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RESEARCH ARTICLE

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Effect of wheat germ oil on phenylhydrazine-induced toxicity in male albino rats

ABSTRACT:
Wheat germ oil (WGO) is a natural source of antioxidant such as glycosylated hydroquinone, tocopherols, and it is an excellent source of polyunsaturated fatty acids. The point of the present study is to see how efficient is the WGO against phenylhydrazine (PHZ) induced hemotoxicity in rats. Rats were divided into five groups (six rats each); control group (normal rats), WGO group (administrated by daily oral dose of WGO, 300 mg/kg b.w.), PHZ group (injected intraperitoneally with a daily dose of PHZ, 60 mg/kg b.w. for 3 consecutive days), treated group (injected with PHZ then after 3 days administered by WGO) and protective group (administrated daily by WGO for 11 days then injected with PHZ). At the end of the experimental period 14 days, blood samples were collected in 2 groups of tubes, one containing EDTA for determination of blood parameters and the second without anticoagulant for separation of serum used for determination of physiological and biochemical parameters. The results showed that the PHZ induced significant decreases in haematological parameters (RBCs, Hb, Hct, and PLT), serum proteins (total protein, albumin and globulin) and antioxidant enzymes (CAT, GPX and SOD) activity and significant increases in white blood cells (WBCs), erythropoietin hormone, serum ferritin level, serum liver function parameters (ALT, AST, TBIL, and ALP), serum kidney function parameters (creatinine, urea and uric acid) and malondialdehyde (MDA) content. Administration of WGO improved the haematological parameters, erythropoietin hormone, ferritin level, serum proteins, liver function parameters, kidney function parameters, antioxidant enzyme activities and MDA content in rats injected with PHZ. In conclusion, the results of the present study revealed that the WGO has therapeutic and protective roles in improving the haemolytic anaemia and toxicity induced by PHZ.

KEY WORDS:
Phenylhydrazine; Anaemia; Wheat germ oil; Haematological parameters; Erythropoietin hormone.

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INTRODUCTION:

Anaemias are common blood illnesses, affecting people of different ages all over the world (Ogbe *et al*., 2010). One of the causes of haemolytic anaemia is the toxic effect of drugs on red blood cells. Phenylhydrazine (PHZ) is a chemical molecule that is used as a chemical intermediate in the pharmaceutical, agrochemical, and chemical industries. Phenylhydrazine was mainly used for experimental induction of anaemia in

animals, it is famous for its toxicity in red blood cells (Spivak, 2002). Phenylhydrazine decreases haemoglobin level, red blood cells (RBCs) count and extracellular haematopoiesis in the spleen and liver (Shukla *et al*., 2012). As a hemotoxic, it causes oxidative stress within erythrocytes, resulting in the oxidation of haemoglobin, leading to the formation of methaemoglobin which is subsequently converted into irreversible hemichromes that lead to the precipitation of haemoglobin in the form of Heinz bodies (Rifkind, 1965). Phenylhydrazine causes damage to skeletal protein, lipid peroxidation, ATP depletion, cation imbalances, and reduced RBCs membrane deformability. All of these signs and symptoms point to haemolytic anaemia (Berger, 2007; McMillan *et al*., 1998 & 2005).

Wheat germ contains 10- 12% oil consisting mainly of oleic, linoleic and linolenic acids (Sjövall *et al*., 2000; Gomez and Ossa, 2002). High lipase and lipoxygenase activities, as well as a high content of unsaturated fatty acids are characteristics of wheat germ (Sjövall *et al*., 2000). Wheat germ is the richest source of antioxidant glycosylated hydroquinone and tocopherols, slight oxidation may destroy essential fatty acids and vitamins (Zhokhov *et al*., 2010). The WGO reduced plasma and liver cholesterol in animals improved physical endurance and delayed aging (Kahlon, 1989). The WGO has been used as a fertility agent, an antioxidant, and an additive in natural food, health and cosmetic products. The WGO can help to reduce oxidative stress (Alessandri *et al*., 2006). Also, it contains policosanol, a substance that can help to lower the raised blood sugar and/or total cholesterol levels (Irmak and Dunford, 2005). The WGO was known to be the richest natural source of alpha, beta and gamma-tocopherols and tocotrienols (Hassanein and Abdel-Razek, 2009). The WGO also contains fat-soluble carotenoids such as lutein, zeaxanthin and beta-carotene, which have antioxidant effects (Leenhardt *et al*., 2008). Zangeneh *et al*. (2019), reported that serum liver function parameters (ALT, AST, TBIL, and ALP) increased in PHZ-treated rats. According to Kale *et al*. (2019), there were disruptions in the kidney and liver function parameters after the injection of PHZ in rats that explained the reduction in serum protein levels. The reduction in serum proteins may be a result of kidney and liver injury caused by PHZ. The present study aimed to explore the beneficial effects of wheat germ oil on blood hemotoxicity induced by PHZ in anaemic rats.

MATERIAL AND METHODS:

Experimental Animals:

Thirty male albino rats (*Rattus norvegicus*), weighing 150 ± 10 g, were obtained from the Helwan Farm of the Egyptian Organization for Vaccine and Biological Preparations. Rats were maintained under standard laboratory conditions (25 ± 2°C, 12 hours light/dark cycle and given food and water) for ten days before the onset of the experiment. Rats were humanely treated according to the ethical guidelines of the Faculty of Science, Benha University (Approval number: ZD/FSc/BU-IACUC/2022-14).

Wheat Germ Oil:

Wheat germ oil was obtained from Sedico Pharmaceutical Company in the form of soft gelatine capsules (Each capsule contains WGO 1000 mg). A daily dose of WGO (300 mg/kg b.w.) was given to the animals by oral gavage using a stomach tube according to Hamed *et al*. (2013).

Phenylhydrazine:

Phenylhydrazine yellow powder was obtained from Sigma Aldrich for Scientific Chemicals, Cairo, Egypt. Phenylhydrazine was dissolved in saline solution for administration (52.2 mg/ 9 ml saline).

Experimental Induction of Anaemia:

In the present study, anaemia was induced by daily intraperitoneal injection of PHZ (60 mg/kg). for three consecutive days (Koffuor *et al*., 2011). Anaemia was considered to be induced when RBCs count, as well as haemoglobin concentration in the blood, were reduced by about 30%.

Experimental Design:

The experimental animals were randomly divided into five groups “six rats each” as follows: (a) control group (normal untreated rats), (b) wheat germ oil group (rats administrated WGO by a daily dose of 300 mg/kg b.w. for 14 days), (c) Phenylhydrazine group (rats injected intraperitoneally with PHZ by a daily dose of 60 mg/kg b.w. for 3 consecutive days), (d) Phenylhydrazine then wheat germ oil group (rats injected with PHZ for 3 days then treated with a daily oral dose of WGO for 11 days), (e) Wheat germ oil then phenylhydrazine group (rats administrated a daily oral dose of WGO for 11 days then injected with PHZ for 3 days). The experimental period was extended for 14 days.

Determination of Haematological and Biochemical Parameters:

Sample Preparations:

At the end of the experimental period, the overnight fasting animals were anesthetized by light inhalation of diethyl ether. Blood samples were collected from the posterior vena cava in two groups of tubes, the first group of tubes containing ethylenediamine tetra acetic acid (EDTA) as an anticoagulant for determination of haematological parameters. The blood in the

second group of tubes was allowed to clot without using any anticoagulant for 1 - 2 h at 37^{O}C then centrifuged at 3000 rpm for 15 minutes. Sera were separated and stored at -20^{O}C for biochemical determinations.

Haematological Parameters:

Red blood cells (RBCs) count, haemoglobin (Hb) content, haematocrit (Hct) value, blood platelets (PLT) count and white blood cells (WBCs) count were determined by an automated haematology cell counter (MS4e Automatic haematology Analyzer).

Biochemical Parameters:

The concentrations of both serum erythropoietin hormone and ferritin were measured by ELISA rat kits purchased from CUSABIO (USA).

Total serum protein and serum albumin levels were determined spectrophotometrically by the methods of Koller (1984) and Webster *et al.* (1974) respectively using Diamond kits (Cairo, Egypt), While serum globulin level was calculated as follows:

Globulin = Total protein- Albumin

Spectrophotometrically serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels were determined by the method of Schumann and Klauke (2003) using Human kits (Germany), Total bilirubin (TBIL) level was determined by the method of Young and Friedman (2002) using Diamond kits (Cairo, Egypt). Serum alkaline phosphatase (ALP) level was determined by the method of Moss (1982) using Spectrum kits (Egypt).

Serum creatinine and urea levels were determined spectrophotometrically according to the methods of Mazzachi *et al*. (2000) and Tabacco *et al*. (1979), respectively by using Diamond kits (Cairo, Egypt), while serum uric acid level was determined by the method of Fossati *et al*. (1980) using Spinreact kits (Barcelona, Spain).

The malondialdehyde (MDA) content and the activities of catalase (CAT), glutathione peroxidase (GPX) and superoxide dismutase (SOD) were determined colorimetrically by the methods of Draper and Hadley (1990), Luck (1965), Ara *et al*. (2018), and Kakkar *et al*. (1984), respectively using kits purchased from BioVision (Milpitas, CA, USA).

Statistical Analysis:

Results are presented as means ± standard deviations of six readings. Statistical analyses of the results were completed by the one-way analysis of variance (ANOVA), followed by Duncan's multiple range test (Duncan, 1957). The significance level was set at (P<0.05) using the Statistical Package for Social Science (SPSS) computer program (version 20.00) produced by IBM Software, Inc. Chicago, USA.

RESULTS:

Haematological Parameters:

The RBCs count, Hb content, Hct value and PLT count indicated significant decreases and significant increases in WBCs (P < 0.05) in the PHZ group compared to those of the control and the WGO groups. Treated PHZ groups (PHZ then WGO and WGO then PHZ groups) have high RBCs count, Hb contents, Hct values and PLT counts and low

WBCs count in comparison with those of the PHZ group but still have low RBCs count, Hb contents, Hct values, PLT counts and high WBCs count in comparison with both the control and the WGO groups (Table 1).

Table 1. Effect of wheat germ oil (WGO) on haematological parameters (RBCs, Hb, Hct, PLT and WBCs) in phenylhydrazine (PHZ) treated male albino rats.

	Animal groups				
	Control	WGO	PHZ	PHZ + WGO	WGO + PHZ
RBCs (M/mm^3)	3.94 ± 0.99^b	4.35 ± 0.14^a	3.38 ± 0.16^c	3.88 ± 0.18^b	3.85 ± 0.16^b
Hb (g/dl)	11.60 ± 0.52^a	12.35 ± 1.01^a	6.12 ± 0.24^c	9.95 ± 0.83^b	9.92 ± 0.82^b
Hct (%)	32.75 ± 1.71^b	38.35 ± 2.61^a	23.00 ± 0.97^c	30.23 ± 2.38^b	31.23 ± 2.80^b
PLT (th/mm^3)	270.00 ± 10.00^a	280.00 ± 5.00^a	190.00 ± 5.00^c	226.33 ± 4.04^b	224.35 ± 4.01^b
WBCs (th/mm^3)	7.00 ± 0.36^b	6.67 ± 0.25^c	8.56 ± 0.51^a	7.83 ± 0.21^b	7.50 ± 0.20^b

M: Million; th: thousand; all data expressed as mean ± standard deviation for six rats; Values with different small letters in the same row were significantly different (P < 0.05).

Serum Erythropoietin and Ferritin Levels:

In the PHZ group, the serum erythropoietin and ferritin levels increased significantly (p < 0.05) compared to those of the control and the WGO groups. Both serum erythropoietin and ferritin levels decreased significantly in treated PHZ groups (P < 0.05) compared to the PHZ group but they still have high levels of erythropoietin and ferritin in comparison with both the control and the wheat germ oil groups (Table 2).

Table 2. Effect of wheat germ oil (WGO) on serum erythropoietin and ferritin levels in phenylhydrazine (PHZ) treated male albino rats.

	Animal groups				
	Control	WGO	PHZ	PHZ + WGO	WGO + PHZ
Erythropoietin (ng/ml)	0.31 ± 0.02[c]	0.27 ± 0.02[c]	1.13 ± 0.07[a]	0.92 ± 0.04[b]	0.86 ± 0.07[b]
Ferritin (ng/ml)	29.13 ± 2.19[d]	30.37 ± 0.86[c]	54.96 ± 1.92[a]	36.46 ± 2.34[b]	36.42 ± 2.34[b]

All data expressed as mean ± standard deviation for six rats; values with different small letters in the same row were significantly different (P < 0.05).

Serum Protein Profile:

Serum protein profile (total protein, albumin and globulin) levels of the PHZ group showed significant decreases (*P* < 0.05) compared to those of the control and the WGO groups. Serum protein levels in treated PHZ groups increased significantly compared to those of the PHZ group, while they were significantly low compared to their values in the control and the WGO groups (Table 3).

Table 3. Effect of wheat germ oil (WGO) on serum protein profile in phenylhydrazine (PHZ) treated male albino rats.

	Animal groups				
	Control	WGO	PHZ	PHZ + WGO	WGO + PHZ
Total protein (g/dl)	7.60 ± 0.10[a]	7.46 ± 0.15[a]	5.00 ± 0.35[c]	6.53 ± 0.31[b]	6.60 ± 0.17[b]
Albumin (g/dl)	4.0 6± 0.12[a]	4.03 ± 0.06[a]	2.73 ± 0.21[c]	3.53 ± 0.21[b]	3.70 ± 0.20[b]
Globulin (g/dl)	3.53 ± 0.15[a]	3.43 ± 0.15[a]	2.26 ± 0.25[c]	3.00 ± 0.10[b]	2.90 ± 0.10[b]

All data expressed as mean ± standard deviation for six rats; values with different small letters in the same row were significantly different (P < 0.05).

Serum Liver Function Parameters:

Serum liver function parameters (ALT, AST, TBIL and ALP) levels indicated significant increases (*P* < 0.05) in the PHZ group compared to those of the control and the WGO groups. Treated PHZ groups have significantly low levels of liver function parameters in comparison with those of the PHZ group but still have high levels in comparison with those of the control and the WGO groups (Table 4).

Table 4. Effect of wheat germ oil (WGO) on serum liver function parameters in phenylhydrazine (PHZ) treated male albino rats.

	Animal groups				
	Control	WGO	PHZ	PHZ + WGO	WGO + PHZ
ALT (U/L)	87.33 ± 1.15[d]	64 ± 2.00[e]	123 ± 3.00[a]	104.66 ± 2.51[b]	97.70 ± 2.17[c]
AST (U/L)	81.40 ± 4.1[d]	60 ± 1.001[e]	136 ± 2.00[a]	124.96 ± 2.95[b]	117.90 ± 2.26[c]
TBIL (mg/dl)	0.70 ± 0.02[c]	0.71 ± 0.01[c]	1.30 ± 0.25[a]	0.91 ± 0.15[b]	0.95 ± 0.10[b]
ALP (U/L)	164.23 ± 4.01[d]	167.34 ± 2.86[d]	214 ± 3.60[a]	195.26 ± 3.29[b]	187.90 ± 2.59[c]

All data expressed as mean ± standard deviation for six rats; values with different small letters in the same row were significantly different (P < 0.05).

Serum Kidney Function Parameters:

Serum kidney function parameters (creatinine, urea and uric acid) showed significant increases (*P* < 0.05) in the PHZ group compared to those of the control and the WGO groups. The treated PHZ groups have significantly low levels of kidney function parameters in comparison with the PHZ group but still have significantly high levels in comparison with both the control and the WGO groups (Table 5).

Table 5. Effect of wheat germ oil (WGO) on serum kidney function parameters in phenylhydrazine (PHZ) treated male albino rats.

	Animal groups				
	Control	WGO	PHZ	PHZ + WGO	WGO + PHZ
Creatinine (mg/dl)	0.90 ± 0.10[c]	0.93 ± 0.06[c]	1.52 ± 0.02[a]	1.17 ± 0.06[b]	1.10 ± 0.00[b]
Urea (mg/dl)	29.00 ± 1.00[c]	28.33 ± 1.52[c]	38.00 ± 1.00[a]	32.66 ± 1.53[b]	32.83 ± 0.76[b]
Uric Acid (mg/dl)	3.66 ± 0.15[c]	3.74 ± 0.10[c]	5.13 ± 0.41[a]	4.23 ± 0.21[b]	4.37 ± 0.15[b]

All data expressed as mean ± standard deviation for six rats; values with different small letters in the same row were significantly different (P < 0.05).

Oxidative Stress Parameters:

In the PHZ group, the serum MDA content increased significantly (*P* < 0.05) while the serum CAT, GPX and SOD activities decreased significantly compared to those of the control and the WGO groups. On the other hand, CAT, GPX, and SOD activities increased significantly and MDA contents

decreased significantly in treated PHZ groups compared to those of the PHZ group but they still have low activities of CAT, GPX and SOD and high contents of MDA in comparison with the control and the WGO groups (Table 6).

Table 6. Effect of wheat germ oil (WGO) on serum oxidative stress parameters in phenylhydrazine (PHZ) treated male albino rats.

	Animal groups				
	Control	WGO	PHZ	PHZ + WGO	WGO + PHZ
MDA (nmol/ml)	3.78 ± 0.27^{d}	3.04 ± 0.08^{e}	8.03 ± 0.43^{a}	7.08 ± 0.17^{b}	6.32 ± 0.39^{c}
CAT (mmol/l)	2.24 ± 0.19^{b}	2.83 ± 0.29^{a}	1.15 ± 0.14^{d}	1.63 ± 0.12^{c}	1.79 ± 0.03^{c}
GPx (ng/ml)	3.26 ± 0.11^{b}	3.57 ± 0.25^{a}	1.29 ± 0.11^{d}	1.74 ± 0.06^{c}	1.86 ± 0.06^{c}
SOD (U/L)	4.09 ± 0.17^{b}	4.75 ± 0.08^{a}	1.88 ± 0.19^{d}	2.56 ± 0.13^{c}	2.81± 0.18^{c}

All data expressed as mean ± standard deviation for six rats; values with different small letters in the same row were significantly different ($P < 0.05$).

DISCUSSION:

Phenylhydrazine is absorbed by inhalation, oral and dermal routes. After absorption, it causes oxidative stress in red blood cells (RBCs) by generation reactive oxygen species (ROS), which react with haemoglobin and changes the oxyhaemoglobin into methaemoglobin, hemichromes and other haemoglobin breakdown products such as Heinz bodies (Rifkind, 1965). The ROS generated by PHZ caused peroxidation of lipids and oxidative degradation of spectrin in the RBCs membrane skeleton (McKie *et al.,* 2001, McMillan *et al*., 2005; Shukla *et al*., 2012). After that PHZ translocates the phosphatidyl serine from the inner to outer of the plasma membrane and causes membrane lipid peroxidation due to lipid peroxidation RBCs to enter the spleen and are uptake by the macrophages (Maines, 1997). It is a signal for Phagocytosis of the cell under programmed death by macrophages. Phenylhydrazine also alters iron metabolism by increasing the expression of ferrous transporter (DMT1) in the spleen, duodenum and liver (Pandey *et al.,* 2014). Phenylhydrazine is widely known for its ability to cause haemolysis in both rats and humans (Shukla *et al.,* 2012). It has also been shown that PHZ lowered Hb concentration and RBCs count (Berger, 2007; Shwetha *et al.,* 2019). Phenylhydrazine also caused pronounced anaemia and a concomitant increase in the numbers of circulating leukocytes in Long-Evans rats. Leucocytosis was caused mainly by an elevation in mononuclear cells, most notably in the lymphocyte population (Naughton *et al*., 1990). The PHZ can cross-link red cell band 3 protein (senescent antigen), resulting in the binding of autologous immunoglobulin G (IgG). Recognition of this complex by macrophage Fc receptor mechanisms triggers rapid erythrophagocytosis in the spleen and possibly the liver (Dornfest *et al*., 1986). In the results of the current study rats treated with PHZ showed significant declines in haematological parameters (RBCs, Hb, Hct, PLT, and WBCs) indicating the presence of haemolytic anaemia PHZ- treated rats. The haemolytic anaemia induced by PHZ may be due to the overproduction of free radicals caused by PHZ indicated by the high content of MDA. When free radicals are produced during oxidation, they enter the circulation and induce lipid peroxidation and damage to the RBCs membrane skeleton, resulting in haemolysis and anaemia.

Administration of WGO increased haematological parameters in rats treated with PHZ, it may be due to the ability of WGO to reduce MDA content (free radical indicator) and increase the activities of antioxidant enzymes. The WGO is a rich source of natural antioxidants such as tocopherols, sterols and B complex vitamins (Liu, 2007). Vitamins E and C reduced the production of Heins bodies and methaemoglobin induced by PHZ (Berger, 2007). Also, vitamin E present in WGO serves as an inhibitor of oxidation processes occurring in tissues and protects cells from free radicals (Traber and Atkinson, 2007).

Erythropoietin, a hormone secreted by the kidneys, stimulates the formation of erythrocytes in the bone marrow (Suresh *et al*., 2020). The results of our study showed significant increases in serum erythropoietin and ferritin levels in rats treated with the PHZ. Zangeneh *et al*. (2019) mentioned that injection of PHZ increased both serum ferritin and erythropoietin levels in rats. Animals treated with PHZ produce more erythropoietin, which enhances stimulated hemopoietic activity (Latunde-Dada *et al*., 2006).

Wheat germ oil is a source of vitamin E that helps in enhancing the efficacy of therapeutic iron for infants and toddlers who have a dietary iron deficiency, potentially by reducing iron-induced inflammation and this lead to decreasing in ferritin level (Tang et al., 2016). In our study, the administration of WGO decreased serum ferritin and erythropoietin levels in rats treated with PHZ and this may be due to the ability of WGO to ameliorate haemolytic anaemia induced by PHZ.

In the present study, the PHZ group showed a significant decrease in serum total protein, albumin and globulin and these results are in agreement with those of Zangeneh *et al*. (2019) and Ayoade *et al*. (2020). According to the study results, there were disruptions in the kidney and liver function parameters after the injection of PHZ in rats that explained the reduction in serum protein levels. The reduction in serum proteins may be a result of kidney and liver failure caused by PHZ. According to Kale *et al*. (2019), after PHZ intoxication, serum albumin and total protein concentrations decreased. Amama *et al*. (2022) reported that the concentrations of urinary albumin, globulin and total protein increased after PHZ injection into rats and this explained their lower serum concentrations and indicated the incidence of liver and kidney injury.

Administration of WGO increased serum proteins in rats treated with PHZ and this may be due to the ability of WGO to ameliorate kidney and liver failure induced by PHZ. Alamery *et al*. (2022) demonstrated that WGO could improve the liver and kidney damage caused by thioacetamide in mice.

In the current study, elevated levels of serum liver function parameters (ALT, AST, TBIL, and ALP) in PHZ-treated rats were indicators for the liver injury induced by PHZ. Haemolysis caused by PHZ may be the reason for the inability of the liver to perform its functions. Zangeneh *et al*. (2019) reported that PHZ-induced haemolysis caused hepatomegaly and chronic liver failure. Haemolysis also increased the production of bilirubin (Mejia *et al*., 2008). Phenylhydrazine has been reported to induce haemolytic anaemia and hyperbilirubinemia in animals due to its haemolytic activity (Nawaz *et al*., 2016). Haemolytic anaemia and hyperbilirubinemia are closely interlinked with each other as the breakdown of red cells lead to jaundice (hyperbilirubinemia) and hyperbilirubinemia in turn triggers anaemia by inducing death of red cells (Lang *et al*., 2015). Elimination of haemolytic waste after haemolysis by the liver caused stress on the liver (Chakrabarti *et al*., 1990).

Serum liver function parameters (ALT, AST, TBIL, and ALP) level decreased after WGO administration to PHZ-treated rats that reflated the hepatoprotective effect of WGO. Akool (2015) reported that WGO is an effective hepatoprotective agent that prevents a variety of liver injuries. Also, the administration of WGO increased vitamin E in the liver providing liver tissues with high antioxidant defence (Mehranjani *et al*., 2007).

The induction of anaemia with PHZ caused significant increases in serum kidney function parameters (creatinine, urea and uric acid) in PHZ-treated rats. These results are in agreement with Ezeigwe *et al*. (2020). Haemoglobin and heme, is a common event in the pathogenesis of numerous diseases with heterogeneous etiologic factors and clinical features, such as microangiopathic haemolytic anaemias (Merle *et al*., 2018). Excessive or chronic intravascular haemolysis leads to renal injury (Schaer *et al*., 2013). Elevated serum uric acid, urea, and creatinine levels in the PHZ-treated rats indicated kidney damage induced by PHZ (Parvaz *et al*., 2022). PHZ-induced haemolysis can cause oxidative stress, which leads to renal injury (Leicht *et al*., 2018).

The study proved the effective role of WGO against PHZ-induced renal damage which is represented in improving kidney function parameters. Nagib (2018) demonstrated that oral administration of WGO improved renal function in rats. Hafez *et al*. (2019) attributed the protective effect of WGO against gentamicin-induced nephrotoxicity to its antioxidant and free radical scavenging properties.

In the present study, The PHZ treated rats showed significant decreases in CAT, GPX, and SOD activities with a significant increase in MDA content compared to the control rats' group. These results agree with Esterbauer and Cheeseman (1990) and also agreement with Bansode *et al*. (2019). Inhibition of the activities of antioxidant enzymes and the increased production of free radicals induced by PHZ resulted in oxidative stress (Kale *et al*., 2019).

The study demonstrated the ability of WGO to lower serum MDA content and increase CAT, GPX, and SOD activities in PHZ-treated rats reflecting the antioxidant activity of WGO. Wheat germ oil pre-administration significantly reduced the content of MDA and increased the levels of catalase and superoxide dismutase (Mohamed and Hamad, 2017). Vitamin E present in WGO serves as an inhibitor of oxidation processes occurring in tissues and protects cells from free radicals (Young and woodside, 2001). Vitamin E is also a powerful anti-inflammatory and antioxidant agent that protects humans from several diseases (Siekmeier *et al*., 2007). Vitamins E and C help to reduce the oxidative damage generated by PHZ in vitro (Claro *et al*., 2006). Additionally, WGO has a lot of unsaturated fatty acids, which may reduce oxidative stress (Alessandri *et al*., 2006).

In conclusion, the results of the present study revealed that wheat germ oil reduced phenylhydrazine toxicity by improving the haematological parameters, oxidative stress, liver function parameters, and kidney function parameters.

REFERENCES:

Akool E. 2015. Molecular mechanisms of the protective role of wheat germ oil against cyclosporin A-induced hepatotoxicity in rats. Pharm. Biol., 53(9): 1311-1317.

Alamery S, Zargar S, Yaseen F, Wani TA, Siyal A. 2022. Evaluation of the effect of wheat germ oil and olmutinib on the thioacetamide-induced liver and kidney toxicity in mice. Life (Basel), 12(6): 900. doi: 10.3390/life12060900.

Alessandri C, Pignatelli P, Loffredo L, Lenti L, Del Ben M, Carnevale R, Perrone A, Ferro D, Angelico F, Violi F. 2006. Alpha-linolenic acid-rich wheat germ oil decreases oxidative stress and CD40 ligand in patients with mild hypercholesterolemia. Arterioscler. Thromb. Vasc. Biol., 26(11): 2577-2578.

Amama EA, Udefa AL, Beshel FN, Archibong EA, Okpa S, Nwangwa JN, Felix NI, Akronyi G. 2022. Fresh palm oil improves impaired renal function in phenylhydrazine-induced anaemic Wistar rats via its anti-anaemic effect and modulation of expressions of pro-oxidant/antioxidants, inflammatory cytokines and caspase-3 in the kidneys. Physiol. Pharmacol., 26(3): 299-312.

Ara J, Fadriquela A, Ahmed MF, Bajgai J, Sajo MEJ, Lee SP, Kim TS, Jung JY, Kim CS, Kim SK, Shim KY, Lee KJ. 2018. Hydrogen Water Drinking Exerts Antifatigue Effects in Chronic Forced Swimming Mice via Antioxidative and Anti-Inflammatory Activities. Biomed. Res. Int., 2018:2571269. doi: 10.1155/2018/2571269.

Ayoade AA, Ogunware AE, Odekunle II, Adedigba PA. 2020. Effects of aqueous *colocasia esculenta* extracts on selected biochemical parameters in phenyl hydrazine induced male anemic albino rats. Asian J. Biochem. Genet. Mol., 3(4): 13-23.

Bansode FW, Arya KR, Meena AK, Singh RK. 2019. Haematenic and anti-haematenic effects of the methanol extract of *Saraca Indica* stem bark against phenylhydrazine induced anaemia in rats. World J. Pharm. Res., 8(9): 1176-1201.

Berger J. 2007. Phenylhydrazine haematotoxicity. J. Appl. Biomed., 5: 125–130.

Chakrabarti S, Naik AA, Reddy GR. 1990. Phenylhydrazine mediated degradation of bovine serum albumin and membrane proteins of human erythrocytes. Biochim. Biophys. Acta, 1028(1): 89-94.

Claro LM, Leonart MS, Comar SR, do Nascimento AJ. 2006. Effect of vitamins C and E on oxidative processes in human erythrocytes. Cell Bioch. Funct., 24(6): 531–535.

Dornfest BS, Lapin DM, Naughton BA, Adu S, Korn L, Gordon AS. 1986. Phenylhydrazine-induced leukocytosis in the rat. J. Leukoc. Biol., 39(1): 37-48.

Draper H, Hadley M. 1990. Malondialdehyde determination as index of lipid peroxidation. Methods Enzymol., 186: 421-431.

Duncan BD. 1957. Multiple range tests for correlated and heteroscedastic means. Biometrics, 13(2): 164-176.

Esterbauer H, Cheeseman KH. 1990. Determination of aldehydic lipid peroxidation products: malondialdehyde and 4- hydroxynonenal. Methods Enzymol., 186: 407-421.

Ezeigwe OC, Nzekwe FA, Nworji OF, Ezennaya CF, Iloanya EL, Asogwa KK. 2020. Effect of aqueous extract of F. capensis leaves and its combination with C. aconitifolius leaves on essential biochemical parameters of phenylhydrazine-induced anemic rats. J. Exp. Pharmacol., 12: 191-201.

Fossati P, Prencipe L, Berti G. 1980. Use of 3,5-dichloro 2hydroxybenzenesulfonic acid/4-aminophenazone chromogenic system in direct enzymic assay of uric acid in serum and urine. Clinic. Chem., 26(2): 227-231.

Gomez A, Ossa E. 2002. Quality of wheat germ oil extracted by liquid and supercritical carbon dioxide. JAOCS, 77: 969-974.

Hafez L, Ali F, El-Ghoneimy A, Abdel-Aziz M. 2019. Nephro-Protective Effect of Wheat Germ Oil on Gentamicin-Induced Acute Nephrotoxicity in Wistar Albino Rat. SVU-IJVS, 2(1): 51-67.

Hamed BM, Awadin WF, EL-Seady YY, Abu-Heakal N. 2013. Biochemical and histopathological effect of wheat germ oil against atherosclerosis risk in hyperlipidemic rats. Egypt. J. Comp. Path. Clinic. Path., 26(2): 45-60.

Hassanein MMM, Abedel-Razek AG. 2009. Chromatographic quantitation of some bioactive minor components in oils of wheat germ and grape seeds produced as by-products. J. Oleo. Sci., 58(5): 227-233.

Irmak S, Dunford NT. 2005. Policosanol contents and compositions of wheat varieties. J. Agric. Food Chem., 53(14): 5583-5586.

Kahlon TS. 1989. Nutritional implications and uses of wheat and oat kernel oil. Cereal Foods World, 34(10): 872–875.

Kakkar P, Das B, Viswanathan PN. 1984. A modified spectrophotometric assay of superoxide dismutase. Indian J. Biochem. Biophys., 21(2): 130-132.

Kale OE, Awodele O, Akindele AJ. 2019. Protective effects of *Acridocarpus smeathmannii* (DC.) Guill. & Perr. root extract against phenylhydrazine-induced haematotoxicity, biochemical changes, and oxidative stress in rats. Biochem. Insights, 12:1178626419883243. doi: 10.1177/1178626419883243.

Koffuor GA, Amoateng P, Andey TA. 2011. Immunomodulatory and erythropoietic effects of aqueous extract of the fruits of *Solanum torvum Swartz* (*Solanaceae*). Pharmacognosy Res., 3(2): 130–144.

Koller A. 1984. Total serum protein. in: “Clinical chemistry: theory, analysis, correlation. (Kaplan LA, Pesce AJ. Eds)”. CV Mosby Co., St. Louis, pp. 1316-1324.

Lang E, Gatidis S, Freise NF, Bock H, Kubitz R, Lauermann C, Orth HM, Klindt C, Schuier M, Keitel V, Reich M, Liu G, Schmidt S, Xu HC, Qadri SM, Herebian D, Pandyra AA, Mayatepek E, Gulbins E, Lang F, Häussinger D, Lang KS, Föller M, Lang PA. 2015. Conjugated bilirubin triggers anaemia by inducing erythrocyte death. Hepatology, 61(1): 275-284.

Latunde-Dada GO, McKie AT, Simpson RJ. 2006. Animal models with enhanced erythropoiesis and iron absorption. Biochim. Biophys. Acta, 1762(4): 414-423.

Leenhardt F, Fardet A, Lyan B, Gueux E, Rock E, Mazur A, Chanliaud E, Demigné C, Rémésy C. 2008. Wheat germ supplementation of a low vitamin E diet in rats affords effective antioxidant protection in tissues. J. Am. Coll. Nutr., 27(2): 222-228.

Leicht HB, Weinig E, Mayer B, Viebahn J, Geier A, Rau M. 2018. Ceftriaxone-induced hemolytic anaemia with severe renal failure: a case report and review of literature. BMC Pharmacol. Toxicol., 19(1): 67. doi: 10.1186/s40360-018-0257-7.

Liu RH. 2007. Whole grain phytochemicals and health. J. Cereal Sci., 46(3): 207-219.

Luck H. 1965. Catalase. In: "Method of Enzymatic Analysis. (Bergmeyer HU. Ed.)". Academic Press, New York and London, pp. 885-894. http://dx.doi.org/10.1016/B978-0-12-395630-9.50158-4

Maines MD. 1997. The heme oxygenase system: a regulator of second messenger gases. Annu. Rev. Pharmacol. Toxicol., 37: 517– 554.

Mazzachi BC, Peake MJ, Ehrhardt V. 2000. Reference range and method comparison studies for enzymatic and Jaffé creatinine assays in plasma and serum and early morning urine. Clin. Lab., 46(1-2): 53-55.

McKie AT, Barrow D, Latunde-Dada GO, Rolfs A, Sager G, Mudaly E, Mudaly M, Richardson C, Barlow D, Bomford A, Peters TJ, Raja KB, Shirali S, Hediger MA, Farzaneh F, Simpson RJ. 2001. An iron-regulated ferric reductase associated with the absorption of dietary iron. Science, 291(5509): 1755-1759.

McMillan DC, Jensen CB, Jollow DJ. 1998. Role of lipid peroxidation in dapsone-induced hemolytic anaemia. J. Pharmacol. Exp. Ther., 287(3): 868-876.

McMillan DC, Powell CL, Bowman ZS, Morrow JD, Jollow DJ. 2005. Lipids versus proteins as major targets of pro-oxidant, direct-acting hemolytic agents. Toxicol. Sci., 88(1): 274-283.

Mehranjani MS, Abnosi MH, Mahmodi M. 2007. Preventing effects of wheat germ oil on sex hormones, liver enzymes, lipids and proteins in rat serum following treatment with pnonylphenol. J. Biol. Sci., 7(8): 1408–1411.

Mejia GB, Sanz CR, Avila MM, Peraza AV, Guzmán DC, Olguín HJ, Ramírez AM, Cruz EG. 2008. Experimental hemolysis model to study bilirubin encephalopathy in rat brain. J. Neurosci. Methods, 168(1): 35-41.

Merle NS, Grunenwald A, Figueres ML, Chauvet S, Daugan M, Knockaert S, Robe-Rybkine T, Noe R, May O, Frimat M, Brinkman N, Gentinetta T, Miescher S, Houillier P, Legros V, Gonnet F, Blanc-Brude OP, Rabant M, Daniel R, Dimitrov JD, Roumenina LT. 2018. Characterization of renal injury and inflammation in an experimental model of intravascular hemolysis. Front. Immunol., 9: 179. doi: 10.3389/fimmu.2018.00179.

Mohamed HRH, Hamad SR. 2017. Nullification of aspirin induced gastrotoxicity and hepatotoxicity by prior administration of wheat germ oil in *Mus musculus*: histopathological, ultrastructural and molecular studies. Cell Mol. Biol. (Noisy-le-grand), 63(8): 120-130.

Moss DW. 1982. Alkaline phosphatase isoenzymes. Clin. Chem., 28(10): 2007-2016.

Nagib RM. 2018. Protective effect of wheat germ powder and oil on metabolic disturbances in rats. J. food dairy sci., 2018(0): 21-27.

Naughton BA, Dornfest BS, Bush ME, Carlson CA, Lapin DM. 1990. Immune activation is associated with phenylhydrazine-induced anaemia in the rat. J. Lab. Clin. Med., 116(4): 498-507.

Nawaz H, Shad MA, Iqbal MS. 2016. Optimization of phenylhydrazine induced hyperbilirubinemia in experimental rabbit. Exp. Anim., 65(4): 363-372.

Ogbe RJ, Adoga GI, Abu AH. 2010. Anti-anaemia potentials of some plant extracts on phenylhydrazine induced anaemia in rabbits. J. Med. Plants Res., 4(8): 680-684.

Pandey K, Meena AK, Jain A, Singh RK. 2014. Molecular mechanism of phenylhydrazine induced haematotoxicity: A review. AJPCT, 2(3): 390-394.

Parvaz N, Amin F, Askari N, Khademalhosseini M, Falahati-pour SK, Falahati-pour SK. 2022. The protective effect of *pistacia vera* pericarp on kidney function in rats with hemolytic anaemia. Res. J. Pharmacognosy, 9(1): 17-28.

Rifkind RA. 1965. Heinz body anaemia: an ultrastructural study. II. Red cell sequestration and destruction. Blood, 26(4): 433-448.

Schaer DJ, Buehler PW, Alayash AI, Belcher JD, Vercellotti GM. 2013. Hemolysis and free hemoglobin revisited: exploring hemoglobin and hemin scavengers as a novel class of therapeutic proteins. Blood, 121(8): 1276-1284.

Schumann G, Klauke R. 2003. New IFCC reference procedures for the determination of catalytic activity concentrations of five enzymes in serum: preliminary upper reference limits obtained in hospitalized subjects. Clin. Chim. Acta, 327(1-2): 69-79.

Shukla P, Yadav NK, Singh P, Bansode FW, Singh RK. 2012. Phenylhydrazine induced toxicity: a review on its haematotoxicity. Int. J. Basic Appl. Med. Sci., 2(2): 86–91.

Shwetha BR, Siddalingaprasad HS, Swamy S, Nagalakshmi NC, Hariprasad MG. 2019. Mechanism of haematotoxicity induced by phenylhydrazine. J. Appl. Pharm. Res., 7(4): 1-6.

Siekmeier R, Steffen C, März W. 2007. Role of oxidants and antioxidants in atherosclerosis: results of in vitro and in vivo investigations. J. Cardiovasc. Pharmacol. Ther., 12(4): 265-282.

Sjövall O, Virtalaine T, Lapveteläinen A, Kallio H. 2000. Development of rancidity in wheat germ analyzed by headspace gas chromatography and sensory analysis. J. Agric. Food Chem., 48(8): 3522- 3527.

Spivak JK. 2002. Polycythemia Vera: myths, mechanisms, and management. Blood, 100(13): 4272-4290.

Suresh S, Rajvanshi PK, Noguchi CT. 2020. The many facets of erythropoietin physiologic and metabolic response. Front. Physiol., 10: 1534. doi: 10.3389/fphys.2019.01534.

Tabacco A, Meiattini F, Moda E, Tarli P. 1979. Simplified enzymic/colorimetric serum urea nitrogen determination. Clin. Chem., 25(2): 336-337.

Tang M, Frank DN, Sherlock L, Ir D, Robertson CE, Krebs NF. 2016. Effect of Vitamin E With Therapeutic Iron Supplementation on Iron Repletion and Gut Microbiome in US Iron Deficient Infants and Toddlers. J. Pediatr. Gastroenterol. Nutr., 63(3): 379-385.

Traber MG, Atkinson J. 2007. Vitamin E, antioxidant and nothing more. Free Radic. Biol. Med., 43(1): 4-15.

Webster D, Bignell AH, Attwood EC. 1974. An assessment of the suitability of bromocresol green with isolated serum globulin fractions. Clin. Chem. Acta, 53(1): 109-115.

Young DS, Friedman RB. 2002. Effects of disease on clinical lab tests. 4th ed, Vol. 1 and 2. AACC, Washington DC. Clin. Chem., 48(4): 682–683.

Young IS, Woodside JV. 2001. Antioxidants in health and disease. J. Clin. Pathol., 54(3): 176-186.

Zangeneh MM, Zangeneh A, Salmani S, Jamshidpour R, Kosari F. 2019. Protection of phenylhydrazine-induced hematotoxicity by aqueous extract of *Ocimum basilicum* in Wistar male rats. Comp. Clin. Pathol., 28: 331–338.

Zhokhov SS, Broberg A, Kenne L, Jastrebova AJ. 2010. Content of antioxidant hydroquinones substituted by β-1, 6- linked oligosaccharides in wheat milled fractions, flours and breads. Food Chem., 121(3): 645-652.

تأثير زيت جنين القمح علي سمية الفينيل هيدرازين في ذكور الجرذان البيضاء

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زيت جنين القمح (WGO) منتج خاص. يتم استخدامه لقيمته الغذائية ، وخاصة لاحتوائه على نسبة عالية من فيتامين هـ. الهدف من هذه الدراسة هو معرفة مدى كفاءة زيت جنين القمح ضد السمية الناجمة عن تناول الفينيل هيدرازين في ذكور الجرذان البيضاء. تم تقسيم الجرذان إلى خمس مجموعات (6 جرذان لكل مجموعة). المجموعة الضابطة، مجموعة زيت جنين القمح (تم معاملتها يوميا بـ 300 مليجرام / كجم من وزن الجسم عن طريق الفم لمدة 14 يوم)، مجموعة الفينيل هيدرازين (تم حقنها داخل الصفاق بجرعة يومية مقدارها 60 مليجرام / كجم من وزن الجسم لمدة ثلاثة أيام متوالية)، المجموعة المعالجة (تم حقنها بالفينيل هيدرازين لمدة ثلاثة أيام ثم معاملتها بزيت جنين القمح لمدة 11 يوم) والمجموعة الواقية (تم معاملتها بزيت جنين القمح لمدة 11 يوما ثم حقنها بالفينيل هيدرازين). وقد استمرت التجربة لمدة 14 يومًا. أظهرت النتائج أن الفينيل هيدرازين قد تسبب في انخفاض قياسات الدم (RBCs, Hb, Hct, and PLT) ، والبروتينات في مصل الدم (البروتين الكلي والألبومين والجلوبيولين) والإنزيمات المضادة للأكسدة (GPX and CAT, SOD) وزيادة في كرات الدم البيضاء (WBCs) وهرمون الإريثروبويتين ومستوى الفريتين وزيادة في وظائف الكبد (ALT, AST, TBIL and ALP) و وظائف الكلي (كرياتينين و البولينا وحمض البوليك) ومحتوى المالونداي الدهيد. من ناحية أخرى، قد احدثت المعاملة بزيت جنين القمح تحسنا في قياسات الدم، وهرمون الإريثروبويتين والفريتين، والبروتينات،ووظائف الكبد ووظائف الكلى وأنشطة الإنزيمات المضادة للأكسدة، ومحتوي المالونداى الدهيد في الجرذان المحقونة بالفينيل هيدرازين. وقد كشفت نتائج الدراسة الحالية أن زيت جنين القمح له دور علاجي ووقائي في معالجة السمية الناجمة عن الفينيل هيدرازين والوقاية منها.