



## Pharmacological Study

Hematinic effect of fruits of *Opuntia elatior* Mill. on phenylhydrazine-induced anemia in ratsSanjay P. Chauhan, Navin R. Sheth<sup>1</sup>, Bhanubhai N. SuhagiaDepartment of Pharmaceutical Chemistry, Faculty of Pharmacy, Dharmsinh Desai University, Nadiad, <sup>1</sup>Department of Pharmaceutical Sciences, Saurashtra University, Rajkot, Gujarat, India

## Abstract

**Introduction:** The fruits of *Opuntia elatior* Mill. are known as prickly pear and folkloric use as hematinic, anti-inflammatory and antiasthmatic action. Previously, the fruit juice of prickly pear was evaluated in reversed anemia induced by HgCl<sub>2</sub> in a dose dependant manner and present study revealed about its effect in acute hemolytic anemia. **Aim:** To evaluate the hematinic activity of fruits of *Opuntia elatior* Mill. **Materials and Methods:** The hematinic activity of an orally administered fruit juice was studied on phenylhydrazine (PHZ)-induced anemic rats. The hematological parameters such as hemoglobin (Hb) content, red blood cell (RBC), packed cell volume (PCV), and reticulocyte count were analyzed as indices of anemia. **Results:** PHZ altered the hematological parameters by hemolysis characterized by a decrease in Hb content, total RBC counts and PCV ( $P < 0.001$ ) on day 3. The Hb content (g%) was significantly increased ( $P < 0.05$ ) at day 7 in 10 and 15 ml/kg fruit juice treated rats, which was a good improvement compared to the standard. **Conclusion:** The speedy and progressive recovery of anemic rats responding to treatment of the *O. elatior* Mill. fruits may be due to increased erythropoiesis and/or antioxidant property of betacyanin.

**Key words:** Hematinic, *Opuntia*, phenylhydrazine, Prickly pear

## Introduction

Two billion people suffer from anemia worldwide and most of them have iron deficiency and hemolytic anemia due to toxicants and oxidants. About 70–80% of the world population, particularly in the developing countries, rely on nonconventional medicine like dietary supplements and herbal remedies in their primary healthcare as reported by the WHO.<sup>[1]</sup> The cactus *Opuntia* (subfamily: Opuntiodae, family: Cactaceae) is a xerophytic plant producing about 200–300 species. In local parlance cactus is called Prickly pear, Slipper thorn, Tuna (English) and has different vernacular names in India such as *Hathlo Thor*, *Chorhthlo* (Gujarati), *Haththathoira*, *Nagphana*, *Nagphani* (Hindi), *Snuhi*, *Vajrakantaka*, *Bahushala* (Sanskrit), *Nagadali*, *Nagakkali* (Tamil), *Nagamulla*, and *Nagajemudu* (Telugu).<sup>[2-5]</sup> As per the Traditional Knowledge Digital Library (TKDL) databases, Formulation ID No. RS/6185<sup>[6]</sup> and RS8/412,<sup>[7]</sup> which consist prickly pear are used for alleviating

*Vata Kapha Dosha* and for treating diseases due to vitiated *Rakta*, cyst, pains, cough, and bronchitis. The fruit juice of *Opuntia elatior* Mill. reversed anemia induced HgCl<sub>2</sub> in a dose-dependent manner.<sup>[8]</sup> The objective of this study is to evaluate the effect of *Prickly pear* in acute hemolysis induced by phenylhydrazine hydrochloride (PHZ).

## Materials and Methods

## Phytochemical analysis

Collection, authentication, and preparation of fruit juice were followed as per the earlier studies.<sup>[8]</sup> Identification of betalains by Spectrophotometric, High-performance liquid chromatography, (HPLC) and liquid chromatography-mass spectroscopy (LC – MS) analysis are already established.<sup>[9]</sup>

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## Hematinic action

### Animal

Albino Wistar rats of either sex (180–250 g body weight) were used in this study. They were housed at ambient temperature ( $22 \pm 1^\circ\text{C}$ ), relative humidity ( $55 \pm 5\%$ ), and 12 h/12 h light dark cycle. Animals had free access to Amrut brand rat pellet diet supplied by Pranav Agro-Industry, Baroda, and water given *ad libitum*. The protocol of the experiment was approved by the Institutional Animal Ethics Committee (IAEC) as per the guidance of the Committee for the purpose of control and supervision of experiments on animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India, vide certificate no. IAEC/RBPMPC/09-10/01 dated 18/07/2009. Acute toxicity studies were performed for *Opuntia* fruit juice (OFJ) as per guidelines 423 prescribed by the OECD.<sup>[8]</sup>

### Experimental design

Anemia was induced by intraperitoneal (i.p.) injection of PHZ at 40 mg/kg for 2 days.<sup>[10]</sup> The dose of OFJ was selected as per toxicity study. Animals were divided into seven groups ( $n = 6$ ) as per followings and treated accordingly.

- Group A: Negative control (saline solution for 15 days)
- Group B: Positive control (PHZ, 40 mg/kg, i.p, for 2 days)
- Group C: PHZ + standard ferrous sulfate containing drug Fefol® (0.0214 mg/kg, per os, treatment started on day 3 up to day 15)<sup>[11]</sup>
- Group D5: PHZ + OFJ (5 ml/kg, per os, treatment started on day 3 up to day 15)
- Group D10: PHZ + OFJ (10 ml/kg, per os, treatment started on day 3 up to day 15)
- Group D15: PHZ + OFJ (15 ml/kg, per os, treatment started on day 3 up to day 15)
- Group E: OFJ (15 ml/kg, per os, treatment started on day 3 up to day 15).

PHZ solution was prepared in dimethyl sulfoxide and injected intraperitoneally for 2 days except Group A and Group E. The vehicle, standard drug, and fruit juice were administered orally from day 3 to day 15 after PHZ administration. Group B animals were allowed to recover naturally. Group E animals were treated only with 15 ml/kg fruit juice as a high dose to check the effect of fruit juice in normal conditions.

Change of body weight in grams of each animal was recorded at day 0, 3, 7, 10, and 15 using ACCULAB digital balance (Model No. ALC-310.3, Sartorius Mechatronics India Pvt., Ltd., Bengaluru, India). Hematological and biochemical parameters were estimated on day 0, 3, 7, 10, and 15.<sup>[8]</sup> Percentage of reticulocytes was carried out by cresyl blue stained on whole red blood cells (RBC).<sup>[10]</sup> On the 15<sup>th</sup> day, rats were sacrificed by the spinal dislocation method under light ether anesthesia and livers, kidneys, and spleens were collected for histopathological study.<sup>[12-14]</sup>

### Statistical analysis

All the values are expressed as a Mean  $\pm$  SEM (standard error of mean). The data were analyzed by one-way ANOVA, followed by Tukey's multiple comparison tests. A level of  $P < 0.05$  was considered as statistically significant. A level of significance was noted and interpreted accordingly.

## Results

### Phytochemical analysis

The Phytochemical screening revealed that the fruits consisted of carbohydrates, polyphenols, flavonoids, and predominant pigment betacyanin. The total betacyanin content (47.10 mg/100 ml) equivalent to betanin obtained from fruits of *O. elatior*.

### Hematinic action

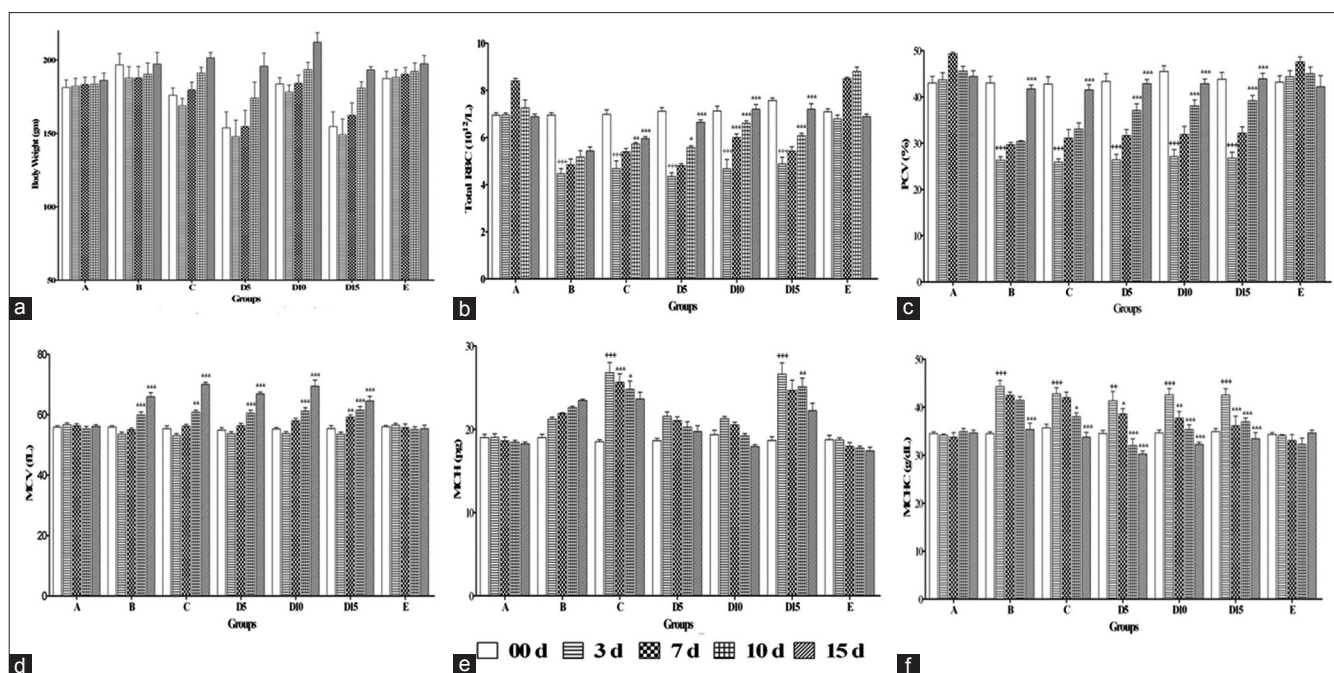
The mean body weight (g) of the albino rats in all groups was recorded on the day 0, 3, 7, 10, 15 [Figure 1]. Statistically insignificant difference in mean body weight was found in different treatment groups.

The mean hemoglobin (Hb) content (g %) of rats at day 3 was decreased significantly ( $P < 0.001$ ) when compared to the negative control (Group A) [Table 1]. In Group D10 and D15, the Hb content (g %) was significantly increased ( $P < 0.05$ ) at day 7, which was a better improvement when compared to Group C and statistically compared with Group B values of the day 3 [Figure 2]. PHZ treated positive control rats demonstrated significant decrease in mean total RBC count ( $P < 0.001$ ), packed cell volume (PCV) ( $P < 0.001$ ), mean cell volume (MCV) and increase in mean cell hemoglobin (MCH) ( $P < 0.001$  in Group C and D15), mean corpuscular hemoglobin concentration and red cell distribution width ( $P < 0.001$ ) on day 3 with respect to the values of negative control (Group A) on day 0. Treatment of anemic rats with standard and OFJ reversed the effect of PHZ on day 7. In Group E rats, there was insignificant change in the hematological parameters during the experimental period [Figure 1].

At day 7, in Group C, D10 and D15, mean reticulocytes were significantly increased  $2.103 \pm 0.249$  ( $P < 0.05$ ),  $2.395 \pm 0.156$  ( $P < 0.001$ ) and  $2.453 \pm 0.253$  ( $P < 0.001$ ), respectively with respect to the values of Group B at the day 3 [Figure 3]. These indicate fruit juice may increase the erythropoietic activity in PHZ-induced anemia.

Liver and kidney function parameters were estimated on day 0, 3, 7, 10, and 15 in PHZ-induced anemia in rats. Statistically, significant increase in mean bilirubin concentration ( $P < 0.001$ ) and decrease in alkaline phosphatase ( $P < 0.05$  in Group C, D10 and D15) and total proteins ( $P < 0.001$ ) in PHZ – treated rats was observed on day 3 with respect to the values of Group A on day 0. There was an elevation in blood urea and creatinine concentration in rats at day 3. The mean blood urea and creatinine concentration was found  $41.47 \pm 1.42$  ( $P < 0.01$ ) and  $0.533 \pm 0.033$  ( $P < 0.01$ ) in Group D10 and  $41.73 \pm 2.24$  ( $P < 0.01$ ) and  $0.483 \pm 0.087$  ( $P < 0.001$ ) in Group D15 at day 10 with comparison to Group B values at day 3. The fruit juice of *O. elatior* reversed the effect of PHZ on liver and kidney function parameters on the day 7 [Figure 4].

Histopathological sections of the kidney in Group A (negative control) rats revealed normal distinct glomeruli and tubules whereas rats of Group B showed shrinkage and acute glomerular nephritis. Fruit juice treated animals exhibited distinct glomerular and tubular structure with improvement compared to Group B kidney sections. In fruit juice treated rats, better



**Figure 1:** Effect of *Opuntia* fruit juice on body weight (a), total red blood cell (b), packed cell volume (c), mean cell volume (d), mean cell hemoglobin (e) and mean corpuscular hemoglobin concentration (f) in phenylhydrazine-induced anemia. Values are Mean  $\pm$  SEM (n = 6), analyzed by one-way ANOVA followed by Tukey's multiple comparison test, \*\*\*P < 0.001, \*\*P < 0.01, \*P < 0.05 for change difference at day 3 versus negative control (Group A) at day 0 and \*\*\*P < 0.001, \*\*P < 0.01, \*P < 0.05 for change difference at day 7, 10 and 15 versus positive control (Group B) at day 3

**Table 1: Estimation of hemoglobin content (g%) of rats in PHZ-induced anemia**

Groups	Days				
	0	3	7	10	15
A	15.32 $\pm$ 0.37	15.38 $\pm$ 0.42	15.78 $\pm$ 0.24	15.3 $\pm$ 0.21	15.12 $\pm$ 0.36
B	15.23 $\pm$ 0.49	11.02 $\pm$ 0.11 <sup>***</sup>	11.27 $\pm$ 0.061	11.58 $\pm$ 0.07	12.53 $\pm$ 0.30
C	15.63 $\pm$ 0.22	10.68 $\pm$ 0.25 <sup>***</sup>	11.97 $\pm$ 0.21	12.98 $\pm$ 0.20 <sup>**</sup>	14.02 $\pm$ 0.29 <sup>***</sup>
D5	15.8 $\pm$ 0.20	11.23 $\pm$ 0.23 <sup>***</sup>	11.73 $\pm$ 0.13	12.57 $\pm$ 0.14	14.98 $\pm$ 0.19 <sup>***</sup>
D10	15.72 $\pm$ 0.27	11.6 $\pm$ 0.42 <sup>***</sup>	12.75 $\pm$ 0.40 <sup>*</sup>	13.85 $\pm$ 0.33 <sup>***</sup>	15.63 $\pm$ 0.34 <sup>***</sup>
D15	15.88 $\pm$ 0.27	11.35 $\pm$ 0.19 <sup>***</sup>	12.7 $\pm$ 0.17 <sup>*</sup>	14.02 $\pm$ 0.31 <sup>***</sup>	15.68 $\pm$ 0.37 <sup>***</sup>
E	15.48 $\pm$ 0.23	15.63 $\pm$ 0.18	15.93 $\pm$ 0.20	16.13 $\pm$ 0.21	16.83 $\pm$ 0.42

Data: Mean $\pm$ SEM (n=6), analyzed by one-way ANOVA followed by Tukey's multiple comparison test, \*\*\*P<0.001 for change difference at day 3 versus negative control (Group A) at day 0 and \*\*\*P<0.001, \*\*P<0.01, \*P<0.05 for change difference at day 7, 10 and 15 versus positive control (Group B) at day 3. SEM: Standard error of mean, PHZ: Phenylhydrazine

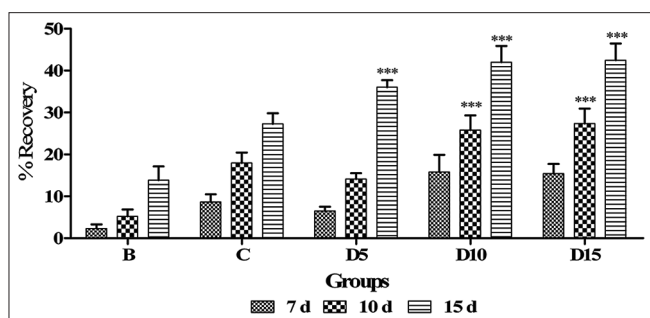
histology was evident, the glomerular and tubular structures were distinct and more improved in comparison to Group B kidney sections. The liver of Group B rats showed distortion of hepatocytes, portal tract dilation, and acute inflammatory infiltration. In standard and fruit juice treated rats, normal histology was seen. Normal cytoarchitecture of the spleen was observed in the control group, whereas cell depletion, acute inflammatory infiltration, fibrosis, and necrosis were observed in the cytoarchitecture of the spleen in Group B rats. In standard and fruit juice treated rat's spleen, mild fatty changes and cell depletion were observed compared to Group B rats [Figure 5].

## Discussion

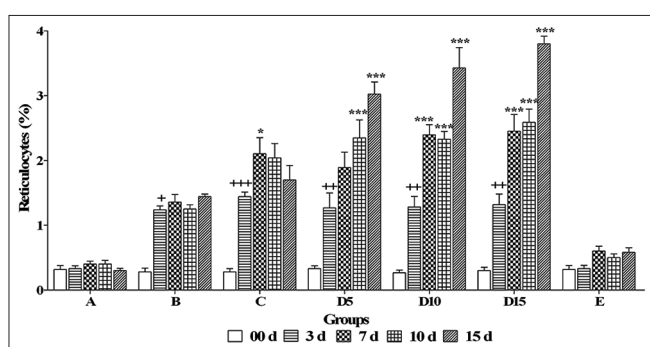
The preliminary phytochemical analysis suggests that the external color of Prickly pear fruits depends on the relative concentration of betacyanins, which has antioxidant potential.

The total betacyanin content (47.10 mg/100 ml) equivalent to betanin obtained from fruits of *O. elatior* was higher compared to *O. ficus-indica* and *O. undulata* Griff. whereas lower compared to *O. stricta* Haw.<sup>[9]</sup>

PHZ produces both aryl and hydroxyl radicals when incubated with rat liver microsomes<sup>[15]</sup> and oxidized by hydrogen peroxide at pH 7.4 and 37°C.<sup>[16]</sup> The radicals induced oxidative stress on the red cell membrane resulting in hemolysis by lipid peroxidation.<sup>[17,18]</sup> The sub-chronic intoxication of rats with PHZ (10 mg/kg/day for 8 days) resulted in a marked hemolytic anemia characterized by decreased RBC, Hb, and PCV.<sup>[19]</sup> Similar results were obtained in the present study when experimental rats were administered PHZ in order to induce anemia. In addition, increased reticulocytosis, methemoglobinemia and hemocatheresis is reported in PHZ intoxicated rats.<sup>[20]</sup> The main function of the RBC is the transportation of oxygen into the tissues of the body. In such pathological or physiological condition the RBC



**Figure 2:** The percentage recovery in hemoglobin content of rats at day 7, 10 and 15 in phenylhydrazine-induced anemia. Values are Mean  $\pm$  SEM ( $n = 6$ ), analyzed by one-way ANOVA followed by Tukey's multiple comparison test, \*\*\* $P < 0.001$ , \*\* $P < 0.01$ , \* $P < 0.05$  for change difference versus positive control (Group B) at day 7, 10 and 15

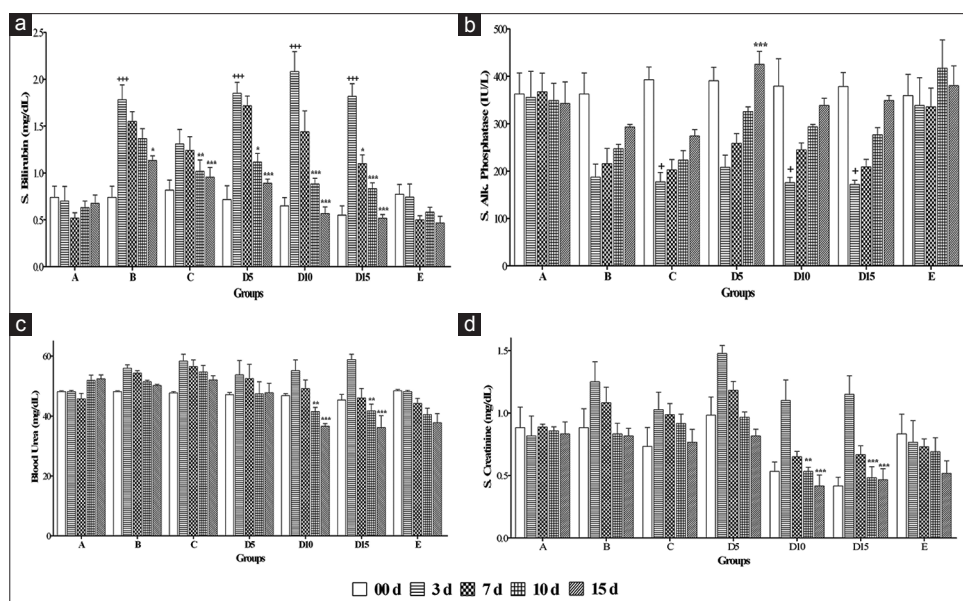


**Figure 3:** Effect of *Opuntia* fruit juice on reticulocyte counts in phenylhydrazine-induced anemia. Values are Mean  $\pm$  SEM ( $n = 6$ ), analyzed by one-way ANOVA followed by Tukey's multiple comparison test, \*\*\* $P < 0.001$ , \*\* $P < 0.01$ , \* $P < 0.05$  for change difference at day 3 versus negative control (Group A) at day 0 and \*\*\* $P < 0.001$ , \*\* $P < 0.01$ , \* $P < 0.05$  for change difference at day 7, 10 and 15 versus positive control (Group B) at day 3

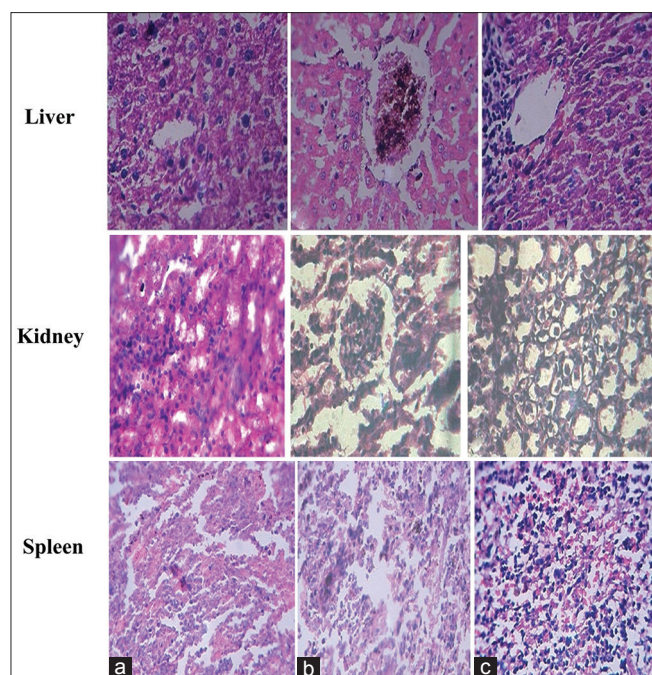
alters its function, and this may be detrimental to the body. In this study, PHZ altered the function of RBC by hemolysis characterized by decreased levels of RBC, Hb and PCV. PHZ increases reactive oxygen species (ROS) and lipid peroxidation and decreases glutathione (GSH). These effects are reversed by N-acetyl cysteine, a known ROS scavenger.<sup>[21]</sup> Thus, PHZ-induced hemolytic injury seems to be derived from oxidative alterations to RBC proteins rather than to membrane lipids.<sup>[22]</sup>

Anemia is a disease characterized by a reduction in the concentration of Hb, circulating RBC and pack cell volume per unit of the peripheral blood below the normal for the age and sex of the patient.<sup>[23,24]</sup> Blood parasites, bacterial infections, viral infections, drugs/chemical agents and metabolic diseases may result in the destruction of RBCs leading to hemolytic anemia.<sup>[25]</sup> Administration of PHZ also resulted in an increase in the MCV and MCH values, which are indicators of macrocytosis thus describing the anemia as macrocytic. This condition is also common in Vitamin B<sub>12</sub> and folate deficiencies probably as a result of an iron deficiency (loss of iron). Macrocytic anemia has also been reported in rats infected with *Trypanosoma brucei* Plimmer and Bradford<sup>[26]</sup> and this has been linked to iron deficiency anemia.<sup>[27]</sup>

In this study, PHZ altered the hematological parameters by hemolysis characterized by a decrease in Hb concentration, total RBC counts, and PCV on day 3. However, the hematological parameters were restored to normal range after treatment with fruit juice of the *O. elatior*. The drug at the dose of 10 ml/kg reduced the recovery time of the blood parameters from 15 days in the anemic control to 10 days. Also, the recovery was progressive such that after 15 days of continuous treatment, the hemoglobin concentration was higher in Group D10, D15 and E treated rats than in the negative control group. It was also observed that the recovery of the treated groups was dose-dependent with the highest dose of 15 ml/kg effecting the



**Figure 4:** Effect of *Opuntia* fruit juice on serum bilirubin (a), serum alkaline phosphatase (b), blood urea (c) and serum creatinine (d) in phenylhydrazine-induced anemia. Values are mean  $\pm$  SEM ( $n = 6$ ), analyzed by one-way ANOVA followed by Tukey's multiple comparison test, \*\*\* $P < 0.001$ , \*\* $P < 0.01$ , \* $P < 0.05$  for change difference at day 3 versus negative control (Group A) at day 0 and \*\*\* $P < 0.001$ , \*\* $P < 0.01$ , \* $P < 0.05$  for change difference at day 7, 10 and 15 versus positive control (Group B) at day 3



**Figure 5: Hematoxyline-eosin sections of kidney, liver and spleen ( $\times 450$ ) of negative control (a), positive control (b) and *Opuntia* fruit juice (c) treated rats in phenylhydrazine-induced anemia model**

highest change. Giving the highest dose 15 ml/kg fruit juice to normal rats did not significantly alter the hematological parameters.

Anemia which resulted from the early loss of RBCs was naturally reversed 7 days later by the regeneration of the blood cells due to an increase of the reticulocytes. The results indicate that the fruit juice (10 ml/kg and 15 ml/kg) increased significantly the number of reticulocytes, mainly 7 days after PHZ administration. The fruit juice could stimulate erythropoiesis process.

The liver is often the primary target for the toxic effects of xenobiotics. It is known that the detoxification of the toxic materials, which enter the body, occurs mainly in the liver. Therefore, the liver can be used as an index for the toxicity of xenobiotics. Total bilirubin may rise in irritation of the liver; this reflects liver cell damage or bile duct damage within the liver itself. Alkaline phosphatase is the marker enzyme produced within the cells of the liver, as the cells are damaged, leaks into the blood stream leading to a rise in the serum levels. It is an enzyme, which is associated with the biliary tract, and it elevated; biliary tract damage and inflammation should be considered.<sup>[13]</sup> From the bilirubin and alkaline phosphatase content observations, it seems that the fruit juice of *O. elatior* improves the liver function significantly.

Urea is the major nitrogen containing metabolic product of protein catabolism in humans. In leukemia and hemolytic anemia, the release of leukocyte protein contributes to high plasma urea. In gastrointestinal diseases, plasma proteins and Hb can be released into the gut and digested. This may contribute to high plasma urea. Creatinine formed as the end product of creatine metabolism is a waste product. The plasma

blood urea and creatinine increases in renal diseases.<sup>[13]</sup> Fruit juice of *O. elatior* showed a tendency toward reversal of these toxicant-induced changes. Reversal of most of these changes by fruit juice administration indicates that they do have some element of cytoprotective activity.

The spleen is the storehouse of dead RBC and the breakdown of Hb also occurs in the spleen. Hemolytic anemia leads to the accelerated breakdown of Hb causing larger iron deposition in the spleen.<sup>[28]</sup> This is likely to be the cause of fibrosis and necrosis observed in the spleen in PHZ treated groups. This disturbance in the cytoarchitecture was significantly reversed by the test drug administration. In this respect, fruit juice was comparatively better because, in addition to attenuating the fibrosis, it restored cellularity to moderate level thus inhibiting the toxicant-induced cell depletion. Based on the biochemical and histopathological results, it can be said that *O. elatior* fruit juice helps in reversing the toxic effects of PHZ on the liver, kidney and spleen.

## Conclusion

The speedy and progressive recovery of anemic rats responding to treatment of *Opuntia elatior* Mill. fruits may be due to increased erythropoiesis. The improvement in the hematological indices exhibited by fruit juice might be connected with the minerals, phenolics, and betacyanin content of the fruits of *O. elatior*. The phenolics and betacyanin have remarkable antioxidant activity. These constituents might have a direct influence on the protection of hemolysis by ROS generated by PHZ. These results support the folklore use of *O. elatior* fruits in the treatment of anemia.

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Nil.

## Conflicts of interest

There are no conflicts of interest.

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## हिन्दी सारांश

### फ़ेनिल हाइड्रेजीन प्रेरित रक्ताल्पता वाले चूहों में नागफनी फल स्वरस का रक्तवर्धक प्रभाव पर अध्ययन

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वर्तमान अध्ययन नागफनी के फल की बढ़ती हीमोग्लोबिन गतिविधियों का मूल्यांकन करने हेतु फ़ेनिल हाइड्रेजीन प्रेरित रक्ताल्पता वाले चूहों को नागफनी के फल स्वरस पिलाकर किया गया। रुधिरविज्ञान संबंधी मानकों जैसे हीमोग्लोबिन (एच.बी.), आर.बी.सी., पी.सी.वी. और रेटिकुलोसाइट की गिनती एनीमिया के सूचकांक के रूप में विश्लेषण किया गया। दिन ३ में फ़ेनिल हाइड्रेजीन ने रक्तापघटन द्वारा रुधिरविज्ञान संबंधी मानकों को बदल दिया जिसमें हीमोग्लोबिन, कुल आर.बी.सी. और पी.सी.वी. (पी < 0.00१) की कमी पायी गयी। १० मि.ली. और १५ मि.ली. प्रति किलोग्राम के हिसाब से नागफनी का फल का रस ७ दिन तक पिलाने पर हिमोग्लोबिन काफी बढ़ गया जो मानक की तुलना में एक अच्छा सुधार था। नागफनी फल के रस की चिकित्सा का यह परिणाम शायद लोहित कोशिका जनन वृद्धि या वीटासाइनिन के एन्टी ऑक्सिडेंट क्रिया की वजह से हो सकता है।