent antioxidant effects that significantly inhibit lipid peroxidation of polyunsaturated fatty acid in animals and in vitro studies **(21, 22).** The aimed of this study to investigate the protective role of aqueous extract of black currant concentrate against oxidative stress through evaluation blood hematological parameters and serum Iron and comparing the prophylactic effect of the aqueous black current extract with that of vit E.

Material and Methods

Twenty-four male Albino rats were randomly divided into four groups (**6 rats/ group**) and treated daily for six weeks as follows:

1- **Group I:** The rats of this group were received 1ml of sun flower oil daily by oral intubations using gavages needle and served as control group.
2- **Group II:** The rats of this group were subjected to ***ad libitum*** supplemented with drinking water containing 0.5% H_2O_2.
3- **Group III:** The rats of this group were administrated daily 400 IU \ kg body weight of vitamin E diluting in sun flower oil using gavages needle **(23)** plus 0.5% H_2O_2 in drinking water.
4- **Group IV:** Animal of this group was intubated daily 60mg/kg body weight of crude aqueous extract of black currant plus 0.5% H_2O_2 in drinking water was prepared according to (**24**).

Collection of blood sampling: At the end of the experiment period (6 weeks), fasting blood samples were collected at 0, 21; 42 days of experiments.

Hematological parameters

Part of blood were kept into tube containing Ethylene Diamine Tetra Acetic Acid (EDTA), for hematological parameter which including (RBCs count, hemoglobin estimation, packed cell volume (PCV) and blood indices (MCV, MCH, MCHC). Red blood cell counts were estimated by light microscopy (**25**).Blood

hemoglobin (Hb) content was determined by cyanomethaemoglobin method (**26)**. Packed cell volume (PCV) was determined by the method of Dacie and Lewis (**25**).

Measurement of Iron concentration

While another part of blood samples were obtained without EDTA, held for not more than four hours before serum was collected by centrifugation (2500 rpm) for 15 minutes, a serum was separated, frozen at-20 until chemical analysis. The level of iron was estimated by atomic absorption (**27)**.

Statistical analysis:

Statistical analysis of data was performed on the basis of two way analysis of variance (ANOVA) using significant level of ($P<0.05$). Specific group Differences were determined using least significant differences LSD (**28**).

The result

The results of the present study showed non significantly statically differences **(P> 0.05)** between groups during the pretreated period, in all means value of **(RBC count, Hb, PCV, MCV, MCH, MCHC and levels of Iron in serum).** Tables (**1, 2, 3,4,5,6 and** 7) illustrated mean values of **(RBC, Hb, PCV, MCV, MCH, MCHC and Iron)** of control and three treated groups along the experiment period.

Table (**1**) showed a significant decrease **(P< 0.05)** in group treated with 0.05% H_2O_2 at 21 and 42 days of experiment compared to control, **GШ** and **IV** groups. with significant increment in **GIV** group at end of experiment compared to **GП** with means value (**5.93±0.21)** so with non significant change in group treated with Vit.E compared to pretreated period and control group .In the table (**2)** notice a significant decline **(P< 0.05)** in **Hb** concentration in **(GП)** at 21and 42 days of experiment compared with control group within means value (**9.25±0.37, 8.40±0.25)** respectively with a significant elevation in groups treated with Vit.E and a aqueous extract of black currant **Ш** and **IV** compared to **GП** with means value (**12 ±0.4, 12.3 ±0.4)** and **(13.38±0.30, 13.60±0.2) .** The result in table (**3**) show a significant decrease **(P< 0.05)** in **PCV** of **GП** with mean value (**20.00±0.77, 19 ± 1.1)** compared to control group, with a significant elevation in **GIV** at the end of experiment compared to H_2O_2 treated period.

The result in table (**4**) showed a significant decrease **(P< 0.05)** in mean value of **(MCV)** in **GП** at 42 day of experiment (**46.3 ±0.70)** compared to control group .with a significant decrease in group treated with Vit.E and 21days period in **IV**compared to **GI, GП** with mean value **(44.6 ± 0.13, 44.8 ±0.14)** respectively, also notice a significant elevation at 42 day of experiment in **GIV** compared to **GП and GШ** with mean value **(49.35±0.9).**

In table **(5)** statically differences were absent **(P> 0.05)** except slight a significant elevation in (**MCH**) at 42 day of experiment in **GIV** compared to other groups. Also clear a significant elevation in **GIV in (MCHC)** at 21 and 42 days of experiment compared to other groups with mean value(46.4±0.6, 48.6±0.5) this clarified in table**(6).**

The result in table **(7)** revealed a significant decrease **(P< 0.05)** in mean values of iron concentration at 21 & 42 days of experiment in **GП** compared to control, **GШ** and **GIV**. Also showed a significant decrease in **GШ** compared to control and **GIV** groups, with no change in mean value in **GIV** compared control group also no change with the time of experiment.

Discussion

The result of the present study showed that administration of 0.05% of H_2O_2 in dinking water caused significant decrease in **RBC count, Hb, PCV, MCV, and levels of serum Iron** compared to control and other treated group as compared to result represented by(**29**).

Recent studies demonstrated that oxidative phenomena plays a significant role in the pathophysiological of anemia because the roles of oxidant in shortness of RBC survival (**30,31**). Its more clear the increase in free radical generation in association with impaired of antioxidant defenses (**30,32**).The oxidative stress induce anemia by increase of lipid peroxidation of fatty acid in cell membrane of RBCs (**30,33,34,35**).The RBC lipid peroxidation can be effected by presence of various oxidant such as H_2O_2 ,the" ROS" formed on the membrane can both damage the red blood cell and then release " ROS" to the vasculature and neighboring tissue contributing to the pathology of anemia, through shortness red blood life-span **(36).** It has been reported the shortness life-span of RBC lead to suicidal death of RBC(eryptosis) this is initiated by an increase in cystolic calcium in RBC which stimulate phosolipids scrambling and increase exposure of phosphatidyle serine to outer surface

of the membrane is recognized by a macrophages and result in removal of RBC from circulation**(34,37**). Also it was noticed the increase of cystolic calcium lead to decrease in deformability with in increase in membrane stiffness of RBC can be attributed to oxidative stress (**31, 35**). It was reported that H_2O_2 generation by membrane bound Hb is not as efficiently scavenged because membrane cytoplasmic catalase not react with H_2O_2 at membrane surfaces also H_2O_2 from RBC has been shown to enter endothelial cell and caused inflammation(**36**). Previous studies found high RBC heme degradation correlate with increased IgG binding to RBC heme membrane, so increased IgG binding through to be triggered by destabilization of structure of Hb facilitating denaturation the denaturated Hb along with heme degradation product and free Iron can induce band 3 protein aggregation and membrane damage that triggers IgG binding and subsequent removal of RBC from circulation ((**38**, **39, 40**).

Also the results showed decrease the in level of Iron in **GП** and this occur because the ability of iron to gain and lose electron i.e. (Fe2+ ↔Fe3+) through Fenton reaction by interaction of iron, copper and H_2O_2 (**41**).This property makes iron and copper two common catalysts of oxidation reaction, so the release of iron can be detrimental to cellular membrane because of pro-oxidation effect it may be have (**42, 43**). Its certified that antioxidant supplementation prevented the reduction of serum iron and iron saturation index in endurance athletes indicating a link between iron metabolism and oxidative stress **(44**).While the result in **GШ** and **GIV** it is reflect the antioxidant properties of Vit.E and aqueous extract of black currant through protection the cell membrane of RBCs from oxidative stress , through ability of Vit.E to extinguish single oxygen species as well as to terminate free radical chain reactions (**45**).It may be act as an antioxidant either by donating hydrogen radical to remove the free lipid radical, reacting with it to form non radical products, or simply trapping the lipid radical (**46**).It is very clear the antioxidant properties of aqueous extract of black currant concentrate due to healthy compound content in different concentration in the skin of grape such as proanthocyanidins PCOS are also found in grape juice and wine ,PCOS in grape seed extract are as much as 50 times more potent than those in vitamin E and up to 20 times more potent than PCos in Vit C **(47).** Beside the black currant contain variety of different photochemical possessing antioxidant and free

radical scavenging called flavonoids .including Quercetin, Myricetin, and Kaempherol **(46, 48, and 49). Sen *et.al*(2005)** It was reported the quercetin in black currant juice has successful inhibiting the oxidation of protein and lipid on the red blood cell membrane in infected animal **(50**).Similar findings were recorded ameliorative effects of black seed for the hematological disturbance could be due to the lowered lipid peroxidation level in cell membranes leading to decreased susceptibility of RBCs to hemolysis **(51).** Another study suggested the aged garlic extract has potential effect as antioxidants in sickle RBC cause significantly improve erythrocytes deformability through stabilization of erythrocyte membrane (**52**).

Table (1) Effect of aqueous black current extract (Vitis Vinfera. L) or vitamin E on mean of Red blood cell count (× 10^6 cell/ml)in H_2O_2 treated rats.

Group / Time days	Group I	GroupП	GroupШ	GroupIV
Pre-treated group	5.20±0.3 Aa	5.25±0.3 Aa	5.26±0.3 Aa	5.23±0.3 Aa
21 days	5.28±0. 3 Aa	4.25±0.1 Bb	5.66±0.2 Aa	5.81±0.3 Aa
42days	5.2±0.3 Aa	4.1±0.1 Bb	5.80±0.3 Aa	5.93±0.21 Ab

Values are Means ± S.E for 6 rats

GI= Control group

GП= H_2O_2 treated group

GШ= Vitamin E +H_2O_2 treated group

GIV=Black currant +H_2O_2 treated group

Capital letters denote between groups differences, $p<0.05$ vs. control.

Small letters denote within group differences, $p<0.05$ vs. pretreated period.

Table (2) Effects of aqueous black current extract (Vitis Vinfera. L) or vitamin E on haemoglobin (Hb) content (g/dl) in H_2O_2 treated rats.

Values are Means ± S.E for 6 rats

GI= Control group

GП= H_2O_2 treated group

GШ= Vitamin E +H_2O_2 treated group

GIV=Black currant +H_2O_2 treated group

Capital letters denote between groups differences, $p<0.05$ vs. control.

Small letters denote within group differences, $p<0.05$ vs. pretreated period.

Group / Time days	Group I	GroupП	GroupШ	GroupIV
Pre-treated group	11.33±0.60 Aa	11.26±0.60 Aa	11.16±0.60 Aa	11.16±0.60 Aa
21 days	11.30±0.60 Aa	9.25±0.37 Bb	12 ±0.4 Cb	13.38±0.30 Cb
42days	11.25±0.60 Aa	8.40±0.25 Bc	12.3 ±0.4 Cb	13.60±0.2 Db

Group / Time days	Group I	GroupП	GroupШ	GroupIV
Pre-treated group	25.83±0.87 Aa	25.83±0.87 Aa	25.83±0.87 Aa	25.83±0.87 Aa
21 days	25.16±0.80 Aa	20.00±0.77 Bb	25 ±0.98 Aa	26 ±0.5 Aa

Table (3) Effects of aqueous black current extract (Vitis Vinfera. L) or vitamin E On packed cell volume (PCV %) in H_2O_2 treated rats.
Values are Means ± S.E for 6 rats

GI= Control group

42days	25.80±0.80 Aa	19 ± 1.1 Bb	26 ±0.5 Aa	29.1 ± 0.4 Cb

Group / Time days	Group I	GroupП	GroupШ	GroupIV

GП= H_2O_2 treated group

GШ= Vitamin E +H_2O_2 treated group

GIV=Black currant +H_2O_2 treated group

Capital letters denote between groups differences, p<0.05 vs. control.

Small letters denote within group differences, p<0.05 vs. pretreated period.

Table (4) Effects of aqueous black current extract (Vitis Vinfera. L) or vitamin E On mean corpuscular volume (MCV) by (femtoliters) in H_2O_2 treated rats.

Pre-treated group	48.40± 0.10 Aa	48.40±0.10 Aa	48.50± 0.10 Aa	48.40± 0.10 Aa
21 days	48.50± 0.10 Aa	47.6±0.75 Aa	44.6 ± 0.13 Bb	44.8 ±0.14 Bb
Group / Time days	Group I	GroupП	GroupШ	GroupIV

Pre-treated group	**21.70±0.12 A a**	**21.50±0.12 Aa**	**21.50±0.12 Aa**	**21.70±0.12 Aa**
21 days	**21.70±0.12 Aa**	**21.9 ±0.13 Aa**	**21. 4±0.10 Aa**	**22.4±2.36 A a**
42days	**21.50±0.12 Aa**	**20.4 ±0.32 Ab**	**21.2±0.10 Aa**	**23 ±0.5 Bb**

Values are Means ± S.E for 6 rats

GI= Control group

GП= H_2O_2 treated group

GШ= Vitamin E +H_2O_2 treated group

GIV=Black currant +H_2O_2 treated group

Capital letters denote between groups differences, p<0.05 vs. control.

Small letters denote within group differences, p<0.05 vs. pretreated period.

Group / Time days	Group I	GroupП	GroupШ	GroupIV

Table (5) Effects of aqueous black current extract (Vitis Vinfera. L) or vitamin E on mean corpuscular hemoglobin (MCH) by (picogram) in H_2O_2 treated rats.

Values are Means ± S.E for 6 rats

GI= Control group

GП= H_2O_2 treated group

GШ= Vitamin E +H_2O_2 treated group

GIV=Black currant +H_2O_2 treated group

Capital letters denote between groups differences, p<0.05 vs. control.

Small letters denote within group differences, p<0.05 vs. pretreated period.

Table (6) Effects of aqueous black current extract (Vitis Vinfera. L) or vitamin E On mean corpuscular hemoglobin concentration (MCHC) by (%) in H_2O_2 treated rats.

Pre-treated group	44.8 ±0.11 Aa	44.4 ±0.11 Aa	44.33±0.11 Aa	44.33±0.11 Aa
21 days	44.8 ±0.11 Aa	45.70±0.13 Aa	45.7±0.11 Aa	48.4±0.6 Bb
42days	44.2 ±0.11 Aa	44.0 ±0.11 Aa	44.66±0.11 Aa	46.6±0.5 Bc

Group / Time days	Group I	GroupП	GroupШ	GroupIV
Pretreated period	10.13±0.03 Aa	10.35±0.07 Aa	10.23±0.08 Aa	10.23±0.08 Aa
21 days	10.23±0.07 Aa	7.25±0.28 Bb	7.95±0.16 Bb	9.95±0.19 Aa
42days	10.38±0.12 Aa	6.65±0.23 Bc	7.81±0.12 Cb	10.40±0.23 Aa

Values are Means ± S.E for 6 rats

GI= Control group

GП= H_2O_2 treated group

GШ= Vitamin E +H_2O_2 treated group

GIV=Black currant +H_2O_2 treated group

Capital letters denote between groups differences, p<0.05 vs. control.

Small letters denote within group differences, p<0.05 vs. pretreated period.

Table (7) Effects of aqueous black current extract (Vitis Vinfera. L) or vitamin E On iron (PPM) concentration in serum in H_2O_2 treated rats.

Values are Means ± S.E for 6 rats

GI= Control group

GП= H_2O_2 treated group

GШ= Vitamin E +H_2O_2 treated group

GIV=Black currant +H_2O_2 treated group

Capital letters denote between groups differences, p<0.05 vs. control.

Small letters denote within group differences, p<0.05 vs. pretreated period.

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التأثير المخفف للمستخلص المائى للزبيب الاسود او فيتامين هـ ضد الاجهاد التأكسدي المؤدي الى اختلالات فى المعايير الدميه ومستوى الحديد فى ذكور الجرذان

عامر حسن عبد الكاظم الزبيدي
كلية التمريض- جامعة ذي قار- قسم تمريض البالغين

الخلاصة: تهدف الدراسة الحالية الى تقييم بعض المعايير الدمية(تعداد خلايا الدم الحمر, تقدير مستوى خضاب الدم, حجم الخلايا المرصوصة, دليل الحالة الدموية " معدل حجم الكرية, معدل هيموكلوبين الكرية, معدل تركيز هيموكلوبين الكرية") ومستوى 0% مع ماء الشرب والمعاملة بالمستخلص المائي للزبيب .تركيز الحديد في الجرذان المعرضة للاجهاد التاكسدي والمعاملة بـ 5 الأسود ومقارنة ذلك مع فيتامين هـ. تم استخدام 24من ذكور الجرذان البالغة و قسمت عشوائياً إلى أربعة مجاميع متساوية) الماء العادي مع Gأعطيت الجرذان في المجموعة الأولى (I (ستة حيوانات/مجموعة) وتمت معاملتها كالتالي لمدة 42يوم, وعدت كمجموعة سيطرة في حين أعطيت حيوانات المجموعة الثانية ( )التجريع الفموي لزيت زهرة الشمس (1مل /حيوان 0%) .) فقد أعطيت (5ПG0%) أما المجموعة الثالثة (.) الماء الاعتيادي مضافا إليه بيروكسيد الهيدروجين بتركيز (5ПG من بيرو كسيد الهيدروجين بماء الشرب بالإضافة إلى التجريع الفموي لفيتامين هـ (400وحدة دولية/كغم) يومياً مذاباً في زيت بالمستخلص المائي للزبيب الأسود بجرعة 60ملغم/كغم من وزن GIV) (زهرة الشمس في حين جرعت المجموعة الرابعة و21 و0الجسم إضافة إلى الماء الحاوي على بيرو كسيد الهيدروجين بنفس التراكيز أعلاه. تم جمع عينات الدم في الأيام من التجربة, وتم أخذ جزء من عينة الدم وحفضها في انابيب حاوية على مواد مانعة للتخثر الدم (حامض الاثلين ثنائي 42 الامين رباعي الخليك) لغرض اجراء المعايير الدمية ومصل الدم لقياس مستوى الحديد.

وقد اظهرت تنائج الدراسة الحالية الى ان المجموعة المعاملة ببروكسيد الهيدروجين اعطت انخفاضاً معنوياً عالياً في(تعداد خلايا الدم الحمر, تقدير مستوى خضاب الدم, حجم الخلايا المرصوصة, ومعدل حجم الكرية) مع عدم حدوث تغير في معدل هيموكلوبين الكرية, ومعدل تركيز هيموكلوبين الكرية في المجموعة الرابعة مقارنة بمجموعة السيطرة والمجاميع الاخرى, بالاضافة الى حصول انخفاضاً معنوياً في مستوى الحديد في مصل الدم. كذلك لوحظ حصول ارتفاع معنوي عالي في (تعداد خلايا الدم الحمر, تقدير مستوى خضاب الدم, حجم الخلايا المرصوصة, معدل حجم الكرية, ومعدل هيموكلوبين الكرية) في المجموعة المعاملة بالمستخلص المائي للزبيب الاسود وبيروكسيد الهيدروجين مقارنة بمجموعة السيطرة والمجموعة المعاملة بالبيروكسيد, مع عدم حصول اي تغيير في تركيز الحديد في المجموعة الثالثة والرابعة في نهاية التجربة.

نستنتج من ذلك وضوح الدور الوقائي لمركز الزبيب الاسود ضد الاجهاد التأكسدي المستحدث وكان اكثر فعالية مقارنة بفيتامين هـ . ولذلك توصي الدراسة بضرورة تقييم المعايير الدمية في الأشخاص المصابين ببعض الأمراض المزمنة وعلاقتها بمستويات الأجهاد التاكسدي .