

Amelioration of Lipid Peroxidation and Antioxidant Enzymes Status in the Serum and Erythrocytes of Phenylhydrazine-Induced Anemic Male Rats: The Protective Role of Artichoke Extract (*Cynara scolymus* L.)

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Abstract

BACKGROUND: Hemolytic anemia is a disorder characterized by the premature erythrocytes destruction. Phenylhydrazine (PHZ) induces oxidative stress and reactive oxygen species (ROS) formation, which causes hemolytic anemia. *Cynara scolymus* due to its antioxidant compounds, has been used for various therapeutic purposes in traditional medicine.

OBJECTIVES: The present study was designed to evaluate the effects of *Cynara scolymus* extract on PHZ-induced anemia in male rats.

METHODS: Hemolytic anemia was induced by intraperitoneal injection of PHZ (40 mg/kg) for 2 days. Thirty male Wistar rats were divided into five groups. Group 1 (normal control). Group 2 (anemic control) received only PHZ. The groups 3 to 5 were injected with 100, 200, 400 mg/kg of the *Cynara scolymus* by gavage, respectively, daily from day 2 to day 15 after PHZ administration. At the end of the treatment period, blood samples were collected to assess hematological parameters, malondialdehyde (MDA) level and antioxidant enzymes activity, including superoxide dismutase (SOD) and total antioxidant capacity (TAC) in the serum and erythrocytes.

RESULTS: In anemic rats, serum and erythrocytes MDA level increased, but SOD and TAC activity decreased significantly when compared with control group ($P \leq 0.05$). These changes were ameliorated by treatment with *Cynara scolymus* at different doses ($P \leq 0.05$). Also, improvement in several hematological parameters was observed in anemic rats after administration of *Cynara scolymus* ($P \leq 0.05$).

CONCLUSIONS: *Cynara scolymus* extract exhibits protective property against PHZ-induced oxidative stress presumably due to antioxidative activity.

KEYWORDS: *Cynara scolymus*, Hemolytic Anemia, Oxidative Stress, Phenylhydrazine, Rat

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Introduction

Anemia is defined as a condition in which the number of red blood cells (RBCs) or their oxygen-carrying capacity is inadequate to meet the physiological needs of the body (Prasad *et al.*, 2018). There are several kinds of anemia classified by a variety of underlying causes. Hemolytic anemia is the most frequent form of anemia (Lee *et al.*, 2012). Hemolytic anemia is categorized as acquired or hereditary. Common acquired causes of hemolytic anemia are autoimmunity, microangiopathy, infection and drug (Dhaliwai *et al.*, 2004; Paul *et al.*, 2018).

Phenylhydrazine (PHZ) is a strong oxidant agent which has toxicity on various tissues at various levels. It is known to shorten life-span of erythrocytes resulting in hemolytic anemia, increased erythropoietic activity, enhanced iron absorption and tissue iron overload (Luangaram *et al.*, 2007). PHZ has been used experimentally for the induction of hemolytic anemia in animal models. The auto-oxidation of PHZ leads to generation of ROS and PHZ-derived radicals, which causes a wide variety of deleterious cellular response including hemolytic anemia (Sung *et al.*, 2013). The observed haematotoxicity is a result of the reaction of PHZ with oxygenated hemoglobin to form oxygen radicals and methemoglobin (Shetlar and Hill, 1985).

Medicinal plants and natural products have been used as traditional treatments for numerous diseases particularly in developing countries because of several reasons including therapeutic effects, affordability, accessibility and fewer side effects (Asase *et al.*, 2008). Several studies have demonstrated that natural medicinal plants with potent antioxidant activity and their potential protective effects can alleviate the damage of oxidative stress-associated diseases through

inhibition of ROS generation and improvement of antioxidant defense mechanisms (Forni *et al.*, 2019; Banerjee *et al.*, 2018).

Cynara scolymus L. (Artichoke), a member of Asteraceae family, is an ancient herbaceous perennial plant, originating from the Mediterranean area, which today is widely cultivated all over the world because of its nutritional benefits and medicinal purposes (Salekzamani *et al.*, 2019). Phytochemicals analysis of *Cynara scolymus* has been found to contain powerful polyphenolic compounds which have therapeutic options including remarkable antioxidant activity against ROS and preventing the formation of free radicals (El-Boshy *et al.*, 2017). Previous studies have reported that artichoke extract has important activities such as hepatoprotective (Gebhardt and Fausel, 1997), hypoglycemic (Salem *et al.*, 2017), antibacterial (Shimoda *et al.* 2003), antioxidant (Salekzamani *et al.*, 2019), anti-inflammatory and immunomodulatory (El-Boshy *et al.*, 2017).

There is little evidence to indicate artichoke is useful to alleviate hemolytic anemia. Therefore, this study was undertaken to evaluate the putative antioxidant action of *Cynara scolymus* extract in an experimental model of PHZ-induced toxicity in Wistar rats.

Materials and methods

Chemicals and preparation of extract

The ethanolic extract of artichoke that was used in this study was purchased from Dineh Iran Industries Complex (Pharmaceutical Company, Tehran, Iran). Phenylhydrazine (PHZ) was purchased from Sigma-Aldrich (St. Louis, MO, USA).

Animals housing conditions

This study was carried out on 30 mature male Wistar rats, weighing approximate-

ly 210-220 gr that were obtained from the Animal Care Unit of Faculty of Veterinary Medicine, Razi University, Kermanshah, Iran. The rats were acclimatized for approximately one week before use. The animals were housed in stainless steel cages under controlled environmental conditions with temperature (22 ± 2 °C), relative humidity of $55\pm 5\%$ and lighting (12-h light/12-h dark cycle). All rats were fed with a standard laboratory pelleted chow diet and fresh water *ad libitum*. Animal experiments used in this study were approved by the Animal Ethics Committee of Razi University following the Guidelines for the Care and Use of Laboratory Animals in Research (Animal Ethical Approval Number: 397-2-008).

Experimental procedure

After one week of acclimatization, the rats were randomly assigned into five groups with six animals per group. In group 1, animals were orally and daily injected with normal saline and served as the normal control. In groups 2 to 5, anemia was induced by Intraperitoneal (IP) injection of PHZ at 40 mg/kg for 2 consecutive days. Rats that developed anemia with hemoglobin (Hb) concentration lower than 14 g/dl were used for the study. The anemic rats were randomly divided into four groups and treated as follows: In group 2, rats were given normal saline orally and served as the anemic control. Groups 3, 4 and 5, anemic rats daily received the hydroalcoholic extract of *C. scolymus* at doses of 100, 200, 400 mg/kg body weight by oral gavage, respectively. The normal saline and hydroalcoholic extract of *C. scolymus* was administered from days 2 to 15 after PHZ injection. The experimental period was 14 days. In this study, dose of PHZ to induce anemia and *C. scolymus* doses were determined according to previous studies (Diallo *et al.*, 2008; Lee *et al.*, 2012; Salem *et al.*, 2017).

Sample collection

At the end of the study period, all rats were weighed and then anesthetized using diethyl ether. Blood samples were taken from the heart to determine the hematological and antioxidant parameters. For erythrocyte preparation, the erythrocytes were sedimented by centrifuge at 500 g for 10 min at 4 °C. The erythrocytes were washed three times (5 ml, each) with cold isotonic saline then the buffy coat was discarded. RBCs and sera were stored at -20 °C until assayed.

Determination of hematological parameters

For hematological analyses, blood was examined using an automatic hematology analyzer (Celltac, Alpha Vet MEK-6550; Nihon Kohden Co, Tokyo, Japan).

Measurement of total antioxidant capacity (TAC) levels in serum and erythrocytes

Spectrophotometer analysis with the aid of colorimetric assay kit (Naxifer™, Navandsalamat Co., Iran) was used to estimate the concentrations of TAC in RBCs and serum by the ferric reducing ability (FRAP) method. This procedure is based on the ability of serum or RBC lysis to reduce iron III (Fe^{3+}) to iron II (Fe^{2+}) in the presence of 2,4,6-Tripyridyl-S-triazine (TPTZ). A complex with blue color and maximum absorbance appeared at 593 nm with reaction of Fe^{2+} and TPTZ. The serum level of TAC was expressed in nanomoles per milliliter (nmol/ml) and nmol per gr of hemoglobin (nmol/grHb) for RBC lysis.

Measurement of malondialdehyde (MDA) levels in serum and erythrocytes

The levels of lipid peroxidation in the serum and RBCs lysis were measured as TBARS using a Nalondi™ assay kit (Navandsalamat Co., Iran). Sera were assayed directly using the kit. The RBCs were first lysed in deionized water containing butylated hydroxytoluene (BHT) provided in the kit,

and were subsequently centrifuged at 10,000 g for 5 min to collect the supernatant. Serum MDA level was expressed as nmol/ml and it is also expressed in nmol/gr Hb in RBCs.

Measurement of superoxide dismutase (SOD) levels in serum and erythrocytes

Copper, zincsuperoxide dismutase (SOD) was assessed by the pyrogallol assay of Nasdox™ kit (Navandsalamat Co., Iran). The method depends on the spontaneous autoxidation of pyrogallol at alkaline pH, resulting in the production of superoxide anion radicals which cause enhanced autoxidation of pyrogallol. Autoxidation is manifested as an increase in absorbance at 420 nm. Specific activity of SOD was calculated as units/ml (unit/gr Hb for RBCs), in which one unit of enzyme gave 50% inhibition of pyrogallol autoxidation.

Statistical analysis

The obtained data were analyzed by the One-way Analysis of Variance (ANOVA) and Tukey's HSD post-hoc test using IBM SPSS software version 21.0 for Windows. The results were expressed as mean val-

ues±SD. The level of statistical significance was set at $P \leq 0.05$.

Results

Effect of *cynara scolymus* extract on hematological parameters

The effects of *Cynara scolymus* extract on hematological parameters in anemic rats are presented in Table 1. The PHZ group showed significant decrease in PCV, Hb, RBC, MCV and MCHC levels compared with the control group ($P \leq 0.05$). Administration of HECS (100-400 mg/kg) significantly improved the decreased levels of above mentioned parameters (mainly at doses of 200 and 400 mg/kg) compared to PHZ-treated rats ($P \leq 0.05$). There was also a significant increase in levels of WBC and lymphocyte in PHZ group when compared with control group ($P \leq 0.05$). Treatment with artichoke extract only at 400 mg/kg has an increasing impact on WBC level compared to PHZ-exposure group ($P \leq 0.05$). Furthermore, we did not observe significant differences in neutrophil levels among all groups ($P > 0.05$).

Table 1. Effect of *Cynara scolymus* Extract on Hematological Parameters in PHZ-Induced Anemic Rats

Hematological Parameters	Control	PHZ	PHZ+HECS (100 mg/kg)	PHZ+HECS (200 mg/kg)	PHZ+HECS (400 mg/kg)
PCV (%)	41.5 ± 2.11	28.2 ± 3.27*	29.7 ± 1.28	32.3 ± 2.29	38.8 ± 2.96 [#]
Hb (gr/dl)	14.2 ± 1.62	9.4 ± 1.36*	10.2 ± 1.71	11.8 ± 1.26	12.6 ± 2.16 [#]
RBC (×10 ⁶ /μL)	6.3 ± 0.87	3.9 ± 0.52*	4.2 ± 0.88	4.8 ± 1.15	5.9 ± 0.73 [#]
MCV (fl)	87.4±2.71	60.2±3.10*	68.3±2.01 [#]	80.2±2.34 [#]	85.2±4.55 [#]
MCHC (gr/dl)	32.1±0.75	26.6±1.15*	27.6±0.60	29.8±0.85 [#]	29.5±1.35 [#]
WBC (×10 ³ /μL)	10.2±1.70	13.4±1.35*	13.5±1.05	13.7±1.25	15.0±1.00 [#]
Neutrophil (×10 ³ /μL)	1.4±0.35	1.2±0.25	1.5±0.20	2.2±0.50	2.5±0.85
Lymphocyte (×10 ³ /μL)	7.9±0.50	11.4±1.95*	12.2±1.05	10.6±1.00	11.7±1.05

Data are expressed as mean±SD (n=6). * $P \leq 0.05$ as compared with control group within each row; # $P \leq 0.05$ as compared with PHZ--treated group within each row. PHZ: Phenylhydrazine; HECS: Hydroethanolic Extract of *Cynara scolymus*; PCV: Packed Cell Volume; Hb: Hemoglobin; RBC: Red Blood Cell; MCV: Mean Corpuscular Volume; MCHC: Mean Corpuscular Hemoglobin Concentration; WBC: White Blood Cell.

Effect of *Cynara scolymus* extract on TAC levels in serum and erythrocytes

PHZ-induced anemic rats showed a significant reduction of serum and erythrocytes TAC level as compared to the healthy control group ($P \leq 0.05$). Administration of *Cynara scolymus* extract at doses of 200

mg/kg and 400 mg/kg considerably increased these parameters toward the control value ($P \leq 0.05$). Significant difference in the TAC level between the groups receiving the HECS in anemic rats induced with PHZ was also observed in the current study ($P \leq 0.05$) (Figure 1 and Figure 2).

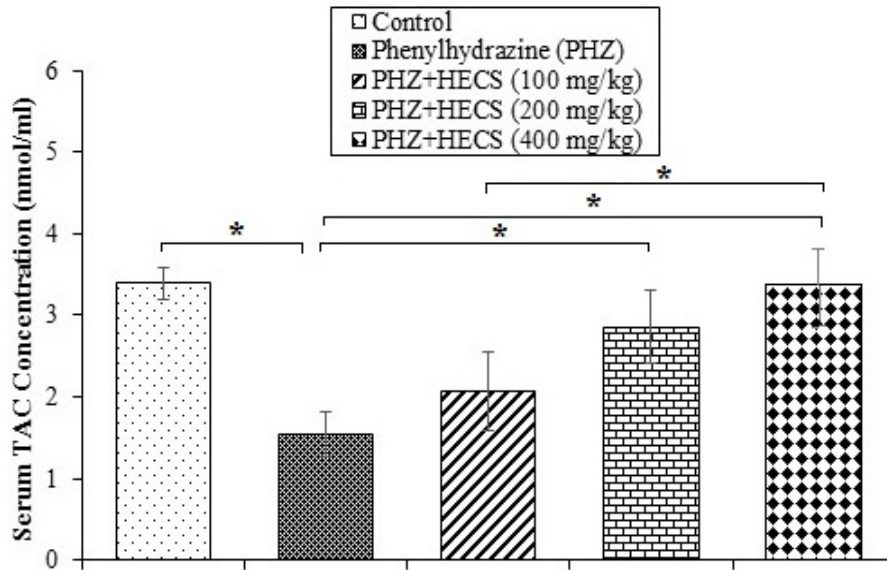


Figure 1. Effect of *Cynara scolymus* extract at different doses on serum TAC concentration in PHZ-induced anemic rats. Data are expressed as mean \pm SD (n=6). * $P \leq 0.05$. PHZ: Phenylhydrazine; HECS: Hydroethanolic Extract of *Cynara scolymus*

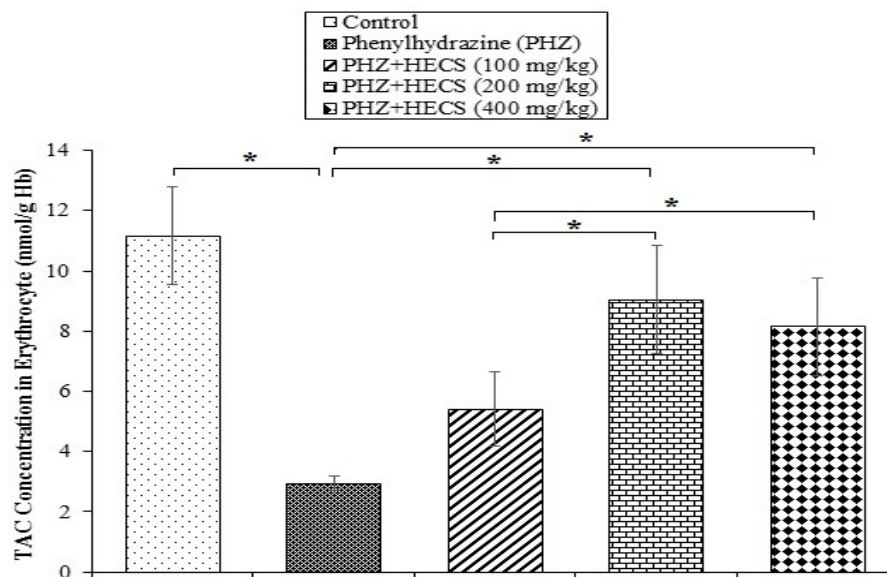


Figure 2. Effect of *Cynara scolymus* extract at different doses on TAC concentration in erythrocyte in PHZ-induced anemic rats. Data are expressed as mean \pm SD (n=6). * $P \leq 0.05$. PHZ: Phenylhydrazine; HECS: Hydroethanolic Extract of *Cynara scolymus*

Effect of *Cynara scolymus* extract on MDA levels in serum and erythrocytes

A marked increase in the MDA level was found in the serum and erythrocytes of PHZ-exposed rats relative to normal rats ($P \leq 0.05$). The treatment of anemic rats with increasing concentrations of *Cynara scolymus* extract (100-400 mg/kg)

was significantly effective in decreasing the elevated level of MDA ($P \leq 0.05$). No significant difference was observed in serum MDA level between HECS treated groups ($P > 0.05$) but a decline in efficacy of HECS in MDA concentration in RBCs was in a dose-dependent manner ($P \leq 0.05$) (Figure 3 and Figure 4).

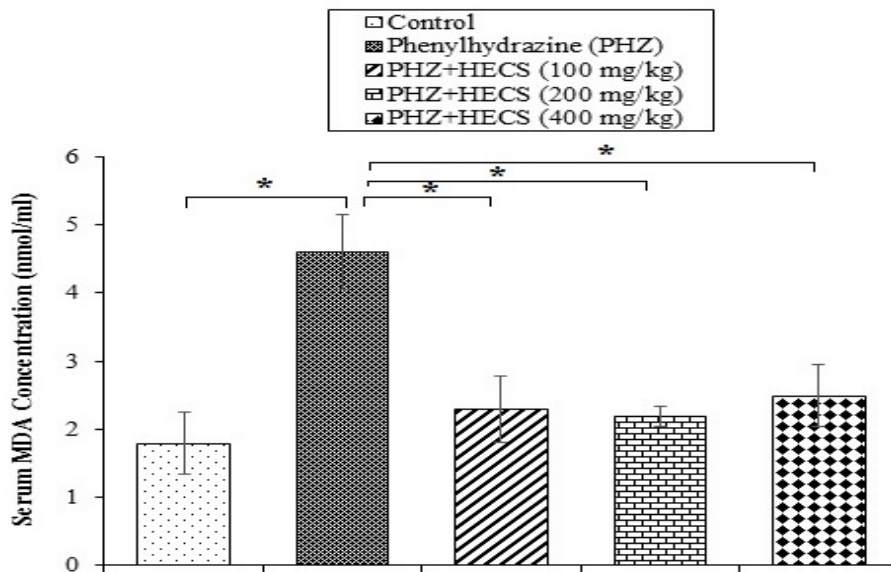


Figure 3. Effect of *Cynara scolymus* extract at different doses on serum MDA concentration in PHZ-induced anemic rats. Data are expressed as mean±SD (n=6). * $P \leq 0.05$. PHZ: Phenylhydrazine; HECS: Hydroethanolic Extract of *Cynara scolymus*

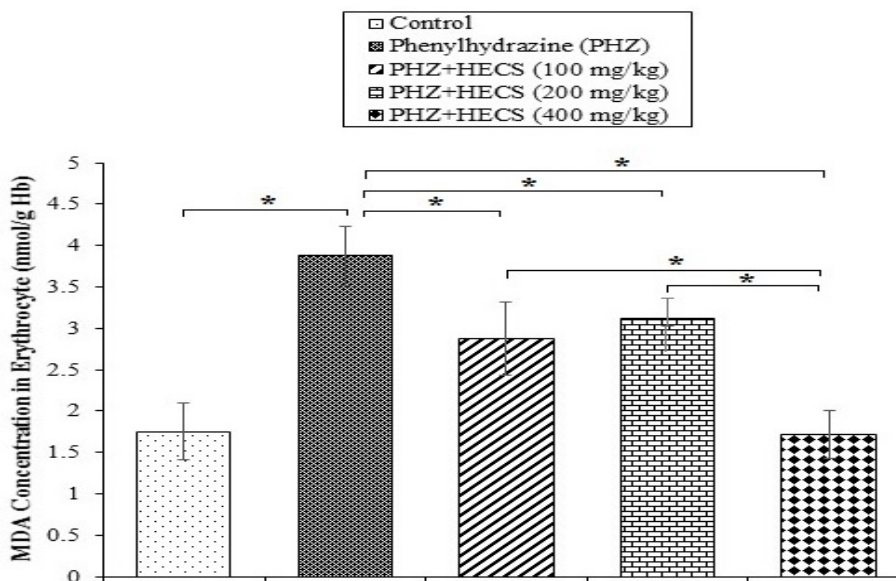


Figure 4. Effect of *Cynara scolymus* extract at different doses on MDA concentration in erythrocyte in PHZ-induced anemic rats. Data are expressed as mean±SD (n=6). * $P \leq 0.05$. PHZ: Phenylhydrazine; HECS: Hydroethanolic Extract of *Cynara scolymus*

Effect of *cynara scolymus* extract on SOD levels in serum and erythrocytes

The SOD activity in serum and erythrocytes has significantly decreased in PHZ group when compared with the normal control group ($P \leq 0.05$). Significant increase of serum and erythrocytes SOD level was determined in rats

treated with *Cynara scolymus* extract at different doses, especially at 200 mg/kg and 400 mg/kg, as compared to untreated anemic rats. Also, statistically significant difference in the SOD level was ascertained between the rats in the various groups treated with HECS ($P \leq 0.05$) (Figure 5 and Figure 6).

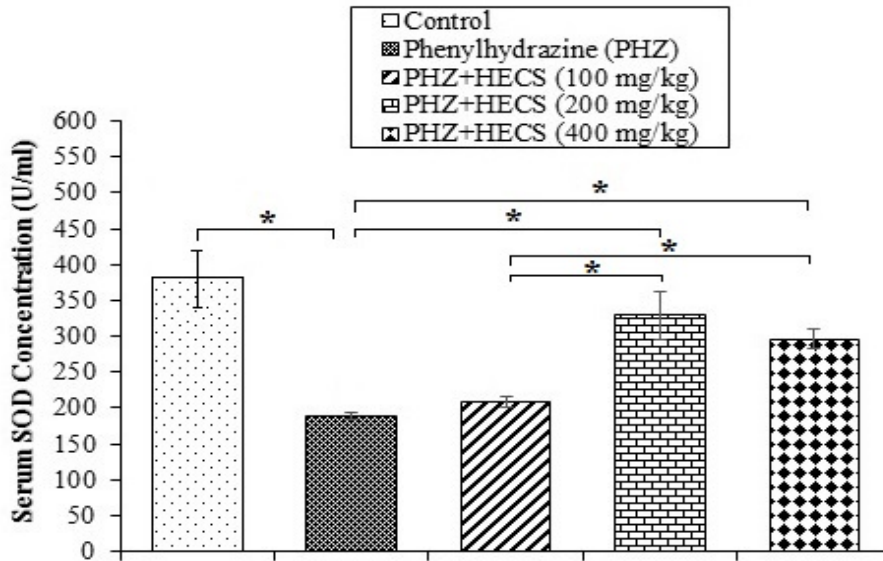


Figure 5. Effect of *Cynara scolymus* extract at different doses on serum SOD concentration in PHZ-induced anemic rats. Data are expressed as mean±SD (n=6). * $P \leq 0.05$. PHZ: Phenylhydrazine; HECS: Hydroethanolic Extract of *Cynara scolymus*

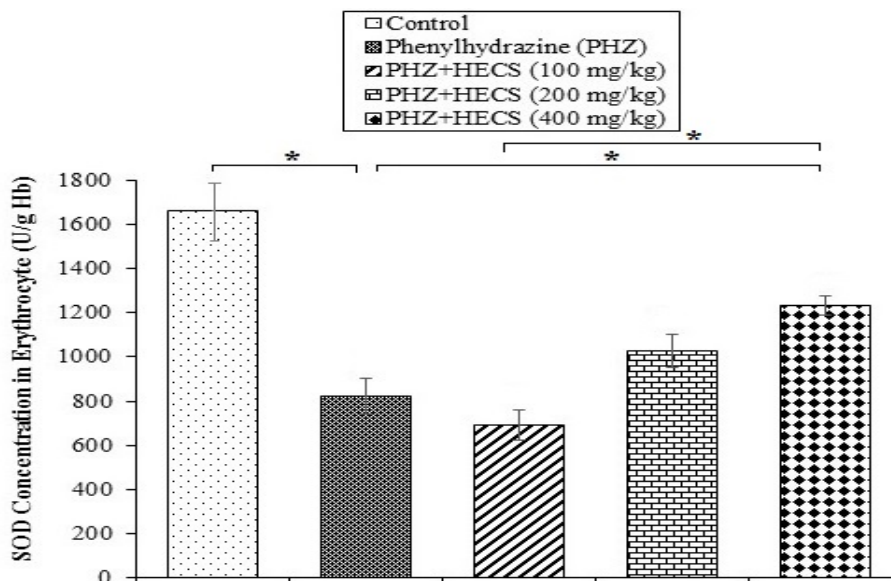


Figure 6. Effect of *Cynara scolymus* extract at different doses on SOD concentration in erythrocyte in phenylhydrazine-induced anemic rats. Data are expressed as mean±SD (n=6). * $P \leq 0.05$. PHZ: Phenylhydrazine; HECS: Hydroethanolic Extract of *Cynara scolymus*

Discussion

The purpose of the study was to verify the antioxidant effects of artichoke extract on serum and erythrocytes antioxidant capacity following hemolytic anemia induced by PHZ injection. Our results indicated that the injection of PHZ significantly decreased Hb and PCV levels in comparison with the control group. In PHZ-induced anemia, ROS-mediated damage of RBCs membrane components is thought to increase lipid peroxidation, erythrocyte membrane rigidity and fragility. In this regard, intravascular hemolysis was increased and higher levels of hemoglobin released into the serum (Pandey *et al.*, 2014). Extract-treated animals in this study have enhanced Hb, PCV levels and RBCs numbers in comparison with PHZ group. Therefore, the beneficial impact of ethanol extract of artichoke in improving hematinic profile in PHZ-induced anemia may be due to reducing ROS which leads to establishing fragility of RBCs and restoring the total Hb concentration in the blood (Avci *et al.*, 2006; Rezazadeh *et al.*, 2012, Salekzamani *et al.*, 2019). On the other hand, it was reported that *Cynara scolymus* is rich in phenolic compounds belonging to different classes such as benzoic and cinnamic derivatives, flavonoids and tannins (Lattanzio *et al.*, 2009). Salem *et al.* (2017) indicated that flavonoids could induce secretion of erythropoietin, which stimulates stem cells in the bone marrow to produce RBCs.

The present study demonstrated that administration of PHZ to rats results in a marked leukocytosis in comparison with control group. This leukocyte response is due to an increase in circulating mononuclear cells, primarily lymphocytes. The results of present study are in line with the results of Borley *et al.* (2010) and Xia *et al.*

(2014) which indicated that leukocytosis in PHZ-treated animals occurred by increasing oxidative and nitrosative stress. The use of high doses of artichoke extract increased the number of leukocytes compared to the PHZ group, which may be due to its antioxidant capacity and its ability to stimulate the production of leucocytes and could serve as immune booster (Pérez-García *et al.*, 2000; Zapolska-Downar *et al.*, 2002).

The antioxidant capacity of whole blood is driven by the circulating RBCs that act as mobile scavengers and exert their antioxidant properties (Siems *et al.*, 2000). If oxidative stress persists, the production of free radicals is greater than the antioxidant capacity of erythrocytes or serum. Thus, incidence of hemolytic anemia or sickle cell anemia is imminent (Chan *et al.*, 1999). Measurement of antioxidant molecules is difficult and requires time and cost. Therefore, the total antioxidant capacity of erythrocytes can be one of the reliable indices to determining oxidative stress in erythrocytes and serum (Singh *et al.*, 2018). Administration of PHZ causes ROS production which leads to the hemolytic anemia. There are several ways to reduce the effects of ROS on haematotoxicity, the most common of which are medicinal plants. Natural antioxidants found in foods and vegetables are one of several factors that increase the antioxidant capacity of erythrocytes (Jimenez-Escrig *et al.*, 2003). The collection data of the present study revealed that TAC level of serum and erythrocytes in PHZ group decreased in comparison with control group, which is attributed to increased oxidative stress and ROS generation following PHZ administration (Shukla *et al.*, 2012). Administration of *Cynara scolymus* extract considerably increased TAC level as compared to the PHZ group. This effect of arti-

choke extract might be dependent on its flavonoids. According to previous research, it was demonstrated that phenolics and flavonoids compounds have a highly antioxidant effect by reducing ROS (Petropoulos *et al.*, 2017; Sahebkar *et al.*, 2018).

Evaluation of MDA concentrations in serum and erythrocytes showed a significant increase in PHZ group compared to the control group, which is in line with the results of Mozafari *et al.* (Mozafari *et al.*, 2016). The level of lipid peroxide is a measure of membrane damage and alterations in the structure and function of cellular membranes (Salem *et al.*, 2017).

One of the important oxidation products is MDA, which is considered as the main marker of lipid peroxidation in oxidative stress, inflammation and various health disorders (Singh *et al.*, 2014). Recent studies have suggested that increase in MDA concentrations is one of the most important mechanisms of PHZ in development of hemolytic anemia (Gaschler *et al.*, 2017; Gheith *et al.*, 2018). Erythrocytes and erythrocyte membrane are more vulnerable to lipid peroxidation and oxygen radical formation because of constant exposure to high oxygen tension and richness in polyunsaturated fatty acid. Alterations in lipid composition of RBC membranes result in morphologically abnormal erythrocytes with decreased life span (Revin *et al.*, 2016). It has been suggested that the membrane lipids play crucial roles in cellular homeostasis including the maintenance of erythrocytes structure, cellular function and viability. Increased ROS, as stated, increases lipid peroxidation and produces substances that are toxic to cells and disrupt cells structure (i.e., hemolysis) (Pandey *et al.*, 2014; Mladenov *et al.*, 2015). The present work showed that *Cynara scolymus* extract resulted in a significant reduction of the MDA concentrations in

PHZ-exposed rats. Hence, it is possible that the mechanism of this protection may be due to its antioxidant activity. In this regard, recent study has shown that *Bidens pilosa* extract with its antioxidant properties, could prevent oxidative damage and reduce MDA level in erythrocytes (Yang *et al.*, 2006).

Our results revealed that SOD activity in serum and erythrocytes has significantly decreased in PHZ group when compared with the control group. The reduction in the SOD activity may be due to the oxidation of cysteine in this enzyme by superoxide anion during its transformation to hydrogen peroxide. In other words, the decrease in SOD activity in PHZ-induced anemic rats may be due to the use of this enzyme in converting the superoxide anion ($O_2^{\bullet-}$) to hydrogen peroxide (H_2O_2) (Plestina-Borjan *et al.*, 2015). In addition, PHZ generates free radicals and ROS such as superoxide anion and hydrogen peroxide (Kamisah *et al.*, 2014). Moreover, increased lipid peroxidation induced by PHZ in the serum and erythrocytes was accompanied by a decrease in SOD activity, which was also reported by recent studies (Maity *et al.*, 2013; Anbara *et al.*, 2015). Erythrocytes contain several biological defense mechanisms against free radical-induced injury, including many antioxidant enzymes such as SOD (Kolanjiappan *et al.*, 2002). Furthermore, SOD could inhibit the accumulation of membrane proteins, which might prevent the disruption or destruction of erythrocyte wall proteins. Also, SOD activity in the cytosol of erythrocytes is about 3 times more than serum, so it could help modulate the oxidant effects of PHZ in erythrocytes. Given these points, measuring SOD can be an important criterion for the prognosis of diseases that cause hemolysis or damage to erythrocytes (Valenzuela *et al.*, 1977; Pandey and Rizvi,

2011; Anbara *et al.*, 2018). Remarkable increments in SOD level of serum and erythrocytes were determined in anemic rats treated with *Cynara scolymus* as compared to untreated anemic rats. These positive results are consistent with another study which has demonstrated natural medicinal herbs could boost SOD activity due to presence of antioxidant compounds (Fukai *et al.*, 2011). In accordance with these findings, the study by Salem *et al.* (2017) showed that administration of ethanol extract of *C. scolymus* increased the SOD level in another oxidative stress-related disorder like diabetes, which indicates the antioxidant activity of extract.

In conclusion, PHZ caused marked oxidative stress and lipid peroxidation in rats by inhibiting the antioxidant enzymes activity. The results revealed the ameliorating effect of *Cynara scolymus* extract through declining the levels of MDA and raising the SOD and TAC activity in PHZ-induced hemolytic anemia in rats. Protective property of *Cynara scolymus* extract against PHZ-induced toxicity might be due to the presence of various phytochemical constituents with potent antioxidant activity.

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Conflict of interest

The authors declared that there is no conflict of interest.

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بهبود وضعیت پراکسیداسیون لیپید و آنزیم‌های آنتی‌اکسیدانی در سرم و گلبول‌های قرمز رت‌های نر مبتلا به آنمی القاء شده توسط فنیل‌هیدرازین: نقش محافظتی عصاره کنگر فرنگی

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چکیده

زمینه مطالعه: آنمی همولیتیک اختلالی است که با تخریب زودرس گلبول‌های قرمز مشخص می‌شود. فنیل‌هیدرازین سبب القاء استرس اکسیداتیو و تشکیل گونه‌های واکنش‌پذیر اکسیژن شده که منجر به بروز آنمی همولیتیک می‌شود. کنگر فرنگی بدلیل ترکیبات آنتی-اکسیدانی خود برای اهداف مختلف درمانی در طب سنتی استفاده شده است.

هدف: مطالعه حاضر جهت بررسی اثرات عصاره کنگر فرنگی بر آنمی القاء شده توسط فنیل‌هیدرازین در رت‌های نر طراحی شد.

روش کار: آنمی همولیتیک با تزریق داخل صفاقی فنیل‌هیدرازین (۴۰ mg/kg) به مدت ۲ روز القاء شد. ۳۰ سر رت نر نژاد ویستار به پنج گروه تقسیم شدند. گروه اول (کنترل نرمال). گروه ۲ فقط فنیل‌هیدرازین را دریافت کرد (کنترل آنمیک). گروه‌های ۳ تا ۵ با کنگر فرنگی به ترتیب با دوزهای ۱۰۰، ۲۰۰ و ۴۰۰ میلی‌گرم به ازای کیلوگرم وزن بدن از روز ۲ تا ۱۵ پس از تزریق فنیل‌هیدرازین گاوآژ شدند. در پایان دوره درمانی، نمونه‌های خون جهت ارزیابی پارامترهای هماتولوژی، سطح مالون دی‌آلدئید (MDA) و فعالیت آنزیم‌های آنتی‌اکسیدانی مانند سوپراکسید دیسموتاز (SOD) و ظرفیت آنتی‌اکسیدانی تام (TAC) در سرم و گلبول‌های قرمز جمع‌آوری شد.

نتایج: در رت‌های کم‌خون، سطح MDA سرم و گلبول‌های قرمز افزایش یافت، اما فعالیت SOD و TAC در مقایسه با گروه کنترل بطور معنی‌داری کاهش یافت ($P \leq 0/05$). این تغییرات با درمان توسط کنگر فرنگی در دوزهای مختلف بهبود یافت ($P \leq 0/05$). همچنین، چندین پارامتر هماتولوژیکی بهبود یافته پس از تجویز کنگر فرنگی در رت‌های کم‌خون مشاهده شد ($P \leq 0/05$).

نتیجه‌گیری نهایی: عصاره کنگر فرنگی دارای خاصیت محافظتی در برابر استرس اکسیداتیو ناشی از فنیل‌هیدرازین بوده که احتمالاً به دلیل فعالیت آنتی‌اکسیدانی عصاره این گیاه است.

واژه‌های کلیدی:

استرس اکسیداتیو، آنمی همولیتیک، رت، کنگر فرنگی، فنیل‌هیدرازین.

Canine Mammary Gland Cancer Stem Cell and its Potential Role in Malignant Biologic Behavior

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Abstract

BACKGROUND: Canine mammary gland cancers are the most prevalent malignancies in dogs. There are different challenges regarding management of these cancers in dogs and human, one hypothesis is related to small cellular subset of tumor mass called cancer stem cell. These cells are therapy resistant and cause metastasis and relapse even after primary successful treatment. The well-identified phenotypes for detecting this population are ALDH1+/CD44+/CD24-/Low biomarkers.

OBJECTIVES: A study to evaluate existence of cancer stem cells in canine malignant mammary glands tumor and assess effects of these cells on clinicopathological parameters of tumors were designed.

METHODS: In this study forty cases of canine mammary glands tumors were collected. All cases were tested via H&E and then Immunohistochemistry (IHC) methods. All samples were evaluated immunohistochemically for common markers of these tumor-initiating cells. Monoclonal antibodies against ALDH1, CD44 and CD24 were used. Some tumor aggressiveness-related parameters, including lymphovascular invasion, tumor grades and histotypes were assessed.

RESULTS: The present study revealed that 17.5% of cases were enriched with cancer stem cells and all of them were diagnosed as grade II and III ($P \leq 0.05$). Other findings showed all cancer stem cell-positive cases were significantly lymphovascular invasion positive ($P \leq 0.05$). The most common histotypes in this research were tubular, tubulopapillary and intraductal carcinomas.

CONCLUSIONS: Our results illustrated that cancer stem cells can be considered as reliable prognostic factors to predict severity of malignant behavior of canine malignant mammary glands cancer, which is comparable with human breast cancer.

KEYWORDS: Cancer, Canine, Malignant tumor, Mammary Glands, Stem Cells

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