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Evaluate the protective effect of *Plantagoovata* against the toxic effect of phenylhydrazine on male rat sex hormones and sperm

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Abstract

The aim of present study is to evaluate the protective role of *Plantagoovata* seeds against the toxic effect phenylhydrazine on male sex hormones. 35 animals (Albino male rats *rattusnorvegicus*) were used. The animals were distributed into 7 groups for each group of 5 rats, randomly as follows: the control group. Group second: rats injected (Intraperitoneal) 40 mg/kg phenylhydrazine. Third group: rat treated with alcoholic extract of *P. ovata* seeds (100 mg / kg). Group fourth: rat administrate Hemavir at 1ml orally/kg daily. The fifth group: rat administrates phenylhydrazine and treated with the alcoholic extract. Group six: rat administrates phenylhydrazine and haemavir. Group Seven: phenylhydrazine as well as the extract in addition to and haemavir. The concentrations of testosterone, SSH, and ICSH were measured using the TOSOH AIA-360 device of Japanese origin 2019. The finding show the PHZ led to a significant decrease ($P \leq 0.05$) in Testosterone, SSH and ICSH levels compared with the control group, while treated with alcoholic extract of *P. ovata* seeds and the drug Hemavir. Also, other groups indicated a significant increase in testosterone, SSH and ICSH. Also, the microscopic examination of sperm slides shows different changes.

Keywords: Testosterone; *Plantagoovata*; phenylhydrazine; Hemavir.

Introduction

Anemia is a decrease in the number of red blood cells and decrease in the concentration of hemoglobin [1]. The hemoglobin rate is less than 13.5 g / dl for a male, and less than 12 g / dl for a female [2]. The symptoms of hemolytic anemia are early fatigue, fatigue and other symptoms including headache, numbness and cold extremities, dizziness, chest pain, short nails, Rapid and irregular heartbeat, paleness of the mucous membranes and paleness of the skin, general weakness and loss of physical activity [3]. In addition, anemia leads to impaired sperm formation

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associated with hemolytic anemia [4]. Anemia leads to a lack of oxygen in the various organs and tissues, including the testicles. Males produce a large amount of sperm every day, which indicates that sperm formation in the testicular tubes takes place at a high reproduction rate, which requires a large consumption of oxygen [5-7].

Because of the harmful effects of anemia, many drugs are used. Medicinal drug such asHemafer drug, (called IPC- hydroxide polymaltose complex), which is one of the treatments that are used in iron deficiency in the body [8]. Medicinal plants have been used because they are effective preventively, have low cost, imperceptible toxic effects, and are easily accessible to all [8-9]. The secondary metabolites of these plants, such as Flavonoid, Phenolic and many other phytochemicals, have clear hematological activity in the treatment of many blood disorders [10-11].

Plantagoovata is a medicinal plant that is rich in many phenolic compounds, flavonoid, alkaloid, terpinoid and vitamin C. These compounds act as antioxidant and anti-inflammatory effect, hypercholesterolemia, hypoglycemia, anti-inflammatory, anti-viral, analgesic, antioxidant, anti-cancer, immune modifier, anti-hypertensive agent [12-13]. It is used to treat many diseases including: hepatitis, skin diseases, infectious diseases, diseases and problems related to the organs of the digestive system, respiratory system, reproduction and circulation [14-15].Therefore, the present study aimed to evaluate the protective role of plantagoovata seeds against the toxic effect phenylhydrazine on male sex hormones.

Materials & methods

Collecting seeds of *Plantagoovata*

The seeds of Plantagoovata were obtained from the rural areas adjacent to the city of Tikrit, to the north of Baghdad, and then the seeds were left for a period of 10 days to dry well.

Alcoholic extract of Plantagoovata seeds

50g of *P. ovata* seed powder were weighed and then placed in a 1000 ml conical flask, then 500 ml of Absolute methanol was added to it and left for the mixing process for one hour with continuous shaking by a Stirrer magnatic device at a speed of 100 rpm Then, the beaker was covered with a black cover (opaque) and its mouth

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was blocked to prevent contamination, and then left on the shaker incubator for 24 hours, and the next day the solution was filtered by several layers of medical gauze until the mixture was filtered and until the mixture was confirmed Remove poorly crushed plankton and then filter through filter papers, put the filter in glass Petri dishes after washing and sterilize well, and then put the dishes in the electric oven at a temperature of 40 degrees Celsius, and after a time (3-4) hours it was done Obtaining the extract as it was collected from the dishes and placed in opaque, black glass bottles, after which it was preserved in the refrigerator until the extract was used in the experiment [16].

Detection of active substances

The analysis of *P. ovata*seeds was carried out in the laboratories of the Department of Environment and Water / Ministry of Science and Technology in order to estimate the total concentrations of minerals, according to the method reported by Wodaje Addis2017 using atomic absorption spectroscopy (SHIMADZU Model AA7000).

Animal model

In the experiment, 35 animals (Albino male rats *rattusnorvegicus*) were used, which were obtained from the animal house of Veterinary Medicine College, Tikrit University with weights ranging from (160-210g) and ages ranging from (11-13) weeks. Food and water were given continuously and in sufficient quantities throughout the period of breeding and treatment. The study period continued from October 13 to November 13, 2020.

Experience Design

The experimental animals were distributed into 7 groups for each group of 5 rats, randomly as follows:

- The first group, the control group: The rats in this group were given a standard feed with drinking water and dosed with distilled water during 30 days.
- Group II: Rats in this group were (Intraperitoneal) injected under the skin at a concentration of 40 mg / kg of body weight / 48 hours [20] in addition to the standard diet with drinking water throughout the trial period.

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- Third group: This group was treated with alcoholic extract of *P. ovata* seeds (100 mg / kg) in addition to the standard diet with drinking water throughout the experiment period.
- Group 4: This group was given Hemavir at 1ml orally/kg daily [21] in addition to the standard diet with drinking water throughout the trial period.
- The fifth group: This group was given phenylhydrazine as above, in addition to oral dosing with the alcoholic extract of the seeds of *P. ovata* at the same concentration mentioned above, in addition to the standard diet with drinking water throughout the trial period.
- Group six: With the same concentrations above, this group was given phenylhydrazine in addition to the dose of haemavir, in addition to the standard diet with drinking water throughout the trial period.
- Group seven: With the same concentrations above, this group was given phenylhydrazine as well as the extract in addition to the drug, in addition to the standard ration with drinking water throughout the trial period.

Biochemical Measurement

The concentrations of testosterone, SSH, and ICSH were measured using the TOSOH AIA-360 device of Japanese origin 2019 which contains (25) pits numbered in sequence, where samples were placed in each specific well (numbered) Inside the device and placing the reagent in a special container next to the wells and measuring tools designed for this device for each one of the hormones.

Semen collection

The abdomen of the animal was opened using a dissection kit to obtain the testicles that the epididymis is attached to. Then the epididymis was separated from the testicles and placed in a glass petri dish, and using a sharp scalpel, the epididymis was cut into parts and a normal saline was added to them according to the method of [17].

Microscopic Examination

A drop of semen was mixed with a drop of eosin dye and Necrosin dye, and then a sperm film was prepared. After that, the slide was washed by running water (tap), then left to dry and imaging under the microscope. Samples were examined with an

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Olympus light microscope, where images of the sperm were taken with a Samsung digital camera.

Results

Active compounds in *P. ovata*

After a process of estimating the active substances by using atomic absorption spectroscopy, table (1) showed active compounds in *P. ovata* seeds

Table (1): Results of the active elements in *P. ovata* seeds

Result (ppm)	Element
96.7	**Calcium (Ca)**
86.6	**Iron (Fe)**
78.9	**Potassium (K)**
53.6	**Selenium (Se)**
46.9	**Copper (Cu)**
41.6	**Zinc (Zn)**

Sex hormone

Table (2) shows that the treatment of male rats with PHZ led to a significant decrease ($P \leq 0.05$) in the concentration of the hormones Testosterone, SSH and ICSH compared with the control group, while treatment of rats treated with PHZ using the alcoholic extract of *P. ovata* seeds and the drug Hemavir The medical results indicated a significant increase in the concentration of testosterone, SSH and ICSH compared with the group treated with PHZ, as well as when the healthy rats administrate with alcoholic extract of *P. ovata* seeds and Hemavir, we notice a significant increase in testosterone and SSH, ICSH compared with the control group.

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Table (2): The effect of treatment with phenylhydrazine at a dose (40 mg / kg / 48 hours) and the alcoholic extract of *P. ovata* seeds at a dose (1 ml / animal at a concentration of 100%) and hemavir drug (1 ml / kg / day) on the concentration of sex hormones for male white rats

Hormones / Groups	ICSH(mIU/ml)	SSH(mIU/ml)	Testosterone (ng/ml)
Control	0.209 ± 0.009 D	0.212 ± 0.019 a	314.0 ± 25.4 C
PHZ	0.199 ± 0.005 E	0.186 ± 0.009 B	234.0 ± 33.2 C
Plantagoovata	0.211 ± 0.005 C	0.230 ± 0.01 A	712.0 ± 27.9 A
Hemafer	0.201 ± 0.004 D	0.217 ± 0.017 A	598.3 ± 44.5 B
PHZ + P. ovata	0.213 ± 0.003 C	0.225 ± 0.023 A	541.0 ± 36.7 B
PHZ + Hemafer	0.217 ± 0.014 A	0.215 ± 0.007 A	335.2 ± 19.7 C
PHZ +P.ovata + Hemafer	0.214 ± 0.008 B	0.222 ± 0.008 A	434.0 ± 33.2 B

Sperm form

In the current study, the microscopic examination of sperm slides shows different changes. Where the control group shows the normal form of sperm (fig: 1). thetreatment with phenylhydrazine at a dose (40 mg / kg / 48 hours) show abnormal form of sperm (fig: 2). treatment with phenylhydrazine at a dose (40 mg / kg / 48 hours) and the alcoholic extract of *P. ovata* seeds show normal sperm (fig: 3). treatment with phenylhydrazine at a dose (40 mg / kg / 48 hours) and andhemavir drug (1 ml / kg / day) show abnormal form of sperm (fig: 4). treatment with phenylhydrazine at a dose (40 mg / kg / 48 hours) and the alcoholic extract of *P. ovata* seeds at a dose (1 ml / animal at a concentration of 100%) and hemavir drug (1 ml / kg / day) shows the normal form of sperm (fig: 5). Finally, alcoholic extract of *P. ovata* seeds show normal sperm (fig: 6).

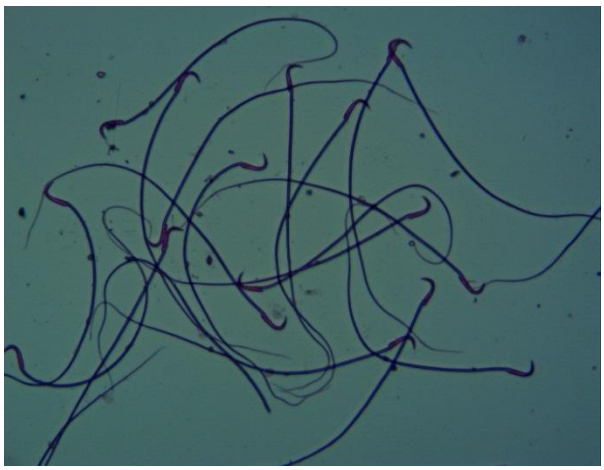

Figure (1): sperm of control group.

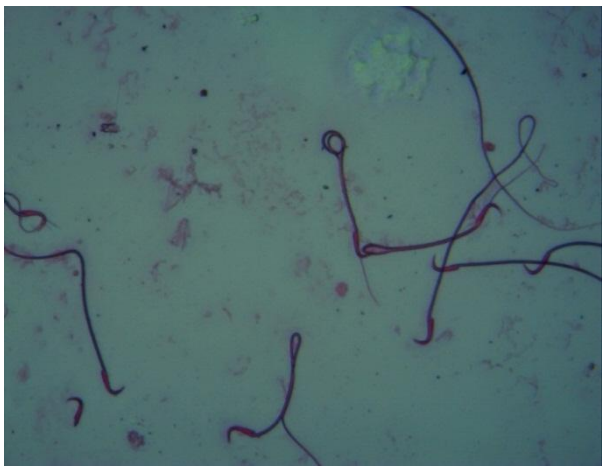

Figure (2): abnormal sperm of PHZ group.

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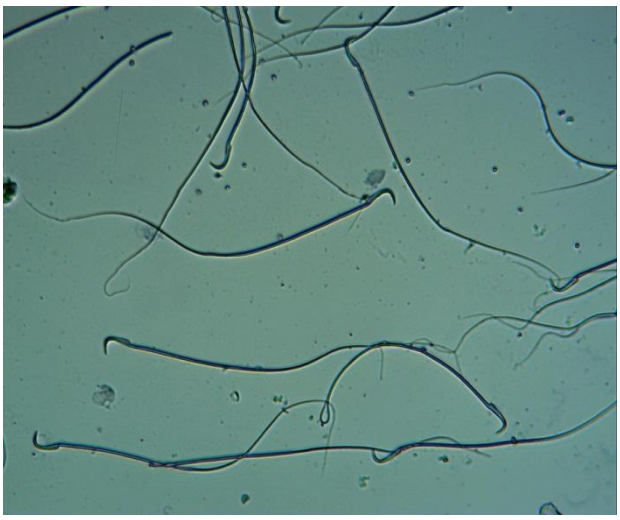

Figure (3): normal sperm of PHZand ethanolic extract group.

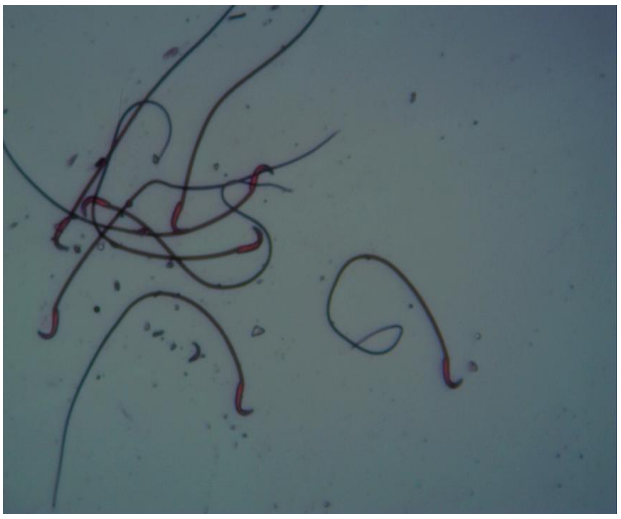

Figure (4): abnormal sperm of PHZand hemavirgroup.

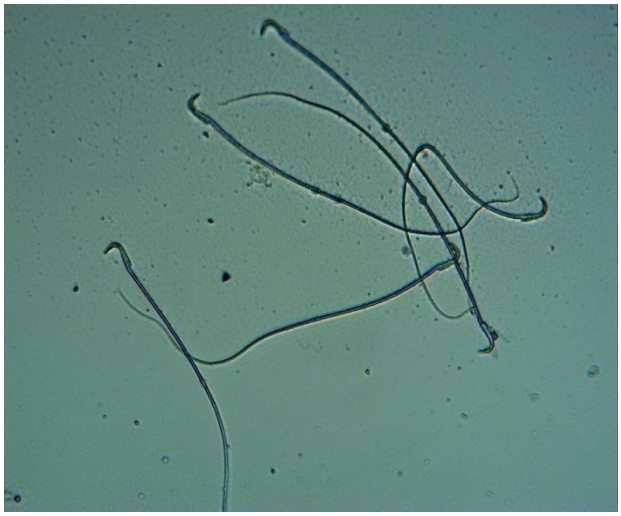

Figure (5): normal sperm of PHZand hemavirwith ethanolic extract group.

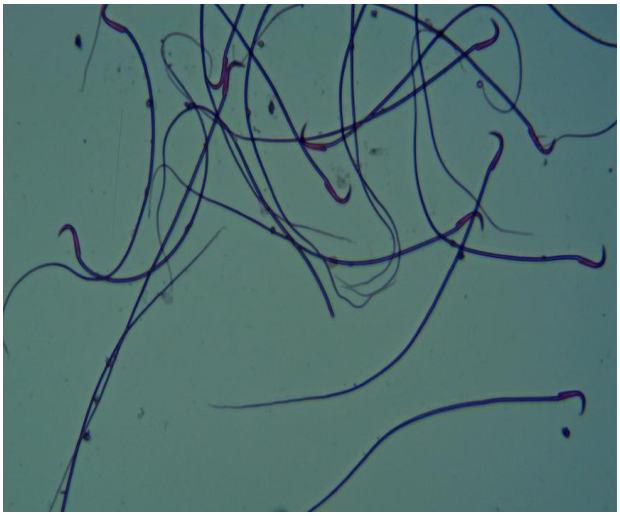

Figure (6): normal sperm of ethanolic extract group.

Discussion

In the current study, a decrease in the levels of testosterone, SSH and ICSH hormones was found in the group administered with phenylhydrazine compared to the control group.The decrease in testosterone level in the phenylhydrazine group may be associated with increased production of free radicals due to phenylhydrazine which in turn causes disturbances in the functions of Leydig and Sertol cells [23]. In Leydig cells, an increase in oxidative stress leads to a decrease in the synthesis and secretion of testosterone hormone, as the reduction of this hormone is an effective factor in inducing disturbance of spermatogenesis and a decrease in the number of sperms in

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the epididymis [24].The results of the current study are in agreement with the study of [20], who indicated that the level of testosterone in phenylhydrazine group had decreased compared to the control group, they suggest that phenylhydrazine causes damage to testicular tissues through oxidative stress that causes the formation of free radicals, which in turn lead to the destruction of Leydig cells, and therefore, due to the decrease in the number of Leydig cells, the amount of testosterone decreased, and this explains the results of the current study and the reason for the decrease in the level of this hormone in the phenylhydrazine group compared to the control group.

About the role of *P. ovata*in the current study, the results showed a significant improvement in the levels of testosterone, SSH and ICSH when used to treat the toxic effect of phenylhydrazine, and in a study conducted to prove the effective role of the *P. ovata*against the toxicity of carbon tetrachloride, which caused a decrease in testosterone levels. There was a significant improvement in the levels of the testosterone after using the alcoholic extract of *P. ovata*, and they suggestthat the components in the extract, represented by cyclosides, flavonoids, tropionides and alkaloids that maintain testicular tissue, sexual vitality and characteristics of sperm. [25]. In terms of the role of the iron supplement Hemafer used in the current study, there was no significant improvement in the levels of androgenic hormones. In sperm results, a morphological deformation was found after it was induced by anemia with Phenylhydrazine, and this result is consistent with the study of [20] when introducing anemia with this substance PHZ, as this study showed that there is a clear relationship between ROS production and apoptosis, which caused damage to DNA in the sperm. After using the seed extract in the treatment, and through our study and examination of the seeds, the results showed that it contains effective mineral elements such as Selenium. Selenium is one of the elements that maintain the natural shape of the sperm [19].

Conclusion

The current study showed that *Plantagoovata* has an antioxidant effect and enhances sex hormones and sperm.

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