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EFFECT OF DEHYDRATED WATER EXTRACT OF FRUITS OF *OPUNTIA FICUS INDICA* ON EXPERIMENTALLY-INDUCED HEMOLYTIC ANEMIA IN RATS

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ABSTRACT

Traditional oral report indicates that fruits of opuntia ficus indica are used in the treatment of anemia in India. Malaria is the on of the leading cause of the Hemolytic anemia, Phenyl hydrazine induced Hemolytic anemia model is similar to Hemolytic anemia occur due to malerial parasite infection. For this purpose, the dehydrated water extract of opuntia ficus-indica is evaluated on Hemolytic anemia model of rat induced by intra peritoneal injection of phenyl hydrazine at 40 mg/kg for 2 days. Oral administration of extract at 150 mg/kg/day and 250 mg/kg/day, to the rats previously treated with phenyl hydrazine, increased the concentration of hemoglobin, number of red blood cells, hematocrit and reticulocytes rate. Moreover, the extract of opuntia ficus indica shows the inhibition on hemolysis of red blood cells exposed to hypotonic solution in vitro. These results support partially the traditional use of fruits of opuntia ficus indica in the treatment of Hemolytic anemia.

Keywords O*puntia ficus-indica,* hemolytic anemia, phenyl hydrazine, Membrane stabilization activity

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INTRODUCTION

Anemia is characterized by the decrease of the hemoglobin rate less than 13 g/dl in male or 12 g/dl in female [8]. In the tropical area, between 10 to 20% of the population presents less than 10 g/dl of hemoglobin. There are many types of anemia such as aplastic anemia, metabolic anemia, regulatory anemia and hemolytic anemia. Amongst them hemolytic anemias are due to an increased rate of RBC destruction and is of greatest clinical concern when the rate of RBC destruction exceeds that of erythropoiesis. Hemolytic anemias can be categorized as either inherited or acquired disorders. Inherited hemolytic anemias include defective globin synthesis, erythrocyte membrane defects, and erythrocyte enzyme deficiencies, while the acquired hemolytic anemias are either

immune-mediated, due to physical stress on the RBC, or are induced by certain infections [22].

Hemolysis can develop from biochemical, immunologic, physical and/or chemical disturbances in the blood and more specifically from extrinsic factors such as immune disorders, infection, drugs, toxin exposure and mechanical red cell trauma [11]. Drug-induced haemolysisis considered as an acquired form hemolytic anemia and results as a consequence of clinical treatment. The causes of drug-induced haemolysis have been categorized as either immune or non-immune in origin[12]. The hypothesis in non-immune associated haemolysis is that the drug of concern causes lysis by damaging membrane integrity [15], drug-induced oxidative damage of the cell membrane[18], and/or unpredicted toxic effects of the drug on cell volume control processes, leading to cell swelling [14].

The plant is bitter, laxative, stomachic, carminative, diuretic, antipyretic, alexiteric; cures biliousness, burning, leucoderma, urinary complaints, tumors, ascites, loss of consciousness, piles, inflammations, vesicular calculi, anaemia, ulcers, cures bronchitis of children, ophthalmia, liver complaints lumbago and enlargement of the spleen. The cladodes are very tasty, stomachic; cure inflammations, ascites, tumors, pains. In South Africa and in Australia a decoction of the stem has been used as a diabetes remedy. The baked fruit is said to be given in whooping cough and syrup of the fruit is said to increase the secretion of bile and control spasmodic cough and expectoration [19]. Mexican fig is used to treat whooping cough, diabetes, prostate problems, rheumatism, nosebleed, and in dentistry in central Mexico [20]. Dried flowers are also ground into a paste and applied to the skin for measles [21]. Fruits of *opuntia ficus indica* are reported as an antioxidant.[3]

Fruits of *opuntia ficus indica* have protecting action on blood cells. it may have protective action on RBC and can be used for the treatment of hemolytic anemia. Anti-anemic activity of fruits of *opuntia ficus indica* has not reported. Thus *opuntia ficus indica* can immerge as a potent option to treat hemolytic anemia and other blood disorders. This work is focused on the ability of the plant fruits to treat hemolytic anemia occur due to drug-induced oxidative damage of the cell membrane.

MATERIALS AND METHODS:

Plant

Fruits of plant *opuntia ficus indica* were collected from rural area near Rajkot. Plant has been authentified and Herbarium submitted at Maliba Pharmacy College, Uka Tarsadia University, Bardoli. Authantification No. UTU/MPC/2012/02

Animals

Wistar rat sex (150 – 300 g), provided by the Department of Pharmacology, Maliba Pharmacy College, were used. They were housed in a standard environmental condition and fed with standard diet and water ad libitum.

Extraction

Fruits were dried at room temperature and powdered. 100 gm of powder was mixed with 200ml of distill water and filtered through muslin cloth. Water evaporated from filtrate at less than 50˚C temperature and residue was collected.[7]

Biological procedure

Phenyl Hydrazine induced Hemolytic anemia in vivo

Rats were divided in total three groups of six rat each. First Group, Model control received Phenyl hydrazine 40mg/kg for first 2 days by intra peritoneal rout dissolved in DMSO vehicle and Normal saline 1 ml for day 1 to 7 from oral route. second group, received phenyl hydrazine 40mg/kg for first 2 days intra peritoneally dissolved in DMSO vehicle and extract of *opuntia ficus indica* 150 mg/kg/day for day 1 to 7 from oral route. third group, received phenyl hydrazine 40mg/kg for first 2 days intra-peritoneally dissolved in DMSO vehicle and extract of *opuntia ficus indica* 250 mg/kg/day for day 1 to 7 from oral route. On the day 0, day 3 and day 8, blood sample collected from retro orbital cavity of each animal and analyzed for various hematological parameters [9].

Membrane stabilization activity *in vitro*

Preparation of erythrocyte suspension: Whole blood was obtained with heparinized syringes from rats through retro orbital cavity. The blood was washed three times with isotonic buffered solution (154 mM NaCl) in 10 mM sodium phosphate buffer (pH 7.4). The blood was centrifuged each time for 10 minutes at 3000 g [4].

Hypotonic solution-induced rat erythrocyte hemolysis: Membrane stabilizing activity of the extract was assessed using hypotonic solution-induced rat erythrocyte hemolysis. The test sample consisted of stock erythrocyte (RBC) suspension (0.50 ml) mixed with 5 ml of hypotonic solution (50 mM NaCl) in 10 mM sodium phosphate buffered saline (pH 7.4) containing different concentration of extract (0.5 mg/ml, 1mg/ml, 1.5mg/ml, 2mg/ml). The control sample consisted of 0.5 ml of RBC mixed with hypotonic-buffered saline solution alone. The mixtures were incubated for 10 min at room temperature and centrifuged for 10 min at 3000 g and the absorbance of the supernatant was measured at 540 nm. The percentage inhibition of hemolysis or membrane stabilization was calculated with the formula [4,5,10].

% Inhibition of hemolysis = 100 x $\{OD_1-OD_2/OD_2\}$

OD_1= *Optical density of hypotonic-buffered saline solution alone*

OD_2= *Optical density of test sample in hypotonic solution*

Analysis of hematological parameters

Rate of hemoglobin, number of red blood cells, haematocrit, MCV, MCHC, RDW and reticulocyte counts were determined on day 8 using automatic counter (Nihon Kohden MEK6410 K, Japan).

Study of the Reticulocytes

At the day 0, day 3 and day 8, slides of blood cells were made and stained by new methylene blue and the % of concentration of reticulocyte is determined based on the whole red blood cells [6].

Statistical analysis

The results are expressed as mean ± S.E.M and statistical analysis was performed by analysis of variance (ANOVA) followed by Tukey's test using GraphPad InStat 3. Results are significant if the probability $P < 0.05$.

RESULTS:

Hemoglobin concentration

As shown in figure 1, In the model control group, hemoglobin level was found to be 15.53g/dl on day 0. It decreased after intraperitoneal administration of phenyl hydrazine to 7.73 g/dl on day 3 and 8.98 g/dl on day 8. In the low dose extract group hemoglobin level was found to be 15.73g/dl on day 0. It decreased after intraperitoneal administration of phenyl hydrazine and oral administration of extract 150 mg/kg/day to 9.7 g/dl and increased after oral administration of extract 150mg/kg/day to 12.95 g/dl on day 8. In the High dose extract group hemoglobin level was found to be 15.67g/dl on day 0. It decreased after intraperitoneal administration of phenyl hydrazine and oral administration of extract 250mg/kg/day to 11.58 g/dl and increased after oral administration of extract 250mg/kg/day to 14.27 g/dl on day 8.

RBC count

As shown in figure 2, In the model control group, RBC count was found to be 7.122 cell * 10^6 /mm^3 on day 0. It decreased after intraperitoneal administration of phenyl hydrazine to 2.5 cell * 10^6 /mm^3 on day 3 and 3.44 cell * 10^6 /mm^3 on day 8. In the low dose extract group RBC count was found to be 6.96 cell * 10^6 /mm^3 on day 0. It decreased after intraperitoneal administration of phenyl hydrazine and oral administration of extract 150 mg/kg/day to 3.417 cell * 10^6 /mm^3 and increased after oral administration of extract 150mg/kg/day to 4.35 cell * 10^6 /mm^3 on day 8. In the High dose extract group RBC count was found to be 7.15 cell * 10^6 /mm^3 on day 0. It decreased after intraperitoneal administration of phenyl hydrazine and oral administration of extract 250mg/kg/day to 4.017 cell * 10^6 /mm^3 and increased after oral administration of extract 250mg/kg/day to 5.215 cell * 10^6 /mm^3 on day 8.

Reticulocyte Count

As shown in figure 3, In the model control group, Reticulocyte count was found to be 8.088% on day 0. It decreased after intraperitoneal administration of phenyl hydrazine to 10.1% on day 3 and 17.917% on day 8. In the low dose extract group Reticulocyte count was found to be 8.117% on day

0. It decreased after intraperitoneal administration of phenyl hydrazine and oral administration of extract 150 mg/kg/day to 15.73% and increased after oral administration of extract 150mg/kg/day to 26.117 on day 8. In the High dose extract group Reticulocyte count was found to be 8.1% on day 0. It decreased after intraperitoneal administration of phenyl hydrazine and oral administration of extract 250mg/kg/day to 19.18% and increased after oral administration of extract 250mg/kg/day to 30.217% on day 8.

Inhibition of Hemolysis

As shown in figure 4, Four different concentration of dehydrated water extract of fruits of opuntia ficus indica were studied for membrane stabilization activity. 0.5 mg/ml concentration has shown 24.37% inhibition of haemolysis, 1mg/ml has shown 32.66% inhibition of haemolysis, 1.5 mg/ml has shown 38.47 % inhibition of haemolysis and 2mg/ml has shown maximum 52.067% inhibition of haemolysis.

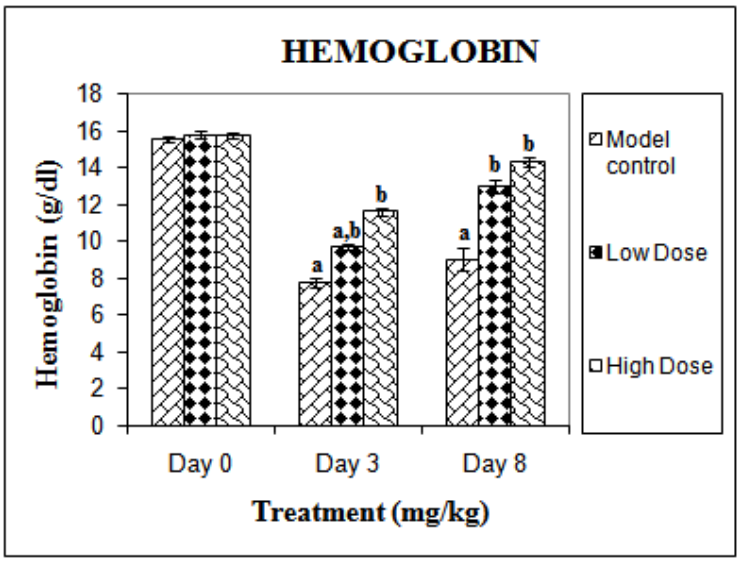

FIG 1 Effect of Dehydrated water extract of *Opuntia ficus indica* on Hemoglobin

Each bar represents the mean ± SEM. Number of animals in each group 6. Day 0 result consider as Normal Control. "**a**" indicate P value<0.001,when compared with normal control group. "**b**" indicate P value<0.01, when compared with disease control group. Statistical analysis was done by One –way ANOVA followed by post hoc Tukey test.

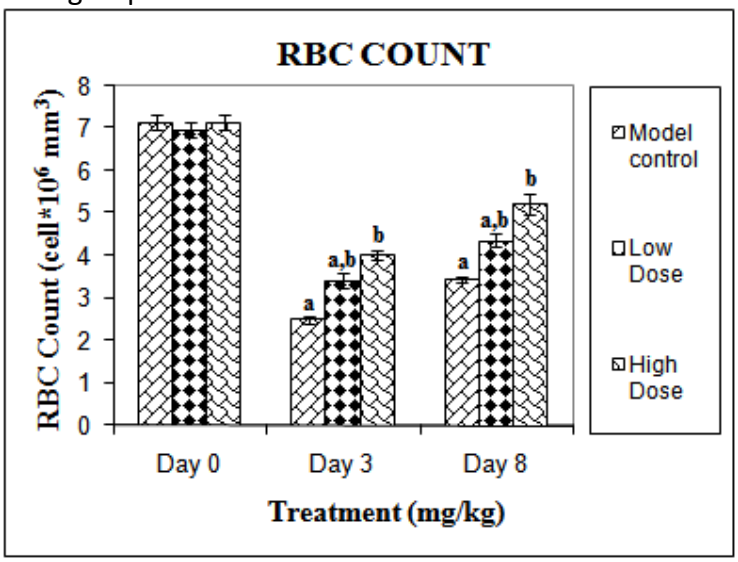

FIG 2 Effect of Dehydrated water extract of *Opuntia ficus indica* on RBC count

Each bar represents the mean ± SEM. Number of animals in each group 6. Day 0 result consider as Normal Control. "**a**" indicate P value<0.001,when compared with normal control group. "**b**" indicate P value<0.01, when compared with disease control group. Statistical analysis was done by One –way ANOVA followed by post hoc Tukey test.

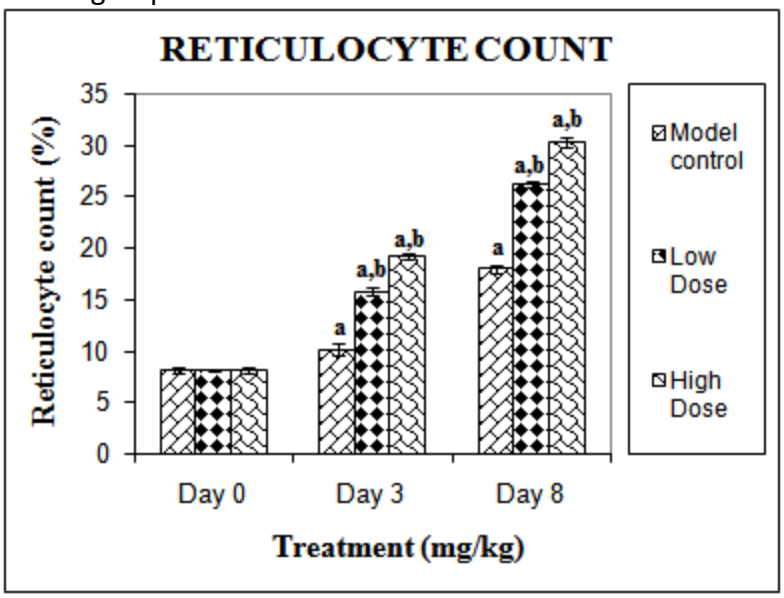

FIG 3 Effect of Dehydrated water extract of *Opuntia ficus indica* on Reticulocyte count

Each bar represents the mean ± SEM. Number of animals in each group 6. Day 0 result consider as Normal Control. "**a**" indicate P value<0.001,when compared with normal control group. "**b**" indicate P value<0.01, when compared with disease control group. Statistical analysis was done by One –way ANOVA followed by post hoc Tukey test.

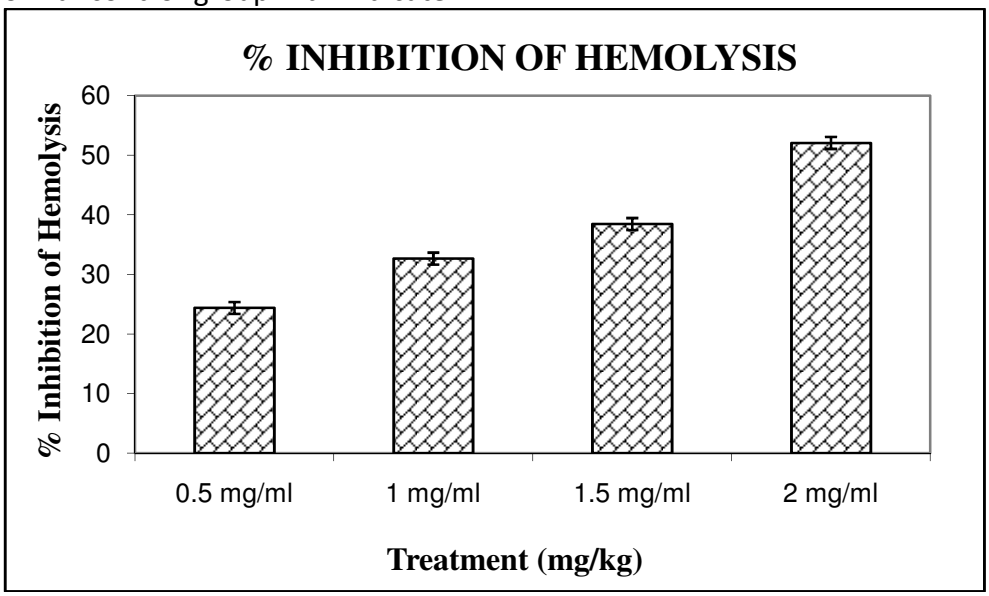

FIG 4 In vitro effect of dehydrated water extract of *Opuntia ficus indica* on % Inhibition of hemolysis of RBC

DISCUSSION:

Anemia is a disease characterized by a reduction in the concentration of hemoglobin, circulating red blood cell and pack cell volume per unit of the peripheral blood below the normal for the age and sex of the patient. Present study aimed to evaluate the hematinic effect of *opuntia ficus indica* fruit on phenyl hydrazine–induced anemia. Phenyl hydrazine is recognized for its capacity to cause hemolysis both *in-vitro* and *in-vivo* by formation of aryl and hydroxyl radicals, which has been demonstrated to be associated with its

interaction with erythrocytes [27]. The detoxification of hydrogen peroxide by way of the glutathione peroxidase pathway has also been described [23,24]. The accumulation of hydrogen peroxide in addition to the detoxifying capacity of the red cell may lead to the oxidation of essential cellular constituents including membrane phospholipids. Such alterations presumably contribute to the eventual hemolysis of affected cells [25]. Intoxication of rats with PHZ (4 mg/kg for 2 days) resulted in a marked hemolytic anaemia characterised by decreased RBC, Hb and hematocrit [1] Similar results were obtained in our study when experimental rats were administered PHZ in order to induce anaemia. The PHZ altered the function of RBC by hemolysis characterised by decreased levels of RBC, Hb and hematocrit. In addition, Ferrali et al. (1997) observed increased reticulocytosis, methaemoglobinemia and haemocatheresis in PHZ intoxicated rats.

This anaemia which resulted from the early lysis of the red blood cells was naturally reversed 7 days later by the regeneration of these blood cells due to the increase of the reticulocytes. Our results indicate that *opuntia ficus indica at 150 mg/kg* dose increased significantly the number of reticulocytes, mainly 7 days after PHZ administration. Moreover, the extract potentiates the increase of the number of reticulocytes. The prompt and progressive recovery of anaemic rats responding to treatment of *opuntia ficus indica* fruit extract may be due to increased erythropoiesis. The improvement in the hematological indices exhibited by test extract might be associated with the minerals, phenolics and betacyanin content of the fruits of *opuntia ficus indica*. These constituents might have direct influence on the protection of haemolysis by reactive oxygen species generated by PHZ.

The aliveness of cells depends on the integrity of their membranes [26]. Exposure of red blood cell to detrimental substances such as hypotonic medium and phenylhydrazine results in lysis of its membrane accompanied by haemolysis and oxidation of haemoglobin [4,26] The haemolytic effect of hypotonic solution is related to excessive accumulation of fluid within the cell resulting in the rupturing of its membrane. Such injury to RBC membrane will further render the cell more susceptible to secondary damage through free radical -induced lipid peroxidation [26]. It is consequently anticipated that compounds with membrane-stabilizing properties, should offer significant protection of cell membrane against injurious substances [5,12]. In our study, on exposure to hypotonic solution the RBCs in the presence of extract showed dose dependent membrane stabilization activity.

CONCLUSION:

In conclusion, the fruits of *opuntia ficus indica* are exhibiting haematinic effect against anaemia induced by phenylhydrazine may be due to its membrane stabilizing and antioxidant properties or due to stimulation of erythropoiesis.

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