University of Thi-Qar Journal of agricultural research

Thi-Qar Journal of agricultural research

ISSN Onlin:2708-9347, ISSN Print: 2708-9339 Volume 14, Issue 1 (2025) PP 73-78

https://jam.utq.edu.iq/index.php/main *https://doi.org/10.54174/utjagr.v13i1.323*

A study to determine the protective effect of the aqueous extract of *beta vulgaris* and the drug hydroxyurea in female laboratory rats induced with hemolytic anemia

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Abstract

The study was conducted to investigate the effect of *beta vulgaris* extract and hydroxyurea on oxidative enzymes in laboratory female rats in which hemolytic anemia was induced using Phenylhydrazine. The groups were divided into seven groups, each group containing 5 females. The first control group was injected with distilled water, the second group was injected with Phenylhydrazine 0.1 mg/kg, the third group was injected with beetroot extract 100 mg/kg. The fourth group was injected with a mixture of beetroot extract and hydroxyurea, the fifth group was injected with Phenylhydrazine and injected with beetroot extract, the sixth group was injected with Phenylhydrazine and injected with hydroxyurea, the seventh group was injected with a mixture of hydroxyurea and injected with Phenylhydrazine and beetroot extract. Through statistical analysis of oxidative enzymes, the results showed an increase in the level of (Superoxide Dismutase) SOD enzyme in the fourth, sixth and seventh groups that were dosed with hydroxyurea compared to the control group, as well as an increase in the level of (Malondialdehyde)MDA enzyme in the fourth, sixth and seventh groups that were dosed with hydroxyurea. The results of the (Glutathion Peroxidase) GPX enzyme also showed no significant differences between the treatment groups at the probability level of $P \leq 0.05$. As for the (Catalase) CAT enzyme after 10 minutes, there was a significant increase in the second group compared to the control group and the rest of the treatment groups. The results also showed that the CAT enzyme after 30 minutes had a significant increase in the sixth group when compared to the control group. Conclusion: The study showed that the best group for inhibiting oxidative enzymes was the one that received the medical drug hydroxyurea.

Key Word: Beta vulgaris, Hydroxyurea, oxidative enzymes

I. Introduction:

Hemolytic anemia is a type of anemia caused by red blood cells being destroyed at a faster rate than they are produced. This results in a low red blood cell count, which causes tissue oxygen deprivation. This type of anemia has genetic or acquired causes, such as defects in the cell membrane or cell enzymes, immune disorders, or toxic effects (Deshpande *et al*., 2020). In recent years, (*Beta vulgaris* Extract) has received attention from researchers due to its antioxidant properties. Beetroot contains active compounds such as betalain, which help reduce oxidative stress and improve blood health. It is also believed that it may contribute to reducing the severity of hemolytic anemia by enhancing enzyme activity and reducing damage caused by oxidative stress (Cotoraci *et al*., 2021). On the other hand, hydroxyurea is used as an effective drug to treat some forms of hemolytic anemia, such as sickle cell anemia. This drug works by enhancing the production of fetal hemoglobin (HbF), which reduces the incidence of hemolysis (Ware *et al*., 2009). Oxidative enzymes, such as glutathione peroxidase and catalase, are essential for protecting red blood cells from damage caused by free radicals. In cases of hemolytic anemia, oxidative stress may increase due to an imbalance between free radicals and the enzymes that neutralize them, which worsens the condition. Antioxidants, such as those

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found in beetroot extract, are thought to help improve enzyme activity and relieve oxidative stress (Winterbourn, 2008).

II. Materials and Methods

Experimental design:

The experiment was conducted on 35 Female laboratory rats, distributed into seven groups, each group containing five rats as follows:

The first group, which is the control group, the rats in this group were given a standard diet with drinking water and were given distilled water for 30 days.

The second group: The rats in this group were injected with PHZ subcutaneously with an intraperitoneal needle at a dose of 0.1 mg/kg over 48 hours, in addition to the standard diet accompanied by drinking water.

The third group: This group was treated with the aqueous extract of beetroot 100 mg/kg orally in addition to the standard diet with drinking water throughout the experiment period.

The fourth group: This group was given hydroxyurea at a rate of one ml/kg orally on a daily basis in addition to the standard diet with drinking water throughout the experiment period.

Group Five: This group was given PHZ at a concentration of 0.1 mg/kg of body weight/48 hours in addition to being given orally with aqueous extract of beetroot at a concentration of 100 mg/kg in addition to the standard feed with drinking water throughout the experiment period.

Group Six: This group was given PHZ at a concentration of 0.1 mg/kg of body weight/48 hours in addition to a dose of hydroxyurea at a rate of one ml/kg orally on a daily basis, in addition to the standard feed with drinking water throughout the experiment period.

Group Seven: This group was given PHZ at a concentration of 0.1 mg/kg 48, in addition to aqueous extract of beetroot at a concentration of 100 mg/kg, in addition to the drug at a rate of one ml/kg orally on a daily basis in addition to the standard feed with drinking water throughout the experiment period.

Collection of beetroot:

Beetroot was obtained from the local market in Nasiriyah city in the winter of 2023, where the plant was taken and washed with sterile distilled water, then dried on filter paper at laboratory temperature, then crushed and ground using an electric grinder, and stored in sterile, tightly sealed containers in the refrigerator (4 ° C) until used for extraction.

Preparation of beetroot aqueous extract Preparation of beetroot aqueous extract:

The extract was prepared by soaking, where 10 grams of the beetroot powder subject to the study were taken and placed in 100 ml of sterile distilled water for two hours, and at the same time soaking for 24 hours and the solids were removed by filtration. The extract was passed over filter paper, then centrifuged using a Hettich universal device at a speed of 3000 rpm for ten minutes, then the filtered liquid was taken and placed in sterile bottles and stored in the refrigerator until use.

Determination of the effective dose:

The most effective dose of the German extract of the beetroot plant Beta vulgaris was determined. 20 animals were used and divided into four groups, with 5 animals in each group, and they were distributed as follows:

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The first group: (the control group was injected with distilled water)

The second group only: (was injected with 50 mg/kg of body weight of the aqueous extract of the plant).

The third group: (was injected with 100 mg/kg of body weight of the aqueous extract of the plant).

Group 4: (Injected with 200 mg/kg body weight of beetroot aqueous extract and given the dose orally via tube feeding for each animal. After forty-eight hours, blood samples were taken using a capillary tube from all groups through the eye cavity, then the serum was separated using a centrifuge at 3000 rpm for five minutes (Hassan and Razooqi, 2021).

This is to measure the hemoglobin concentration. In light of this, the most effective dose of the aqueous extract was chosen: 100 mg/kg body weight, as the effective dose showed a significant effect in reducing the hemoglobin percentage. Then the dose was repeated at the same concentrations above for 72 hours to ensure the effective dose, and the effective dose was at the same concentration of 100 mg/kg body weight.

III. Results:

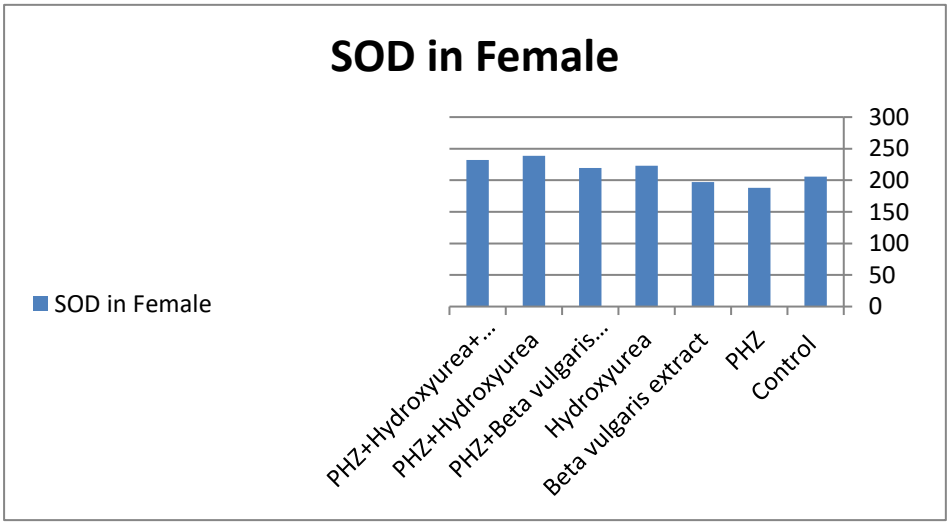

Figure1: showing the level of SOD enzyme in groups treated with PHZ،*Beta vulgaris* extract and hydroxyurea.

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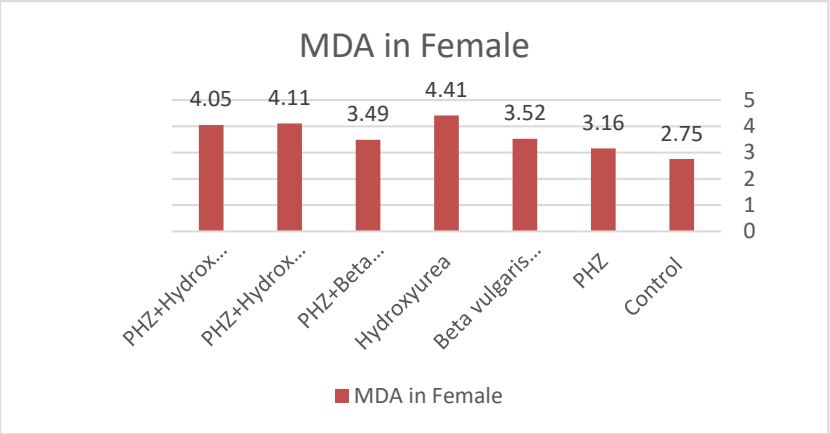

Figure2: showing the level of MDA enzyme in groups treated with PHZ،*Beta vulgaris* extract and hydroxyurea.

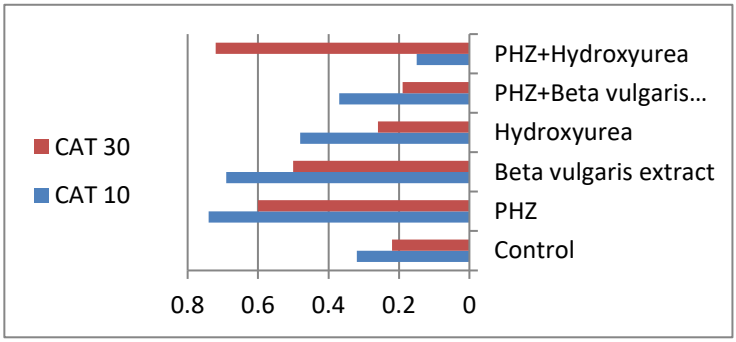

Figure3: showing the level of GPX enzyme in groups treated with PHZ،*Beta vulgaris* extract and hydroxyurea.

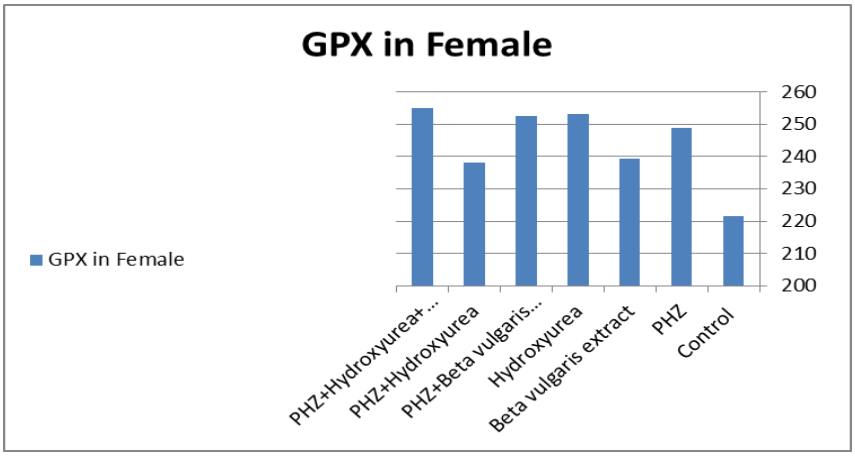

Figure4: showing the level of CAT enzyme in groups treated with PHZ،*Beta vulgaris* extract and hydroxyurea.

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IV. Discussion:

The results of the current study indicated a significant increase in MDA in the serum of Female rats treated with (Hydroxyurea, Phylhydrazine) when compared with the control group at a probability level (P≤0.05), except for the oxidative enzymes SOD, GPX, which did not show any significant difference at a probability level (P≤0.05). While the results of the current study showed a significant increase in SOD, MDA, CAT in the serum of female rats treated with (Hydroxyurea, Phylhydrazine) when compared with the control group at a probability level (P≤0.05), as for the enzyme GPX, it did not show any significant difference at a probability level (P≤0.05).

The results of the current study, as shown in Figure (1,2,4) showed an increase in oxidative enzymes in the group treated with PHZ compared to the control group. The results of the current study are consistent with the study (Banerjee *et al.,* 2020), which indicated that Phylhydrazine (PHZ) causes an increase in MDA due to the stimulation of oxidative stress within cells. The results of the current study showed an increase in MDA in the fourth group treated with Hydroxyurea as shown in Figures (1,2,4). These results are consistent with the study (Vinhaes *et al.,* 2020), which indicated that high doses of hydroxyurea for continuous periods lead to high levels of oxidative stress and thus an increase in the MDA enzyme. The results of the current study are also consistent with the study (Pedrosa *et al*., 2021), which indicated that the group treated with hydroxyurea has an increase in MDA when treated through an exploratory study to investigate systemic oxidative stress in children and adolescents with sickle cell anemia. In addition, they evaluated the potential effect of hydroxyurea on the state of oxidative stress in a comparative study from Brazil, where the results showed that hydroxyurea reduces oxidative stress. The results of the current study also agreed with the study (Hanan *et al.,* 2020), which showed a decrease in SOD, CAT, GPX and an increase in MDA in the serum of patients with anemia. The results of the current study also showed that the treatment of rats with PHZ with the aqueous extract of beetroot plant led to an increase in oxidative enzymes (SOD, CAT, GPX) and a decrease in (MDA) compared to the control group as shown in Figures (1,2,3,4). This is consistent with the results of the study (Naeem, 2018), which showed a significant decrease in MDA in the serum, while the oxidative enzymes SOD, CAT, GPX increased compared to the control group. Given the presence of both total flavonoids and phenols in *beta vulgaris* and juice, these materials represent rich sources of antioxidants, so it is recommended to use beetroot powder.

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