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Proactive role of Adiantum aqueous extract and some supplements in lipid profile and atherogenic indices in anemic rats Induced by phenylhydrazine

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ABSTRACT:

Anemia is a common medical condition that affects millions of people worldwide, and treatment with coriander extract and some dietary supplements is of great importance in various fields. The current study aimed to investigate the negative effect of phenylhydrazine on lipid levels and to demonstrate the role of coriander extract, vitamin C, and Hemafer in the complications of anemia. The experiment was conducted in the animal house at the College of Veterinary Medicine, Tikrit University, where male mice were divided into five groups, each containing six randomly distributed mice. One group served as the control group, while the second group was treated with phenylhydrazine (PHZ) at a dose of 40 mg per kg by subcutaneous injection. As for the third group, PHZ was treated with Hemafer (1 ml per kg per day), while the fourth group was treated with PHZ and vitamin C (250 mg per kg per day). As for the fourth group, PHZ was treated with Adiantum aqueous extract at a concentration of (1 ml per animal at a concentration of 25%). The lipid profile (total cholesterol, triglycerides, low-density lipoprotein, very low-density lipoprotein, high-density lipoprotein, and atherosclerosis indicators) was measured. The results of the current study showed that treating male rats with phenylhydrazine (PHZ) resulted in a significant increase ($P<0.05$) in the levels of cholesterol, triglycerides, low-density lipoprotein (LDL), and very low-density lipoprotein (VLDL), with no significant differences in high-density lipoprotein (HDL). The results of the current study indicated a significant increase ($P<0.05$) in the levels of AIP1, AIP2, and AIP3 in the group treated with phenylhydrazine compared to the normal control group. It also indicated a significant decrease ($P<0.05$) in the levels of cholesterol, triglycerides, LDL, and VLDL, with no significant differences in HDL levels in the groups treated with PHZ+Hemafer, PHZ+C, and the aqueous extract of coriander. It also indicated a significant decrease ($P<0.05$) in the levels of AIP1, AIP2, and AIP3 in the group treated with Hemafer, the group treated with Vitamin C, and the group treated with the Adiantum aqueous extract (AAE)+PHZ. We conclude from the above that treating male rats with the aqueous extract of coriander and the Hemafer drug reduces the side effects of anemia.

Keyword: Atherosclerosis: Oxidative stress: Free radicals: Vitamin C: Hemafer drug:Red blood cell.

الدور الوقائي للمستخلص المائي لنبات الكزبرة وبعض المكملات الغذائية على مرتسم الدهون ومؤشرات تصلب الشرايين في الجرذان البيض المصابة بفقر الدم المستحث بالفينيل هيدرازين

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مستخلص:

فقر الدم هو حالة طبية شائعة تؤثر على ملايين الأشخاص حول العالم ، وان المعالجة بمستخلص الكزبرة وبعض المكملات الغذاء لها أهمية كبيرة في مجالات متعددة. هدفت الدراسة الحالية إلى معرفة التأثير السلبي للفينيل هيدرازين على مستوى الدهون وكذلك إلى إظهار دور مستخلص الكزبرة وفيتامين C والهيمافير في مضاعفات فقر الدم. أجريت التجربة في بيت الحيوان في كلية الطب البيطري / جامعة تكريت حيث قسمت ذكور الفئران إلى خمس مجموعات كل مجموعة تحتوي على 6 فئران موزعة عشوائيا حيث كانت مجموعة هي مجموعة السيطرة اما المجموعة الثانية فعوملت بالفينيل هيدارزين PHZ (40 ملغم لكل كليو) بالحقن الجلدي اما المجموعة الثالثة فعوملت PHZ مع عقار الهيمافير(1 مل لكل كيلو يوما) اما المجموعة الرابعة فعوملت PHZ مع فيتامين c(250 ملغم لكل كيلو يوما) اما المجموعة الرابعة فعوملت PHZ مع بالمستخلص المائي للكزبرة Adiantum aqueous بتركيز (1 مل لكل حيوان بتركيز 25٪).تم قياس مستوى مرتسم الدهون (الكولسترول الكلي و الدهون الثلاثية و البروتين الدهني واطئ الكثافة والبروتين الدهني واطئ الكثافة جدا والبروتين الدهني عالي الكثافة و مؤشرات تصلب الشرايين). أظهرت نتائج الدراسة الحالية أن معاملة ذكور الفئران بالفينيل هيدرازين (PHZ) أظهرت زيادة معنوية ($P<0.05$) في مستوى الكوليسترول والدهون الثلاثية والبروتين الدهني منخفض الكثافة (LDL) والبروتين الدهني منخفض الكثافة جدا (VLDL) وعدم وجود فروق معنوية في البروتين الدهني عالي الكثافة (HDL). كما أشارت نتائج الدراسة الحالية إلى ارتفاع معنوي ($P<0.05$) في مستوى AIP1 و AIP2 و AIP3 في المجموعة المعالجة بالفينيل هيدرازين عند مقارنتها بمجموعة التحكم الطبيعية، كما أشارت إلى وجود انخفاض معنوي ($P<0.05$) في مستوى الكوليسترول والدهون الثلاثية و LDL و VLDL وعدم وجود فروق معنوية في مستوى HDL في المجموعة المعالجة بـ PHZ+Hemafer والمجموعة المعالجة بفيتامين C +PHZ والمجموعة المعالجة بالمستخلص المائي للكزبرة، كما أشارت إلى وجود انخفاض معنوي ($P<0.05$) في مستوى AIP1 و AIP2 و AIP3 في المجموعة المعالجة بـ Hemafer والمجموعة المعالجة بفيتامين C والمجموعة المعالجة بالمستخلص المائي للكزبرة PHZ+(AAE) Adiantum aqueous extract. نستنتج مما سبق ان معاملة ذكور الجرذان بالمستخلص المائي للكزبرة وعقار الهيمافير يؤدي الى تقليل التاثيرات الجانبية لفقر الدم

الكلمات المفتاحية: تصلب الشرايين: الإجهاد التأكسدي: الجذور الحرة: فيتامين سي: عقار هيمافير: كريات الدم الحمراء.

1. Introduction

Anemia is defined as a decrease in hemoglobin, hematocrit, It is indicative of an underlying condition and can be categorized as macrocytic, microcytic, or normocytic anemia. illness. Anemia infective frequently exhibit nebulous symptoms such weakness, weariness, and lethargy. Shortness of breath, syncope, and a reduced ability to exercise can all be signs of severe anemia. This exercise explains how anemia is diagnosed and treated, as well as how the multidisciplinary team manages patients with this illness (1). "Anemia of chronic diseases" (AHZ) refers to anemia that arises in tumors, non-infectious inflammatory disorders, and infectious and inflammatory processes. The shift of iron in the macrophage system's cells, which is triggered During inflammatory processes, including infectious and non-infectious or different malignancies, is one of the primary causes causing anemia in these situations. AHZ is the second most common condition after iron deficiency anemia (IDA). Although the nature and treatment approaches of these anemias differ, it is crucial to isolate AHZ into a distinct pathogenic variety and raise awareness of it because it resembles IDA and certain anemias. (2). The study and use of local medicinal plants with anemia-causing properties is therefore necessary to help manage anemia in both rural and urban areas, particularly in Nigeria, where a high prevalence of anemia among children and pregnant women is caused by insurgency and human displacement. This is because anemia is difficult to manage and control in most parts of Africa due to poverty, ignorance, and a lack of access to health care (3).Interest in a variety of processes catalyzed, mediated, or accelerated by phenylhydrazine has increased over the past ten years due to its numerous academic and industrial applications. (4). A chemical molecule with numerous applications in the chemical, medicinal, and agricultural sectors is phenylhydrazine (PHZ). It has harmful effects, particularly on red blood cells, due to its potent oxidizing properties (5). Most often, phenylhydrazine is used in experimental models. that investigate the hematological effects of new drugs and the mechanism of hemolytic anemia. (6). The vast majority of people treat

their anemia with herbal remedies and nutritional supplements. (7). Hemafer, also known as Iron(III)-hydroxide polymaltose complex, is a type of oral iron preparation that is used to make up for iron deficiencies in the body. (8). The best supplement to improve iron absorption is vitamin C, sometimes referred to as ascorbic acid. Ascorbic acid's boosting qualities were originally shown by Moore and Dubach in 1951. They noted that the upper gastrointestinal lumen had boosting qualities that were both dose-dependent and ascorbic acid-dependent. In the stomach fluid, ascorbic acid increases the absorption of both naturally occurring dietary iron and iron-fortified meals by acting as a non-heme co-ligand. Only when taken with food does it work. When 500 mg of vitamin c was consumed with a test meal, it was absorbed six times, as opposed to less when the same quantity was taken four and eight hours before to the meal. (9). The utilization of medicinal plants and their active ingredients as natural sources of antioxidants has been studied by researchers. Antioxidants are substances that efficiently stop oxygen and nitrogen free radicals(O-N F R) from reacting with biomolecules in a number of ways, minimizing damage or averting cardiovascular disease and cell death. (10). The Pteridaceae family of plants includes the plant Adiantum, which has numerous use in traditional and folk medicine. This plant can be found growing all over the world. References to Persian medicine state that it has numerous beneficial effects for the respiratory system (including shortness of breath), eyes, spleen, genitourinary system, digestive system, and edema.. In several regions of the world, it is used in traditional medicine to treat bronchitis, diabetes, headaches, fever, bronchitis, colds, coughs, abdominal pain, and menstrual issues. Current studies have shown that this plant has anti-inflammatory, antibacterial, and antioxidant properties. (11). Recent findings have demonstrated that erythrocyte macrophages have a distinct anti-inflammatory pattern and regulate wound healing, atherosclerosis, and host defense in a context and disease-dependent manner. (12).

2- Materials and methods:

2-1 Design and experimental **animals:**

Six groups of six male mice were randomly selected from the experimental animals, taking into account the uniformity of weights and ages, as follows: The first group (G1) was given normal feed and drinking water, while the second group (G2) was treated with phenylhydrazine (PHZ) at a concentration of 40 mg/kg, according to (Anbara et al., 2018). The third group (G3) was treated with (PHZ) and Hemafer at a rate of 1 ml per kg per day, according to (13). While the fourth group (G4) was treated with (PHZ) and vitamin C at a rate of 250 mg/kg, according to (Abel et al., 1983). The fifth group (G5) was treated with PHZ and Adiantum aqueous extract (AAE) at a concentration of 25% daily according (14).

2-2 Induction of anemia:

For two days in a row, an intraperitoneal injection of 40 mg/kg of phenylhydrazine caused hemolytic anemia. When hemoglobin and red blood cells dropped by about 30%, anemia was officially diagnosed. (15).

2-3 Blood Sample Collection Biochemical Tests:

After euthanasia, final blood was collected directly from the heart. Serum was obtained by centrifuging blood samples at 5000 rpm for 15 min and stored at -70°C for further analysis. Serum levels of total cholesterol (TC), triglycerides (TG), and high-density lipoprotein cholesterol (HDL-c) were measured using enzymatic colorimetric methods (Pars Azmoon Commercial Kits, Tehran, Iran). LDL-c and VLDL-c were calculated according to (16):

LDL-cholesterol = total cholesterol – triacylglycerol/5 – HDL-cholesterol

VLDL-cholesterol = triacylglycerol/5 [mg/dl]

while atherogenic indices AP1 and APII APIII were calculated according to (17).

$$AIP\ I = \frac{TC}{HDL - C\ Con.}$$

$$AIP\ II = \frac{vLDL - C\ Con. + LDL - C\ Con.}{HDL - C\ Con.}$$

$$AIP\ III = \frac{LDL - C\ Con.}{HDL - C\ Con.}$$

2-4 Statistical analysis:

The SPSS statistical software was used to statistically examine the find-

ings. The ANOVA statistical program was used to statistically evaluate the data, and Duncan Multiple Range(D-MR) is use to compare the arithmetic means, as reported by (18).

3- Results:

When compared to the normal control group, the current study's results showed that the phenylhydrazine-treated group had significantly high levels of total cholesterol, triglycerides, (LDL), and (VLDL), but no discernible changes in high-density lipoprotein (HDL). It also indicated that there was a significant decrease in the levels of Total cholesterol and triglycerides and LDL and VLDL and no significant differences in the level of HDL in the group treated with Hemafer, the groups treated with Vitamin C and the group treated with the Adiantum aqueous extract and phenylhydrazine As in **Table(1).**

Table (1) show a level of lipid profile in rats in the study groups.

Parameterts / Groups	Cholesterol (mg/dl)	Triglycerides (mg/dl)	HDL-C (mg/dl)	LDL-C (mg/dl)	VLDL-c (mg/dl)
Control (n=6)	129.477 ± 1.795 B	154.841 ± 3.62 b	37.766 ± 0.370 a	61.760 ± 1.104 b	29.927 ± 0.651 b
PHZ (n=6)	150.550 ± 1.112 A	179.613 ± 1.497 a	34.696 ± 0.230 a	79.783 ± 1.372 a	35.923 ± 0.299 a
PHZ + hemafer (n=6)	123.207 ± 6.342 B	155.057 ± 2.751 b	35.211 ± 0.917 a	58.060 ± 1.615 b	31.011 ± 0.550 b
PHZ+Vc (n=6)	118.927 ± 0.757 B	143.752 ± 2.560 c	35.879 ± 0.475 a	53.183 ± 1.922 c	30.438 ± 0.631 b
PHZ+AEA (n=6)	118.753 ± 3.845 B	150.677 ± 0.988 b	36.426 ± 0.103 a	50.323 ± 0.898 c	30.135 ± 0.198 b

The results of the current study also indicated a significant increase in the level of AIP1, AIP2, and AIP3 in the group treated with phenylhydrazine when compared to the normal control group. It also indicated a significant decrease in the level of AIP1, AIP2, and AIP3 in the group treated with Hemafer, the group treated with Vitamin C, and the group treated with the Adiantum aqueous extract and phenylhydrazine as shown in **Table 2.**

Table 2 show the values of the atherogenic indices AIP1, AIP2, AIP3.

Parameterts / Groups	AIP I	AIP II	AIP III
Control (n=6)	4.114 ± 0.309 b	2.427 ± 0.094 b	1.634 ± 0.051 b
PHZ (n=6)	5.177 ± 0.117 a	3.335 ± 0.101 a	2.210 ± 0.123 a
PHZ+hemafer (n=6)	4.425 ± 0.382 b	2.552 ± 0.101 b	1.667 ± 0.283 b
PHZ+Vc (n=6)	4.016 ± 0.175 b	2.043 ± 0.514 b	1.482 ± 0.154 b
PHZ+AEA (n=6)	4.138 ± 0.128 b	2.210 ± 0.117 b	1.382 ± 0.093 b

5- Discussion:

Research on toxic anemia produced by PHZ has provided basic insights into the pathogenesis, consequences of anemia on other physiological processes, and related illnesses. (19). Serum cholesterol levels are positively correlated in anemic patients. (20). Furthermore, free radical damage caused by iron suggests that anemic patients may be at increased risk for heart disease (21). Previous studies have reported that hyperlipidemia , hypercholesterolemia , triglycerides , LDL-C and VLDL-C cause an increase in neutrophil count, which is positively correlated with atherosclerotic plaque burden, and thus, an increase in neutrophil count promotes hypercholesterolemia and atherosclerosis in anemic rats .as table 1 (22). In addition, the results of the current study showed that there was an increase in the index of atherogenesis (AIP1, AIP2, AIP3) in the group of rats with induced anemia by phenylhydrazine as table 2 .The results of the case study were consistent with what was stated in (23). However, iron is a form of reactive oxygen species that attack cellular lipids, proteins and nucleic acids. Therefore, iron balance is carefully regulated to avoid the harmful effects of iron deficiency and iron overload (24). Previous studies have shown that iron deficiency and dyslipidemia are very common in patients with anemia. The use of Hemafer stimulates the me-

tabolism and activates fat-producing enzymes (25). Previous studies have indicated that iron deficiency and the need for blood testing for cadmium have been reported (26).This is what the results of our current study demonstrated, as a decrease in the lipid profile and atherogenic indicators was found, as shown in Table 1,2. It has been suggested that vitamin C is the primary component in lipid modulation and regulates the breakdown of cholesterol into bile acids, and High dosages of vitamin C did, in fact, improve blood glucose management and lower serum levels of TG and LDL in patients with type 2 diabetes, according to a clinical investigation (27). In fact, by converting cholesterol to bile acids and increasing hepatocytes' LDL receptors, cholesterol absorption from the bloodstream may be increased and result in a decrease in Levels of LDL in the blood (28). Another trial by Paolisso et al. found that giving 500 mg of vitamin C to patients with type 2 diabetes significantly decreased their plasma levels of insulin, TG, total cholesterol (TC), and LDL. On the other hand, (29) According to earlier research, using vitamin C supplements for four weeks lowers the rise in the atherosclerosis index, TC, TG, LDL-C, and VLDL-C (30). Without a doubt, the active ingredients in hawthorn berry inhibit cholesterol and HMG-CoA reductase in concert. Absorption may show notable hypolipidemic advantages, confirming C. aronia's hypocholesterolemic action in rats fed a high-cholesterol diet (31). For instance, saponins may increase bile acid excretion by blocking the absorption of bound bile acids and lipids (cholesterol). Additionally, saponins may increase LDL receptors, inhibit the synthesis of fatty acids, and activate lecithin-cholesterol acetyl transferase (LCAT) and lipase acetyl-CoA carboxylase (32).

6- Conclusions:

Anemia can be accompanied by other diseases, including high cholesterol and atherosclerosis. Therefore, patients with anemia should have lipid profile tests. In addition, Adiantum aqueous extract can be used to avoid high cholesterol because it contains active ingredients. In addition, vitamin C reduces oxidative stress and thus reduces the incidence of atherosclerosis.

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