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HAEMATINIC EVALUATION OF FRUITS OF *OPUNTIA ELATIOR* MILL. ON MERCURIC CHLORIDE INDUCED ANEMIA IN RATS

Sanjay P. Chauhan[1]*, N. R. Sheth[2], B. N. Suhagia[3]
[1]Associate Professor, Faculty of Pharmacy, Dharmsinh Desai University, Nadiad, Gujarat, India
[2]Professor and Head, Department of Pharmaceutical Sciences, Saurashtra University, Rajkot, Gujarat, India
[3]Dean, Faculty of Pharmacy, Dharmsinh Desai University, Nadiad, Gujarat, India

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***Corresponding author**
Dr. Sanjay P. Chauhan, Associate Professor, Faculty of Pharmacy, Dharmsinh Desai University, Nadiad, Gujarat, India
E-mail: sanjulmcp@rediffmail.com
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ABSTRACT
The fruits of *Opuntia elatior* Mill. (Family: Cactaceae) is known as prickly pear and widely used in several indigenous systems of medicine for the treatment of various ailments, *viz.* Anemia, asthma, inflammatory disorders, and diabetes. The objective of the present work is to screen phytochemical compositions and evaluation hematinic activity of fruits of *Opuntia elatior* Mill. The hematinic activity of an orally administered fruit juice (5, 10 and 15 ml/kg) was studied on mercuric chloride ($HgCl_2$) -induced anemic rats. Phytochemical analysis signifies the presence of betacyanin as an active principle which was confirmed by spectrophotometric, HPLC and LC-MS techniques. The total betacyanin content (47.10 mg/100 ml) equivalent to betanin obtained from the fruits of *O. elatior* Mill. was higher compared to *O. ficus-indica* and *O. undulata* Griff. while lower compared to *O. stricta* Haw. Mercuric chloride altered the hematological parameters by hemolysis characterized by decrease in Hb content, total RBC counts and PCV ($p < 0.001$) on day 30. Fruit juice at the dose of 10 ml/kg and 15 ml/kg showed a good percentage of recovering in hemoglobin, 32.99 % and 38.18 %, respectively, which was higher than standard treated group (29.8 %) indicating the correction of anemia induced by mercuric chloride after 30 days treatment. The speedy and progressive recovery of anemia in the treatment of prickly pear may be due to increased erythropoiesis and/or antioxidant property of betacyanin.
Keywords: Prickly pear, Opuntia, Haematinic, Mercuric chloride

INTRODUCTION

The phytopharmaceutical compound control diseases are an old approach that has led to the discovery of modern pharmaceuticals. The presence of medicinally active nutrients and their multifunctional properties render *Opuntia* spp. fruits a perfect candidate for the production of phytopharmaceutical products. Although traditionally respected for its pharmacological properties by the Native Americans, cactus pear is still hardly acquitted because of insufficient scientific information[1]. The billions of people suffer from anemia worldwide and most of them having iron deficiency and hemolytic anemia due to toxicants and oxidants. About 70 – 80 % of the world populations, particularly in the developing countries, rely on non-conventional medicine in their primary healthcare as reported by the WHO[2]. 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 like Hathlo Thor, Chorhthlo (Gujarati), Haththathoira, Nagphana, Nagphani (Hindi), Snuhi, Vajrakantaka, Bahushala (Sanskrit). It was found that class in India did not all belong to one species as an *O. dillenii*, but three to four species distributed over different regions in India. *O. dillenii* Haw. is found mainly in the southern parts of the India while *O. vulgaris* Mill (Syn *O. monocantha* Haw.) is distributed mainly in the northern parts; *O. elatior* Mill. is found in western India[3,4]. The *Opuntia* species were used as analgesic and anti-inflammatory, anticancer, antidiabetic, anti-hyperlipidemic and – hypercholesterolemic, antioxidant, antiulcer, antiviral, diuretics, immune modulatory, improve platelet function, neuroprotective, wound healing, monoamino-oxidase inhibitor and nutritional important[5,6]. The literature study reveals that the phytochemical composition and hematinic action of fruits of *Opuntia elatior* Mill. was not found in support of folkloric use[7]. In light of this, the present screening was carried out to ascertain the chemical composition and hematinic activity of fruits of *Opuntia elatior* Mill.

MATERIALS AND METHODS

Collection, Authentication and Preparation of Fruit Juice (OFJ)

The fruits of *Opuntia elatior* Mill. were collected from roadside weed near Atkot, Ta: Jasdan, Dist: Rajkot, Gujarat, India at Latitude (22^0 1' 48" N), Longitude (71^0 12' 0" E) and Elevation 193 M (633 ft) and authenticated by Raw Materials Herbarium and Museum, National Institute of Science and Communication and Information Resources, New Delhi (NISCAIR). The herbarium (specimen voucher No.: rbpmpc/museum/herbarium/07-08/01) was preserved in the museum of Department of Pharmacognosy, Smt. R. B. Patel Mahila Pharmacy College, Atkot, India. Mature fruits of *Opuntia elatior* Mill. were collected and immediately taken to the laboratory. Spines and glochides were removed from fruits by just heating on the wire gauge burner and then washed with water. The peel of the fruits was removed manually and pulp subjected to homogenization for 5 minutes using portable blender (Boss appliances, Daman). After homogenization, fruit juice was filtered though glass filter G_4 (Borosil Glass Works Ltd., Mumbai, India) and filtered *Opuntia* fruit juice (OFJ) was used for various estimation and biological studies.

Phytochemical Analysis

Identification of betalains by Spectrophotometric, High performance liquid chromatography (HPLC) and Liquid chromatography – mass spectroscopy (LC – MS) were performed and recently published by Chauhan *et al*.[7].

Haematinic Action

Animal

Albino Wistar rats of either sex (180-250 g body weight) were used for this study. They were housed at ambient temperature (22 ± 1^{0}C), relative humidity (55 ± 5 %) and 12 h/12 h light dark cycle. Animals had free access to rat pellet diet (Amrut brand) supplied by Pranav Agro Industry, Baroda, and water given *ad libitum*. The protocol of the experiment was approved by the Institutional Animal Ethical 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 Study

Acute toxicity studies were performed for OFJ as per guidelines 423 prescribed by the OECD. Female albino rats were used for acute toxicity study. The animals were kept fasting for overnight providing only water. These were divided into two groups of each containing five animals. One group was administered with water and another with OFJ at the dose of 20 ml/kg p.o. The animals were observed for 30 minutes and then periodically for first 24 h special attention during the first 4 h and thereafter daily for 14 days. The observations like sedation, convulsions, tremors, lethargy, death etc. were systemically recorded with individual records of each animal.

Experimental Design

Haematinic action was evaluated by methods previously described by Rathore and Siddiqui[8] and Sarkar *et al*.[9] with some modification. Mercuric chloride ($HgCl_2$) dose was calculated after carrying out initial pilot studies. The dose of OFJ was selected after carrying out acute toxicity study. It was found that maximum dose (20 ml/kg, p.o.) was safe and based on that we had selected three different doses low (5 ml/kg), medium (10 ml/kg) and high (15 ml/kg) for this study. Animals were divided in seven groups (n = 6) as per followings and treated accordingly.

Group A: Negative control (saline solution for 60 days)
Group B: Positive control ($HgCl_2$ solution, 4 mg/kg, p.o., up to 30 days)
Group C: $HgCl_2$ + Standard ferrous sulfate containing drug Fefol® (0.0214 mg/kg, p.o., treatment started on day 31 up to day 60)[10]
Group D5: $HgCl_2$ + OFJ (5 ml/kg, p.o., treatment started on day 31 up to day 60)
Group D10: $HgCl_2$ + OFJ (10 ml/kg, p.o., treatment started on day 31 up to day 60)
Group D15: $HgCl_2$ + OFJ (15 ml/kg, p.o., treatment started on day 31 up to day 60)
Group E: OFJ (15 ml/kg, p.o., treatment started on day 31 up to day 60)

Mercuric chloride (4 mg/kg, p.o.) was given to each rat except for group A and E for 30 days to induce anemia. After mercuric chloride exposure, treatment was given for the next 30 days except in group A and B. Group B animals were allowed to recover naturally. Group E animals were treated with only fruit juice (15 ml/kg) for next 30 days. Hematological and biochemical parameters were estimated on 30th and 60th day. At the end of the study, histopathological study of liver, kidney and spleen were evaluated.

Body Weight and Hematological Investigation

The change of body weight in grams of each animal was recorded at 7 day intervals up to day 60 using ACCULAB digital balance, (Model No. ALC-310.3, Sartorius Mechatronics India Pvt. Ltd., Bangalore, India). Hematological parameters were estimated on day 0, 30 and 60 in $HgCl_2$ –induced anemia. Blood samples were withdrawn from the retro-orbital plexus under light ether anesthesia, collected in heparinized capillary tubes and analyzed for hematological parameters. Hemoglobin (Hb) content (g %) of each animal was estimated at 7 day intervals up to day 60 and others like total red blood cells (RBC), packed cell volume (PCV), mean cell volume (MCV), mean cell hemoglobin (MCH), mean cell hemoglobin concentration (MCHC) and red blood cell distribution width (RDW) were estimated on fully automated fluorescence flow cytometry 5-part different analyzers (Sysmex XS800i, Japan) at day 30 and 60.

Measurement of Liver and Kidney Function

Pre- and post – treated biochemical parameters of liver and kidney were estimated on day 30 and 60 in $HgCl_2$ – induced anemia. For collection of blood and serum separation, blood samples were withdrawn from the retro-orbital plexus under light ether anesthesia without any anticoagulant and allowed for 10 minutes to clot at room temperature. It was centrifuged at 2500 g for 20 minutes. The serum obtained was kept at 4°C until used. Estimation of Liver function test, Alkaline phosphatase and Bilirubin, by p-nitrophenyl phosphate and Diazo method of Pearlman and Lee and Kidney function test, Urea and Creatinine, by urease-glutamate dehydrogenase and modified Jaffe method were carried out using enzymatic kit Erba Diagnostic Germany Limited, Baroda, India, respectively.

Histopathology of Liver, Kidney and Spleen

On 60th day rats were sacrificed by the cervical dislocation method under light ether anesthesia and livers, kidneys and spleens were collected for histopathological study. Tissues were fixed at 10 % neutral-buffered formalin solution was embedded in paraffin and used for histopathological examination. Tissue sections (4-5 μm) were cut on a microtome and taken on glass slides coated with albumin. The hematoxyline-stained sections were stained with eosin for two minutes and quickly passed through ascending grades of alcohol, cleaned with xylene, and mounted in Canada Balsam. The stained sections were examined under an Olympus BX 40 photomicroscope and photographed. The samples were either coded to perform a blind study or expert guidance

was sought from a veteran pathologist to determine histopathological changes[11-13].

Statistical Analysis

All the values are expressed as Mean ± SEM (standard error of mean). The data were analyzed by one way ANOVA followed by Turkey'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

Effect on Body weight and Hemoglobin Content

The mean body weight (g) of the albino rats in different treatment groups was recorded at 7 days interval up to 60 days and presented in Figure 1. Statistically, highly significant decrease in body weight ($p < 0.001$) was found in $HgCl_2$ treated groups on day 30 with respect to the values of the negative control (Group A) group on the same day. On the day 49, significant increase in body weight (g) was obtained 191.2 ± 8.77 ($p < 0.05$) and 201.7 ± 4.82 ($p < 0.001$) in group D10 and D15 treated groups, respectively. However, on the day 60, highly significant enhancement in body weight ($p < 0.001$) was obtained in group C, D10 and D15 treated groups with respect to the values of the group B at the day 30. There was no significant change in group B and D5 treated groups after 30 days. In group E, there was also increasing in body weight after 30 days. Pre and post treated mean Hb content (g %) in all groups were estimated at 7 day interval up to 60 days in rats (Figure 1). $HgCl_2$ treated positive control rat demonstrated significant ($p < 0.001$) decrease in mean Hb content compared negative control on day 30, indicated anemia. The percentage of reduction in Hb concentration was found 25.59 ± 1.274 %, 23 ± 0.84 %, 22.55 ± 2.45 %, 20.87 ± 2.06 % and 22.43 ± 1.57 % in group B, C, D5, D10 and D15 respectively at day 30. We observed a significant increase in Hb concentration in group D5, D10 and group D15 on day 60 ($p < 0.05$, $p < 0.001$, $p < 0.001$, respectively) with respect to positive control on day 30 and it was same as standard (group C, $p < 0.001$). Group D5, D10 and D15 treated rats showed significant ($p < 0.001$) percentage recovery compared to positive control and it was as same as found in the standard group (Figure 2). The mean Hb level in group D15 was recovered significantly ($p < 0.001$) at day 42, 49 and 60 compared to positive control. We did not find a significant change in Hb content in group E (only 15 ml/kg, OFJ) treated animals.

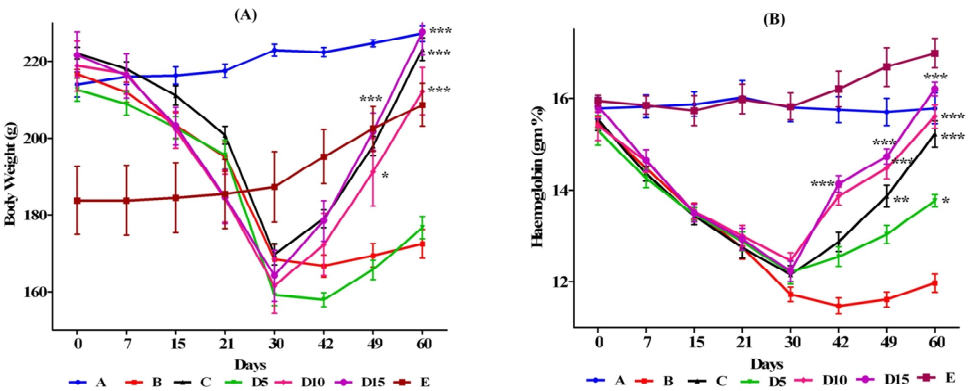

Figure 1: Effect of OFJ on body weight (g) and Hb content (gm %) in $HgCl_2$ induced anemia

Values are Mean ± SEM (n = 6), analyzed by one way ANOVA followed by Turkey's multiple comparison test, ***p < 0.001, **p < 0.01, *p < 0.05 for change difference at day 60 Vs positive control (group B) at 30 days

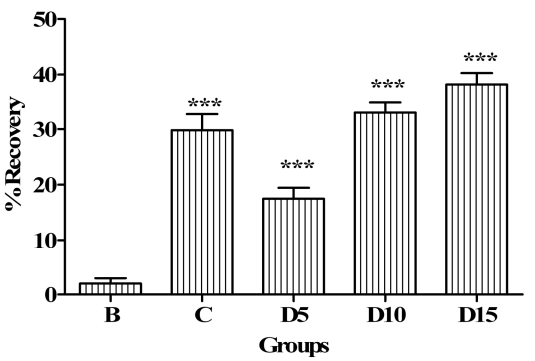

Figure 2: The percentage recovery in Hb content of rats in HgCl2 induced anemia at day 60

Values are Mean ± SEM (n = 6), analyzed by one way ANOVA followed by Turkey's multiple comparison test, ***p < 0.001, **p < 0.01, *p < 0.05 for change difference Vs positive control (group B) at day 60

Effect on Hematological Parameters

Pre- and post – treated hematological parameters in all groups were estimated at day 30 and 60 respectively and have been presented in Figure 3. $HgCl_2$ treated positive control rats at day 30 demonstrated a significant decrease in RBC count ($p < 0.001$), PCV ($p < 0.001$), MCV ($p < 0.001$), MCH ($p < 0.001$) and MCHC ($p < 0.01$), while significant increase was observed in levels of RDW ($p < 0.001$) when compared to non treated negative control. Treatment with OFJ for 30 days showed significant increase in RBC ($p < 0.001$) compared to the positive control at day 30 and its equivalent to standard. We observed a significant increase in PCV ($p < 0.001$) and MCH ($p < 0.001$) in group C and D15, MCV ($p < 0.001$) in group D15 and MCHC ($p < 0.01$) in group C, D10 and D15 treated animals at day 60. It was observed that RDW significantly ($p < 0.001$) reduced in group C, D10 and D15 at day 60 with respect to the values of group B at day 30. There was no significant difference in hematological parameters of group E treated animals.

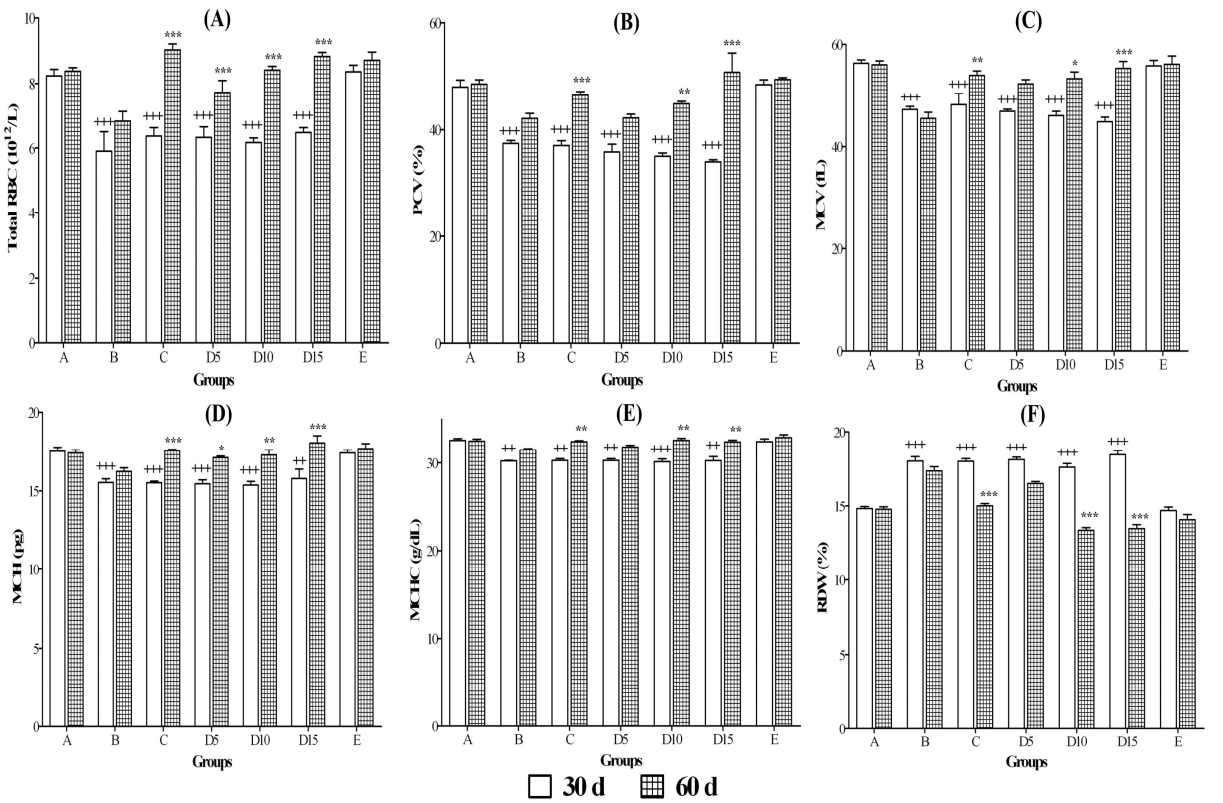

Figure 3: Effect of OFJ on total RBC (A), PCV (B), MCV (C), MCH (D), MCHC (E) and RDW (F) in $HgCl_2$ induced anemia

Values are Mean ± SEM (n = 6), analyzed by one way ANOVA followed by Turkey's multiple comparison test, +++$p < 0.001$, ++$p < 0.01$, +$p < 0.05$ for change difference Vs negative control (group A) at 30 days and ***$p < 0.001$, **$p < 0.01$, *$p < 0.05$ for change difference at day 60 Vs positive control (group B) at 30 days

Effect on Liver and Kidney Functions

Pre and post treated liver and kidney function parameters were estimated at day 30 and 60 respectively in $HgCl_2$ – induced anemia and have been presented in Figure 4. The mean bilirubin level was significantly increased in group B, D5 ($p < 0.01$) and C, D10, D15 ($p < 0.001$) at the day 30 in comparison to the values of group A at the same day. There was not a significant change in alkaline phosphatase in the day 30 in comparison to the values of group A at the same day. The mean Bilirubin (mg/dL) and alkaline phosphatase (IU/L) were found to be 0.513 ± 0.009 and 351.3 ± 42.93 ($p < 0.001$), respectively in group D15 at the day 60 in comparison to the values of group B at the day 30. In group E treated rat, there was not significant change in liver and kidney function parameters. The mean blood urea level was significant increased in group D5 ($p < 0.01$) and in group B, C, D10 and D15 ($p < 0.001$) while mean creatinine (mg/dL) level didn't change in a significant manner at the day 30 with respect to the values of negative control group on the same day. In group D10 and D15 treated animals, the mean blood urea (mg/dL) was found 52.77 ± 1.04 ($p < 0.05$) and 50.72 ± 0.64 ($p < 0.01$) while mean creatinine level was also reduced in OFJ treated animals at the day 60 in comparison to positive control group values at the day 30.

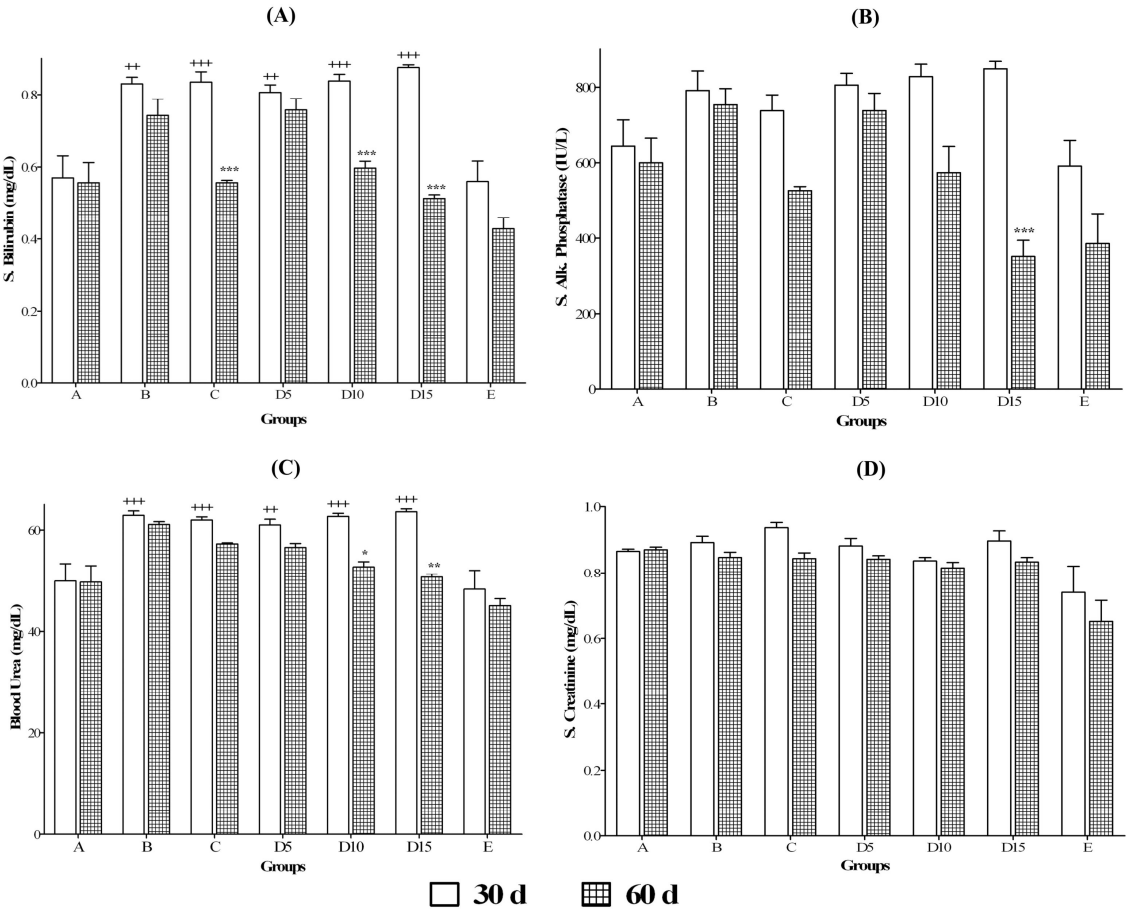

Figure 4: Effect of OFJ on S. Bilirubin (A), S. Alkaline phosphatase (B), Blood urea (C) and S. Creatinine (D) level in $HgCl_2$ induced anemia
Values are Mean ± SEM (n = 6), analyzed by one way ANOVA followed by Turkey's multiple comparison test, +++p < 0.001, ++p < 0.01, +p < 0.05 for change difference Vs negative control (group A) at 30 days and ***p < 0.001, **p < 0.01, *p < 0.05 for change difference at day 60 Vs positive control (group B) at 30 days

Histopathology Study

Histopathological section of kidney, liver and spleen were presented in Figure 5. Kidney section of group A (negative control) rats revealed the normal distinct glomeruli and tubules. In group B rats the kidney showed shrinkage, fibrosis and acute glomerular nephritis. In group C rat fibrosis and acute nephritis were less compared to group B. In fruit juice treated rats better histology was evident, the glomerular and tubular structures were a distinct and more improvement compared to group B and C groups kidney sections. The liver was badly damaged in group B rats, showed distortion of hepatocytes, portal tract dilation, and acute inflammatory infiltration. In standard and fruit juice treated rats, quite 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 spleen in group B rat. In standard and fruit juice treated rats spleen mild fatty changes and cell depletion was observed.

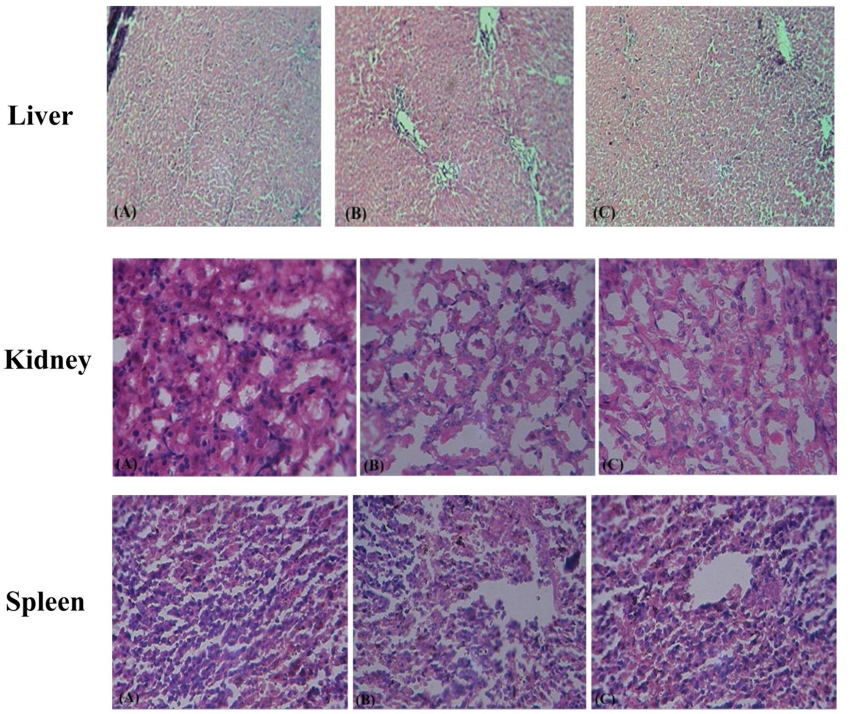

Figure 5: Hematoxyline – Eosin sections of liver, kidney and spleen (450 x) of negative control (A), positive control (B) and OFJ (C) treated rats in $HgCl_2$ induced animal model

DISCUSSION

This study aimed to evaluate the hematinic effect of the *Opuntia elatior* Mill. fruit on $HgCl_2$–induced anemia. Before explaining the possible protective role of fruit, it seems essential to describe the mechanism of Hg induced anemia. Hg ions bind with–SH groups in the bio-membranes, and damage them via lipid peroxidation[14]. Hg also binds with lysosomal membranes and renders them labile[15]. It inhibits protein synthesis[16], alters the tertiary structure of RNA and DNA[17] and affects their synthesis. It disturbs the structure and function of inner mitochondrial[18]. All these effects can be held responsible for anemia due to inorganic Hg –induced cellular damage[19]. Iron deficiency is the commonest cause of hypochromic microcytic anemia. In iron deficiency, the amount of iron lost from the body exceeds the amount absorbed. The physiological demand for iron exceeds iron uptake. First there is depletion of the iron store of the body followed by a reduction in the plasma level of iron and development of hypochromic microcytic anemia. Hypochromic microcytic anemia can be interpreted based on reduction of hemoglobin content, total RBC count, PCV, MCV, MCH, MCHC and increase in RDW values is the indicator of hypochromic microcytosis[12] and that's why we precise the estimation of these hematological parameters. The results obtained after 30 days indicated that $HgCl_2$ –induced hypochromic microcytic or hemolytic anemia was due to iron loss. The results after treatment indicated that the fruit juice of *Opuntia elatior* Mill. increased significantly the hemoglobin, total RBC count, RBC indices (MCV, MCH, MCHC), PCV and decreased RDW. Loss of body weight is a common clinical feature of anemia. It was observed that there was a remarkable increase in body weight in animals treated with OFJ at dose 10 ml/kg (212.2 g) and 15 ml/kg (227.7 g) after continuous treatment for 30 days which was better than standard treated group. Only OFJ (15 ml/kg) treated rat demonstrated a slight increase in body weight (208.7 g) after 30 days but comparatively less than disease treated groups. The reversal of body weight by fruit juice could be considered as a significant effect. It indicates reversal of the toxicant induced tissue degenerative changes. Body weight change is the sum of the effects occurring in different parts of the body and reversal of the toxicant induced decrease is an index of good tissue or cytoprotective activity of the test drugs. Hemoglobin estimation is considered as the marker for evaluating the correction of anemia. At the dose of 10 ml/kg and 15 ml/kg of fruit juice showed a good percentage of recovering in hemoglobin, 32.99 % and 38.18 %, respectively, which was higher than standard treated group (29.8 %) indicating the correction of anemia. The hematinic action of fruit juice was dose dependant manner. It was observed that there was a slight increase in hemoglobin content but not significantly in group E higher dose treated rat. 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, 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. Proteins are synthesized in the liver; low level indicates that the synthetic function of the liver has been markedly diminished. 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[12]. From the bilirubin, alkaline phosphatase and total protein content observations, it seems that fruit juice of *Opuntia elatior* Mill. improves the liver function significantly. The liver showed $HgCl_2$–induced pathological changes[20]. Ashe *et al*. had reported severe hepatic effects in rabbits exposed to metallic Hg vapors[21]. Accidental, fatal Hg vapor inhalation exposures in a young child caused hepatocellular damage and biochemical alterations[22]. Urea is the major nitrogen containing metabolic product of protein catabolism in humans. In leukemia and hemolytic anemia, release of leukocyte protein contributes to high plasma urea. In gastrointestinal disease, plasma proteins and hemoglobin 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[12]. Fruit juice of *Opuntia elatior* Mill. showed tendency towards reversal of these toxicant induced changes. The changes observed after $HgCl_2$ administration can be mainly attributed to the toxicant induced kidney damage. Reversal of most of these changes in fruit juice administration indicates that they do have some element of cytoprotective activity. The kidney is badly damaged by $HgCl_2$ exposure[20]. Fitzhuge *et al*. studied Hg-acetate (25 ppm) –induced changes in kidney of rats and reported a dose dependant change in its structure and function[23]. Among human beings, inorganic Hg salt ingestion result in anuria and uraemia from acute tubular necrosis[24]. The spleen is the storehouse of dead RBC and the breakdown of hemoglobin also occurs in the spleen. Hemolytic anemia leads to accelerated breakdown of hemoglobin causing larger iron deposition in spleen[25]. This is likely to be the cause of fibrosis and necrosis observed in the spleen in $HgCl_2$ 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. Numerous *in vitro* studies have demonstrated the beneficial effect of phenolics and betalains as antioxidant action. These are generally attributed to the ability of antioxidant to neutralize reactive oxygen species such as singlet oxygen, hydrogen peroxide (H_2O_2), or suppression of the xanthine/xanthineoxidase system, all of which may induce oxidative injury i.e. lipid peroxidation[26] and as we know inorganic Hg induced lipid peroxidation, inhibition of protein synthesis and cellular damage which results in anemia.

CONCLUSION

The fruit juice of *Opuntia elatior* Mill. reversed anemia induced by $HgCl_2$ in a dose dependant manner. The antioxidant phenolics and betanin constituents and mineral compositions appear most likely as the active ingredients responsible for hematinic effect of *Opuntia elatior Mill*. fruits. This result supports at least partially the traditional use of fruits in the treatment of anemia.

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