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Clinical evaluation of Beet root and Prickly pear in the management of Anemia: An Observational Study

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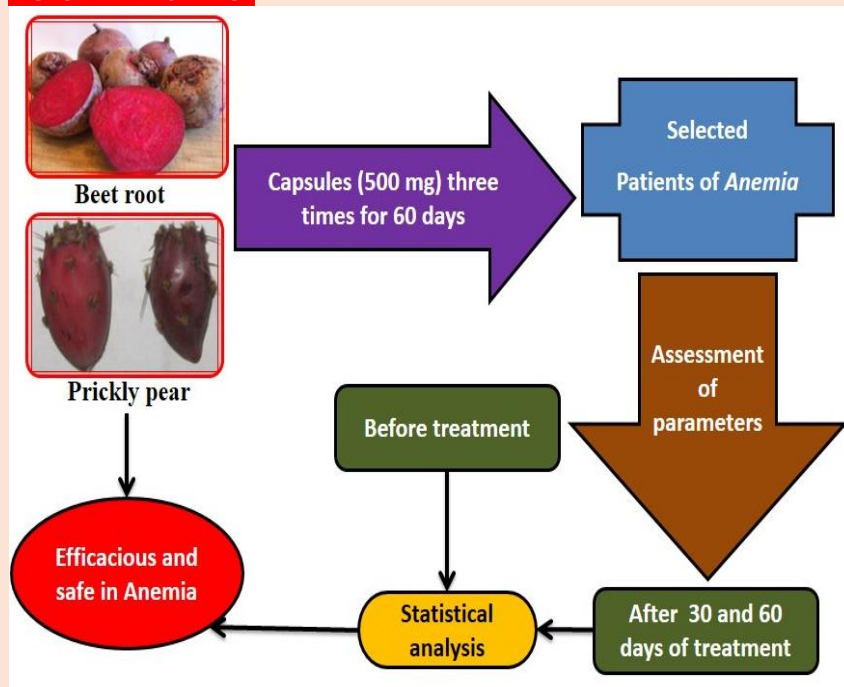
ABSTRACT

Introduction: In conventional medicine, various forms of iron are commonly prescribed, but these therapies have their noted adverse effects like constipation, gastric discomfort and poor bioavailability. The need is felt for search of safe and effective herbal preparation to improve the hemoglobin level. The aim was to evaluate the clinical efficacy and safety of beet root (*Beta vulgaris* L. ssp. *vulgaris*) and prickly pear (*Opuntia elatior* Mill.) as a haematinic agent. **Methods:** Patients were randomly divided into two groups. Group I (BR) (n=11) patients were given beetrootcapsule (00 Size) and group II (PP) (n=18), prickly pear capsule, three times daily after meal for 60 days. Haemoglobin, total RBC, reticulocytes, serum ferritin were assessed initially, mid and at the end of the treatment as primary efficacy variables. Liver function tests and kidney function tests were considered for safety aspect of the treatment. **Results:** There is increase in haemoglobin (Hb) content and total RBC in both treatments after 30 days. On day 60, mean Hb content and total RBC were significantly increased in BR group and in PP group (11.14 ± 0.32 gm%; 9.25 ± 0.39 $10^{12}/L$, $p < 0.001$). **Conclusion:** Beet root and prickly pear confer the folkloric use as haematinic, betacyanin might be responsible for the erythropoiesis and present dose is safe for long term use. Although the concentration of betacyanin compared to beet root is less in prickly pear, but produce more efficacious action on erythropoiesis.

KEYWORDS

Beet root, Prickly pear, Heamatinic, Anemia, Opuntia

PICTORIAL ABSTRACT



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1. Introduction

Anemia is one of the most common health problems in India. The problem is much more in rural than the urban area. The prevalence of anemia in 16 - 70 years age group was 47.9%. The prevalence of anemia was higher among females (50%) than males (44.3%)^[1]. The highest prevalence of anaemia was seen in children <10 years followed by women and older adults. The vast majority of anaemia cases were microcytic, suggesting that iron deficiency was the main cause of anaemia. However, the prevalence of normocytic anaemia increased with age^[2]. The red beet root (*Beta vulgaris* L.) is also an old folk medicine for anemia. Effect of beet juice on haemoglobin among adolescent girls were evaluated and found improvement in haemoglobin level (g%) from 10 to 12.67^[3]. Ethanol extract of beet root effectively raised the level of haemoglobin and erythrocyte count at dose 200 mg/kg in phenyl hydrazine induced anemia in rat^[4]. With respect to antioxidant activity of beet root, it ranked among the ten most potent antioxidant vegetables. Betalains, chief

constituent, provide protection against oxidative stress-related disorders by acting as antioxidants which confirmed by many *in vitro* and *in vivo* study^[5]. Netzel et al. characterised the renal excretion of valuable hydrophilic antioxidant phytochemicals such as phenolics and betalains in healthy humans after red beet juice ingestion^[6]. *Opuntia elatior* Mill. is commonly known as Prickly pear. High levels of betalains, taurine, calcium, magnesium and antioxidants are noteworthy of *Opuntia* species exhibit various pharmacological activities, lending support to the rationale behind several of its traditional uses^[7]. Fruits of *Opuntia elatior* Mill. evaluated for bronchodilatory^[8], mast cell stabilization^[9], analgesic and anti-inflammatory actions^[10]. Author has evaluated haematinic action of prickly pear on HgCl₂ - and phenyl hydrazine - induced anemia in rat^[11,12]. In conventional medicine, various forms of iron viz. ferrous sulfate, ferrous fumarate etc. are commonly prescribed, but these therapies have their noted adverse effects like abdominal pain, constipation, gastric discomfort and poor bioavailability.

Owing to the gravity of the situation, need is felt for search of safe and effective clinically tested herbal nutraceutical oral dosage forms

to improve the haemoglobin level in Anaemia. Based on the traditional and preclinical report, the present study's aim to observe clinical efficacy and safety of beet root (*Beta vulgaris* L. ssp. *vulgaris*) and prickly pear (*Opuntia elatior* Mill.) in the management of anemia.

2. Materials and methods

2.1 Observational trial design

This was a single center, open randomized clinical study conducted at J. S. Ayurveda College & P. D. Patel Ayurveda Hospital Nadiad, Gujarat, India vide IEC approval no. 06/01-2013 and also registered in clinical trial registry of India vide registration no. CTRI/2016/12/007598.

2.2 Participants

Eligible participants were enrolled based on inclusion criteria. Either of gender of age between 18 to 60 years, with haemoglobin between 8 to 10 g%. Exclusion criteria were pregnant, lactating, severe unstable renal, hepatic or any continuing blood loss conditions i.e. haematemesis, melaena. Haemoglobin (g%) investigation was done before the enrolment of participants and signed consent voluntarily to enrol in the trial.

2.3 Study settings

The study took place at J. S. Ayurveda College & P. D. Patel Ayurveda Hospital, Nadiad, Gujarat during September 2014 to June 2016.

2.4 Interventions

Participants (57) were assigned into two groups. Group I patients received beet root capsule (500 mg) and group II, prickly pear capsule (500 mg), administered orally three times daily after meal for 60 days without mentioning special diet.

2.5 Outcomes

Haematological parameters (Hb, total RBC, reticulocytes, ferritin) were assessed initially (day 0), mid (day 30) and end (day 60) of the treatment as primary efficacy variables. Liver function tests (SGOT, SGPT, Alkaline phosphatase) and Kidney function tests (S. Creatinine and Routine Urine Microscopy) were considered for safety aspect of the treatment on 0, 30th and 60th day. During the course of the trial treatment, if any serious condition or serious adverse events that required urgent treatment or if subjects themselves wanted to withdraw from the study, such subjects were withdrawn from the trial.

2.6 Statistical analysis

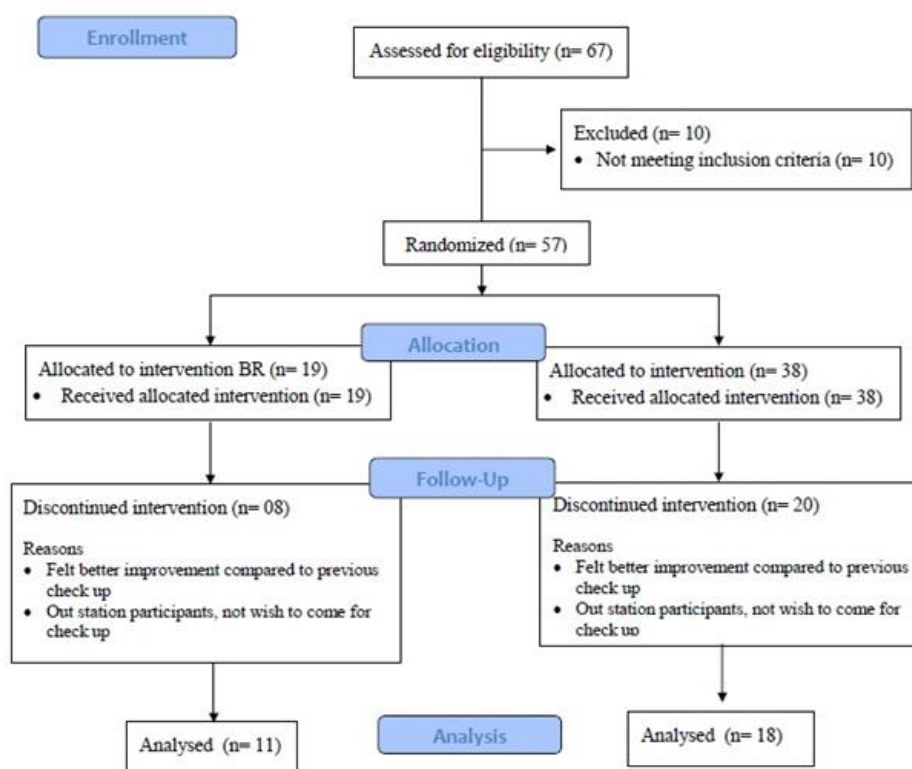
All the values are expressed as Mean \pm SEM (standard error of mean). Data on clinical symptoms and objective tests before and after the treatment will be tabulated and analyzed by one-way ANOVA followed by Turkey's multiple comparison tests. A level of $p < 0.05$ was considered as statistically significant. A level of significance was noted and interpreted accordingly.

3. Results and discussion

Participants flow of efficacious parameters haemoglobin and total RBC presented in Flow chart 1. Pre- and post - treated Haematological parameters (Hb, total RBC, reticulocytes, ferritin) were estimated at day 00, 30 and 60 (Table 1). There is increase in Hb content and total RBC in both treatments after 30 days. On day 60, mean Hb content and total RBC were observed significant rise in BR group and PP group. The PP treated patients were observed little more recovery in Hb and almost double in total RBC compared to BR treated patients. There is no significant change was found in reticulocytes count in both treatments. The ferritin level was reduced on day 30 and 60 in both groups compared to day 00 and significant decreased on day 60

($p < 0.01$) in PP treated patients. The liver function parameters were estimated at the day 00, 30 and 60 and have been presented in Graph 1. After both treatments, there is no significant change in the liver function compared to day 00 readings. Estimation of serum creatinine as kidney function parameter showed no significant change in both treatments (Graph 2). There were not found any adverse effects and complications during study. Participates felt better improvement regarding their complaints like weakness, fatigue, palpitation, effort intolerance and breathlessness which are improved so they did not come for next check-up.

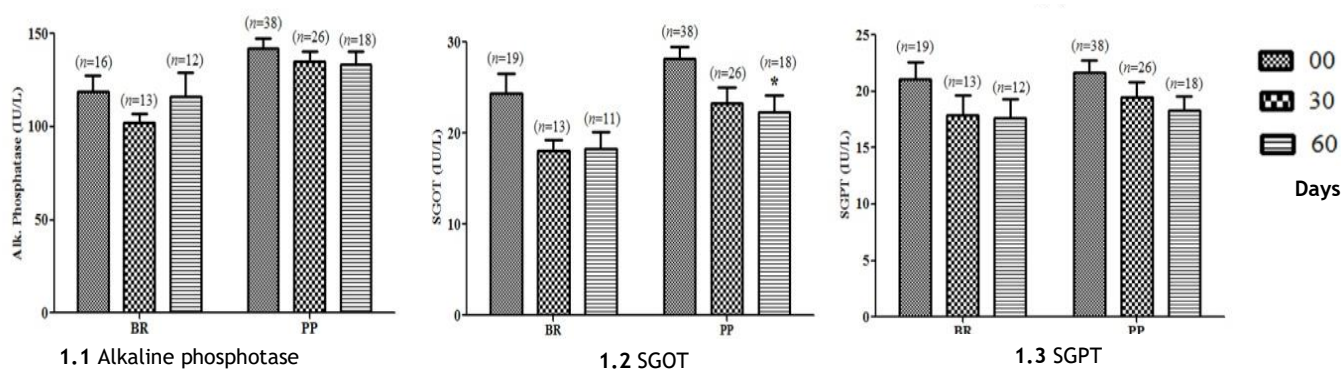
Phytochemicals from food and herbal are continuously investigated for medicinal properties. In conventional medicine, various forms of iron viz. ferrous sulfate, ferrous fumarate etc are commonly prescribed, but these therapies have their noted adverse effects e.g. nausea, vomiting, abdominal pain, gastric discomfort and constipation. The betalain pigments have recently emerged as a novel class of antioxidants. Their presence is limited to a few edible vegetables, such as red beet and prickly pear. In the present study, clinical evaluation of standardized capsules of beet root and prickly pear in the management of anemia have been carried out to establish efficacy and safety in light of folkloric use and preclinical reports. The spectrophotometric and chromatographic analysis suggest that the external color of beet root and prickly pear depends on the relative concentration of betacyanins (red-violet pigments with maximum absorbance at around 535 nm) and estimation of total betacyanin equivalent to betanin is the best method to standardize the capsule. There is a major role of trace elements and vitamins in the erythropoiesis, presence iron and zinc were found in prickly pear and beet root capsules, respectively, while concentration of folic acid was more in prickly pear capsule. So the trace elements as well may be presence of betacyanin play major role in increasing Hb%. Stability studies suggest prickly pear capsule is more stable compared to beet root capsule. At the end of one year, color of beet root capsule powder was faded and turns to brown from red-violet compared to initial, while such degradation was not observed in prickly pear. This indicates prickly pear powder is more stable compared to beet root at room temperature for one year which is confirmed by estimation of total betacyanin. Beetroot is also being considered as a promising therapeutic treatment in a range of clinical pathologies associated with oxidative stress and inflammation. Its constituents, most notably the betalain pigments, display potent antioxidant, anti-inflammatory and chemo-preventive activity *in vitro* and *in vivo*^[13]. The bioavailability of betalains were investigated in the simulated *in vitro* model of the human intestinal epithelium using Caco-2 cell monolayers to mimic a functional barrier^[14-15]. It suggests that betalains can be absorbed in their unchanged form and produce biological action^[16]. The protective action of prickly pear upon alcohol-induced damages in rat erythrocytes was reported^[17]. Prickly pear supplements protect against oxidative damage induced by ethanol in rat's erythrocytes and normalized the impairment of their osmotic stability and morphologic aspect. The progressive recovery of anemic rats responding to prickly pear may be due to increased erythropoiesis and/or antioxidant property of betacyanin. The betalains contents could confer to beet root and prickly pear a potential anti-oxidant activity. In the present study, on day 60, mean Hb content and total RBC were observed significant rise in BR group (10.59 ± 0.32 g%; $4.35 \pm 0.27 \times 10^{12}/L$, $p < 0.001$) and PP group (11.14 ± 0.32 gm%; $9.25 \pm 0.39 \times 10^{12}/L$, $p < 0.001$), respectively. Overall observation showed PP had more efficacious action on Hb, total RBC, reticulocytes and serum ferritin compared to BR. There is a surprisingly significant reduction of ferritin level in PP treatment which may indicate no role of iron in the increment of Hb and total RBC. The liver and kidney functions were normal after the treatment which indicates both are safe for long term treatment. As the intervention was implemented for both sexes and 18 to 60 years aged group, would benefit from beet root and prickly pear in the management of anemia.

Flow chart 1. Participants**Table 1. Effect of BR and PP on Haematological parameters**

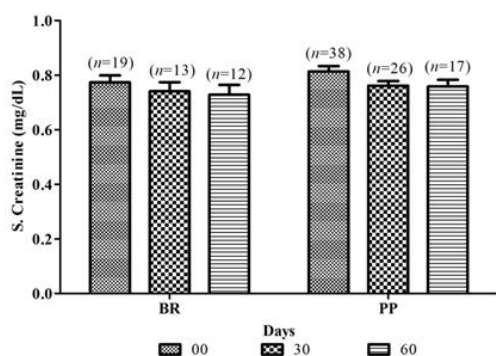
Drug	Haematological Parameter	Days		
		00	30	60
BR	Haemoglobin (g%)	8.50 ± 0.27 (n=19)	9.57 ± 0.33* (n=13)	10.59 ± 0.32*** (n=11)
	Total RBC (10 ¹² /L)	8.50 ± 0.27 (n=19)	4.24 ± 0.10* (n=13)	4.35 ± 0.27** (n=11)
	Reticulocytes (%)	1.29 ± 0.14 (n=15)	1.1 ± 0.12 (n=8)	1.03 ± 0.07 (n=8)
	S. Ferritin (ng/mL)	15.02 ± 5.60 (n=15)	8.40 ± 3.18 (n=8)	7.56 ± 1.92 (n=8)
PP	Haemoglobin (g%)	8.52 ± 0.18 (n=38)	9.46 ± 0.22** (n=26)	11.14 ± 0.32*** (n=18)
	Total RBC (10 ¹² /L)	3.83 ± 0.06 (n=38)	8.82 ± 0.21*** (n=26)	9.25 ± 0.30*** (n=18)
	Reticulocytes (%)	1.48 ± 0.13 (n=34)	1.25 ± 0.09 (n=23)	1.26 ± 0.17 (n=17)
	S. Ferritin (ng/mL)	16.16 ± 2.84 (n=37)	7.60 ± 0.19* (n=25)	4.30 ± 0.75** (n=17)

Values are Mean ± SEM, 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 00 Vs 30 and 60 days.

Graph 1. Effect of BR and PP on Liver function parameters

Values are Mean ± SEM, analyzed by one way ANOVA followed by Turkey's multiple comparison test., * $p < 0.05$ for change difference at day 00 Vs 60.

Graph 2. Effect of BR and PP on Serum Creatinine

Values are Mean \pm SEM, analyzed by one way ANOVA followed by Turkey's multiple comparison test

4. Conclusion

Beet root and prickly pear confer the folkloric use as haematinic, betacyanin might be responsible for the erythropoiesis and present dose is safe and efficacious. Although the concentration of betacyanin compared to beet root is less in prickly pear, but produce more efficacious action on erythropoiesis which is require further investigation to identify the mechanism of betacyanin on erythropoiesis.

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Conflict of interest None declared

Contributors Dr Chauhan contributed to design, conceptualization of the topic and literature study. Dr Gopani contributed to data acquisition. Dr Suhagia and Dr Gupta contributed to the manuscript review and data analysis. Dr Kalapi Patel and Dr Manish Patel contributed to design, literature study and manuscript editing.

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