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Original Article

Haematinic Activity of *Nagaphani* (*Opuntia elatior* Mill.) *Swarasa* through its Betalain Content on Phenylhydrazine-induced Haemolytic Anaemia in Rats

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ABSTRACT

Aim: Woody shrubs of *Nagaphani* (*Opuntia elatior* Mill.) (Family: Cactaceae), bearing a pear shaped fleshy fruits rich in betalain content, vitamins and has antioxidant activities. Its fruits showed a great demand for its daily consumption in anaemic condition and also for its health benefits in general debilities. The present study aimed to rationalise folklore claim on *Nagaphani* at classical dose of original dosage i.e. *swarasa* (fresh expressed juice) in albino rats. **Materials and Methods:** *Nagaphani* was subjected for quality assurance through physicochemical and phytochemical analysis. *In vitro* antioxidant studies of extracts carried out using DPPH scavenging activity and FRAP assay. *In vivo* hematinic activity of *Nagaphani swarasa* at two dose levels (1.8 and 3.6 mL/kg) assessed against phenylhydrazine-induced haemolytic anaemia in rats. **Results:** *Nagaphani* fruit *swarasa* showed *in vitro* antioxidant activity due to presence of betalain, phenolic and Vitamin C contents. In hematinic study, there was significant increase in the haemoglobin and related parameters, serum ferritin, total iron and antioxidants such as glutathione, glutathione peroxidase and superoxide dismutase in anaemic rats. *Nagaphani* fruit *swarasa* reversed the other biochemical parameters, reticulocyte count and TIBC content in a dose-dependent manner in phenylhydrazine-induced haemolytic anaemia in rats. **Conclusion:** The result of study supports the ethanomedicinal claims of *Nagaphani* fruit *Swarasa* as being potent haemitinic drug to treat haemolytic anaemia in albino rats may be due to presence of betalains as an active constituent and potent antioxidant agent.

Keywords: Betalain, Haemolytic anaemia, Haematinic, *Nagaphani*, Phenylhydrazine, *Opuntia elatior*.

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INTRODUCTION

Anaemia is the most prevalent nutritional deficiency disorder around 30% globally and WHO estimated, 50% of children and in 25% of men in developing countries. The highest ubiquity of anaemia exists in the developing world especially in India compared to other developing countries.[1] In India, around 0.5% of total deaths in 2016 were contributed by nutritional deficiencies. Prevalence of anaemia was reduced up to 16% in past 16 years i.e. 74.3% to 58.5% during 2015-16, whereas recent data highlights the on-going micronutrient deficiencies in Indian population. Studies state that 60-70% population faces low ferritin levels. Among 30% of world's population is anaemic with vast margins in developing countries and thus classified amongst the top ten-selected health risks as per World Health Organization.[2]

Anaemia described as *Pandu roga* has age-old history of occurrence and treatments in classical texts typically characterised by the presence of *Ketaki dhulinibhachaya* that means discolouration resembling the colour of the Pandanus flowers. In every case of disease, reduction in number of circulating RBCs and Haemoglobin is witnessed.[3]

In conventional medicine, various forms of iron viz. ferrous sulfate, ferrous fumerate etc. are commonly prescribed, but these therapies have their noted adverse effects e.g. nausea, vomiting, abdominal pain, gastric discomfort and constipation. Therapeutic agents derived from natural produces for treating anaemia has attracted the world to utilize them as an effective alternative therapeutic tool.[3] Woody shrub of *Nagaphani* (*Opuntia elatior* Mill.) (Family: Cactaceae), bearing a pear shaped fleshy fruits rich in betalain content, vitamins and has antioxidant activities. Its fruits are sold in market of Gujarat showed a great demand for its daily consumption in anaemic condition and also for its health benefits in general debilities.[4] Previous research work reported the haematinic activity of *O. elatior* fruits *swarasa* against mercuric

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chloride-induced haemolysis of RBC in rats,[5] while in another study same model was used but at very high dose of *O. elatior* fruits juice (5, 10 and 15 mL/kg),[6] and further, lack of certain parameters for correlation of mode of action of drug.

In the present study, Phenylhydrazine (PHZ) model was used because PHZ generates free radicals and thus, oxidative stress which in turn increase the aging process of RBC cells, potentially resulting in anaemia and consequential secondary involvement of other tissues, such as the spleen and liver.[7] Considering, drug is rich source in nutrient, betalain, vitamins, iron content and anti-oxidant activity, Phenylhydrazine (PHZ)-induced anaemia is best suited model for hematinic activity of *Nagaphani*. Further, 'Romantic approach' suggested that, traditional medicine is good as such and should be remained as,[8] therefore, present research study executed at classical dose of original dosage form of *Nagaphani* i.e. fruit *swarasa* (fresh expressed juice) as given in folklore.[9]

MATERIALS AND METHODS

Collection and expression of fresh *swarasa*

Fully ripened fruits of *Nagaphani* (*Opuntia elatior* Mill.) were collected after proper identification of morphological characters using Flora,[9] from surrounding of Jamnagar, Gujarat (March-April). Here, the study demands the use of fresh fruit *Swarasa* (expressed juice) every time according to the need. Freshly collected *Nagaphani* fruits made free of glochides and spines, outer skin of fruits was removed after thoroughly washed with tap water and fruit pulp was macerated. *Swarasa* obtained after filtering sludge and seeds, remains fresh used for research study.

Quality compliance of *Nagaphani* fruit *swarasa*

Nagaphani fruit *swarasa* was evaluated for Pharmacopeial parameters for quality compliance such as specific gravity, pH, total solid content, viscosity, ash value as per standards procedure in Ayurvedic Pharmacopeia of India.[10] Thin layer chromatography analysis of *Nagaphani* fruit *swarasa* was carried out using mobile phase as Toluene: Ethyl acetate: Acetic acid (7:3:1). *Nagaphani* fruit pulp rich in betalain, was estimated by homogenized in methanol (1 mg/mL), centrifuged at 3000 rpm at 15°C for 10 min. Filtrate separated was analysed in two concentrations i.e. 200 and 400µg/mL using spectrophotometer at 200-600 nm absorption spectra confirmed by observing λ_{max}. Betalain presence was confirmed by presence of peak at 535 nm.[11]

Quantitative estimations

Nagaphani fruit *swarasa* was estimated for the presence of total phenolic content by Folin's Ciocalteu method, total sugar by anthrone reagent method, protein by Kjeldahl method, fat by Soxhlet extraction method, Vitamin C employing coupling reaction of 2,4-dinitrophenyl hydrazine method and Iron content.[12]

In vitro antioxidant study

Extracts of *Nagaphani* fruit at different concentrations were assessed for free radical scavenging activity by using 1, 1-diphenyl-2-picryl hydrazyl (DPPH) and ferric reducing antioxidant power (FRAP) assays against ascorbic acid as a standard.[13]

Experimental animals

Wistar albino rats of both sexes (200±20 g) obtained from Animal house, Institute of Teaching and Research in Ayurveda, Jamnagar, were used on approval of Institutional Animal Ethics Committee (IAEC/23/2018/11) in conformity with the guidelines of the CPCSEA, India. Rats were exposed to 12 hr light and dark cycle with maintained temperature (22±3°C) and 50-60% relative humidity. Animals were fed with standard pelleted diet and drinking water was given *ad libitum*. All rats were reared under same environmental conditions.

Haematinic activity

For induction of anaemia in albino rats, intraperitoneal injection of freshly prepared Phenylhydrazine hydrochloride (40 mg/kg) in normal saline was given for 2 consecutive days at intervals of 24 hr in overnight fasted rats.[14] Wistar albino rats were randomized into different groups, each contains 6 rats. Group-I received distilled water (5 mL/kg, po) as control group and Group-II kept as anaemic control group. Group-III and Group-IV kept as drug treated groups, received *Nagaphani* Fruit *Swarasa* (NFS) orally at therapeutic dose level (TED, 1.8mL/kg) and TEDx2 (3.6 mL/kg) respectively. The fresh fruit *Swarasa* and water to both control groups were given daily for thirty consecutive days. The rat Therapeutic Equivalent Dose (TED) calculated on the basis body surface area ratio,[15] from classical human therapeutic dose of fruit *Swarasa* i.e. 20 mL/day.

The animals were carefully examined for mortality or morbidity, adverse effects, if any during entire experimental period. The body weight of each rat was noted down at day 0, 7, 14, 21 and 30 i.e. on weekly basis. On 30th day, final body weight was noted down thereafter, blood was collected under light ether anaesthesia from retro orbital puncturing of overnight fasted rats. The blood collected in EDTA tube used for various haematological parameters and in plain tube used for separation of serum for biochemical and antioxidant parameters. Thereafter, animals were sacrificed abdomen was opened through midline incision to dissected out the liver, spleen and kidney. The organs examined for sign of toxicity and weighed.

Automatic cell analyser (Swelab, Sweden) used for estimation of haematological parameters- Red Blood Cells (RBCs) and reticulocyte indices, Haemoglobin (Hb), Mean Corpuscular

Haemoglobin (MCH), Mean Corpuscular Haemoglobin Concentration (MCHC), Mean Corpuscular Volume (MCV), Packed Cell Volume (PCV), White Blood Cells (WBCs) and differential count and platelets.

Fully automated auto analyser (BS-200; Lilac Medicare Pvt. Ltd., Mumbai) was used for estimation of serum biochemical parameters by using biochemistry reagent kit-blood sugar, urea, total cholesterol, triglyceride, HDL-cholesterol, VLDL-cholesterol, bilirubin, serum transaminases (SGOT and SGPT), Alkaline Phosphatase (ALP), total protein, albumin, globulin and A/G ratio, Total Iron Binding Capacity (TIBC), total iron and transferrin. Further, remaining serum stored at -20°C immediately and afterwards subjected to estimation of total protein,[16] Superoxide Dismutase (SOD) activity,[17] total glutathione,[18] and Glutathione Peroxidation (GPx).[19]

Statistical analysis

The data are expressed as mean ± Standard Error of Mean (SEM) (n=6). One way Analysis of Variance (ANOVA) followed by Dunnett's multiple 't' test was used to compare the mean of quantitative variables among groups and Student 't' test for paired and unpaired data were used for statistical analysis. $p<0.05$ was considered as value of significance.

Table 1: Qualitative analysis of betalains in *Nagaphani* fruit *swarasa*.

Conc. (µg/mL)	Wavelength (nm)	Absorption
200 µg/mL	535.50	0.095
	272.00	0.083
400 µg/mL	535.50	0.223
	273.00	0.312

Table 2: Quantitative estimations in *Nagaphani* fruit *swarasa*.

Parameters	Results
Total phenolic (µg/mL)	213.60±32.32
Total sugar (µg/mL)	161.59±17.32
Protein (µg/mL)	19.8
Fat (µg/mL)	2.5
Vitamin C (µg/mL)	12.9
Iron (ppm)	1877.9 ppm = 0.1877%

RESULTS

Quality compliance of *Nagaphani* fruit *swarasa*

The data on physicochemical parameters and HPTLC study complied with prescribed Pharmacopoeial limit. Spectrophotometric analysis of methanolic extract of *Nagaphani* fruit at 200 and 400 µg/mL showed one peak of visible absorption spectra at 484 nm may be due to presence of betaxanthins in low concentration and other peak at 535 nm due to higher concentration of betacyanins (Table 1).

Quantitative estimations

Nagaphani fruit *swarasa* contains higher amount of total phenolic content (213.60 µg/mL) expressed as gallic acid equivalent, sugar (161.59 µg/mL), protein (19.8 µg/mL), fat (2.5 µg/mL), Vitamin C (12.9 µg/mL) and Iron (1877.9 ppm, 0.188%) (Table 2).

In vitro antioxidant activity

DPPH scavenging activity of methanolic and water extracts of *Nagaphani* fruit was determined by the IC_{50} values in various concentrations (100-600µg/mL). Standard ascorbic acid exhibited lowest IC_{50} value and highest activity followed by water extract of *Nagaphani* fruit (501.15 µg/mL) and methanolic extract (666.71 µg/mL) (Table 3). The ferric reducing antioxidant power response of water and methanolic extracts (10-100 µg/mL) of *Nagaphani* fruit increased with concentrations. Lowest IC_{50} value and highest activity was found in water extract (13.62 µg/mL) followed by methanolic extract (15.54 µg/mL) (Table 4).

In vivo haematinic activity

Phenylhydrazine treated rats showed decrease in body weight of rats in comparison to control group. *Nagaphani* fruit *swarasa* at lower dose produced significant increase in body weight of albino rats in comparison to initial values. Other treated rats did not produce any significant effects on body weight of rats. Phenylhydrazine treated rats showed significant increase in liver

Table 3: DPPH free radical scavenging activity of *Nagaphani* fruit.

Concentrations (µg/mL)	% Inhibition			
	Water extracts		Methanol extracts	
	Ascorbic acid	*Nagaphani*	Ascorbic acid	*Nagaphani*
100	93.14	91.42	83.54	84.81
200	84.00	88.00	75.94	77.84
300	69.14	72.57	67.08	68.98
400	56.57	62.85	56.96	65.82
500	43.42	50.28	44.93	61.39
600	33.14	37.14	37.34	53.79
IC_{50} (µg/mL)	456.64	501.15	464.79	666.71

Table 4: Ferric Reducing antioxidant power of extracts of *Nagaphani* fruit.

Concentration (µg/mL)	% Inhibition			
	Water extracts		Methanol extracts	
	Ascorbic acid	*Nagaphani*	Ascorbic acid	*Nagaphani*
10	41.93	64.51	63.70	30.64
20	59.27	75.80	68.54	60.48
40	71.77	90.32	79.03	86.29
60	89.51	103.22	87.09	104.03
80	107.25	120.96	92.74	127.41
100	120.96	129.83	97.58	137.09
IC_{50} (µg/mL)	14.09	13.62	30.71	15.54

Table 5: Effect of test drugs on the body weight and relative organ weight of albino rats.

Parameters	Control	PHZ	NFS, TED	NFS, TEDx2
Body weight				
Initial	217.50±10.39	180.00±4.46	211.83±7.98	206.50±5.62
7th Day	218.33±6.60	221.50±17.08	198.17±10.93	201.67±5.28
21st Day	203.50±8.08	222.17±14.58	241.17±7.12#	213.33±11.67
28th day	212.00±14.35	189.50±5.86	230.83±9.12#	210.67±10.87
Relative organ weight (g/100 g body weight)				
Liver	2.72±0.195	3.99±0.231@	2.82±0.084*	2.95±0.129*
Spleen	0.174±0.010	0.790±0.055@	0.211±0.005*	0.202±0.014*
Kidney	0.663±0.017	0.715±0.023	0.587±0.008	0.711±0.075

All values are expressed in mean±SEM.# *p<0.01* compared with initial values (Paired '*t*' test); @ *p<0.01*, when compared to normal control group; **p<0.01*, when compared to phenylhydrazine control group (Annova followed by Dunnett's multiple '*t*' test).

and spleen weight which was significantly reversed by *Nagaphani* fruit *swarasa* at both dose levels in comparison to phenylhydrazine control group (Table 5).

Phenylhydrazine treated rats showed significant decrease in neutrophils, total RBC count, haemoglobin, PCV and MCHC while significant increase in lymphocyte, MCH and MCV in comparison to control group. Administration of *Nagaphani* fruit *swarasa* at both dose levels showed significant restoration of above haematological parameters except MCHC in comparison to phenylhydrazine control group (Table 6).

Phenylhydrazine adversely affects the carbohydrate and lipid metabolism by significant increase in blood glucose, non-significant decrease in serum cholesterol, HDL-cholesterol while increase in triglyceride and VLDL cholesterol levels in comparison to control group. Phenylhydrazine treated rats exhibited decrease blood urea, increase in SGOT and alkaline phosphatase levels when compared to control group. Administration of *Nagaphani* fruit *swarasa* showed cholesterol lowering effect, significant reversal in SGOT, alkaline phosphatase, total bilirubin and blood urea levels when compared to Phenylhydrazine treated group. Phenylhydrazine in rats resulted into significant increase in TIBC level, while decrease in total iron content in anaemic rats, which was significantly reversed by *Nagaphani* fruit *swarasa* at both dose levels. Transferrin level was detected below <0.0878 in all rats including control and PHZ control groups (Table 7).

Phenylhydrazine administration in rats showed significant decrease in serum superoxide dismutase and glutathione peroxidase while non-significant decrease in glutathione when compared to control group. Antioxidant properties of *Nagaphani* fruit *swarasa* shown by significant increase in superoxide dismutase, glutathione and glutathione peroxidase in serum of rats when compared to phenylhydrazine control group (Table 8).

DISCUSSION

In the present research study, relationship between folklore claim on *Nagaphani* (*Opuntia elatior*) fruit and its antioxidant activities were evaluated through *in vitro* and *in vivo* antioxidant parameters and quantitative estimation of betalain content. DPPH assay, a simple and sensitive antioxidant parameter allows testing of both lipophilic and hydrophilic compounds.[13] DPPH scavenging activity of methanolic and water extracts of *Nagaphani* fruit was determined by the IC_{50} values in various concentrations. An IC_{50} value is the concentration of the sample required to scavenge

Table 6: Effect of test drugs on haematological parameters in albino rats.

Parameters	Control	PHZ	NFS, TED	NFS, TEDx2
WBC (10^3/Cumm)	8983.3± 661.02	8283.3± 912.66	8583.3± 936.45	8333.3±384.4
Neutrophils (%)	21.67±2.04	7.00±1.65@@	17.00±1.73*	21.17±2.05*
Lymphocytes (%)	74.67±2.27	89.00±2.08@@	78.50±2.11*	73.17±1.49*
Eosinophil (%)	2.17±0.31	1.83±0.31	2.17±0.31	2.67±0.33
RBC (10^3/µL)	8.50±0.43	3.95±0.14@@	7.46±0.26*	7.65±0.22*
Hb (g%)	15.58±0.56	11.57±0.29@@	15.82±0.37*	16.32±0.59*
Platelet (10^3/µL)	880.83±60.58	1021.5±72.96	1019.83±46.02	761.5±143.15
PCV (%)	48.67±2.14	40.55± 0.99@	53.50±2.18*	54.67±1.64*
MCV (fl)	57.30±0.91	104.07±1.57@@	71.80±1.92*	71.53±1.13*
MCH (pg)	18.37±0.33	29.58±0.37@@	21.30±0.52*	21.37±0.72*
MCHC (g/dL)	32.08±0.39	28.43±0.19@	29.73±0.99	29.93±1.29

All values are expressed in mean±SEM.@ $p<0.05$, @@ $p<0.01$, when compared to normal control group; *$p<0.01$, when compared to phenylhydrazine control group (Annova followed by Dunnett's multiple '*t*' test).

Table 7: Effects of test drugs on serum biochemical parameters in albino rats.

Parameters	Control	PHZ	NFS, TED	NFS, TEDx2
Blood sugar (mg/dL)	68.00±4.39	68.00±4.39	77.00±3.96*	79.00±4.43*
Urea (mg/dL)	52.83±4.64	34.50±2.06@	45.17±3.83	59.33±5.82**
Cholesterol (mg/dL)	63.17±3.91	53.50±2.70	61.50±2.91	62.00±5.95
Triglyceride (mg/dL)	132.33±25.65	158.50±33.50	129.17±13.05	116.00±8.06
HDL-chol. (mg/dL)	40.33±5.06	31.33±1.73	43.67±3.28	42.17±6.07
T. Bilirubin (mg/dL)	0.267±0.021	0.333±0.021	0.217±0.017**	0.217±0.017**
D. Bilirubin (mg/dL)	0.133±0.021	0.167±0.021	0.100±0.00	0.117±0.017
VLDL-chol. (mg/dL)	26.50±5.14	31.67±6.67	25.83±2.67	23.17±1.62
SGPT (IU/L)	65.83±8.77	57.00±4.02	60.83±4.89	64.83±7.97
SGOT (IU/L)	137.33±13.49	149.17±7.46	114.33±2.49*	108.33±7.99*
ALP (IU/L)	111.00±19.01	229.50±16.64@@	144.00±13.94**	93.67±15.24**
Total proteins (g/dL)	6.783±0.224	6.317±0.224	6.967±0.174	6.717±9.659
Albumin (g/dL)	2.42±0.25	2.45±0.04	2.45±0.09	2.50±0.19
Globulin (g/dL)	4.37±0.07	3.87±0.23	4.52±0.13	4.47±0.09
A:G Ratio	0.567±0.061	0.650±0.050	0.533±0.021	0.567±0.049
TIBC (ng/dL)	251.17±10.45	347.33±4.08@@	277.17±7.96**	269.00±11.74**
Total Iron (ng/dL)	259.17±33.85	104.33±7.42@@	158.17±29.50	189.33±38.94
Transferrin (g/L)	<0.0878	<0.0878	<0.0878	<0.0878

All values are expressed in mean±SEM.@ $p<0.05$, @@ $p<0.01$, when compared to normal control group; *$p<0.05$, **$p<0.01$, when compared to phenylhydrazine control group (Annova followed by Dunnett's multiple '*t*' test).

50% of the free radicals in the system. Standard ascorbic acid revealed highest DPPH scavenging activity as shown by lowest IC_{50} value. *Nagaphani* fruit in water extract has pronounced DPPH scavenging activity followed by methanolic extract in dose dependent manner. Therefore, it is assumed that, *O. elatior* fruit showed hydrogen-donating ability and serve as free radical scavengers and thus serving as primary antioxidants.

Determination of the reductive ability of *Nagaphani* fruit was measured by transformation of Fe^{3+} to Fe^{2+} which has been known to take place when drug having reducing property.[13] In the present study, concentration dependent increase was observed in *Nagaphani* fruit extracts and ascorbic acid. A lowest IC_{50} value was found in water extract followed by methanolic extract of *O. elatior* fruit. It is evident from findings that, the *Nagaphani* fruit swarasa

Table 8: Effects of tests drugs serum antioxidant parameters in albino rats.

Parameters	Control	PHZ	NFS, TED	NFS, TEDx2
SOD (Unit/mg protein)	1.037±0.04	0.634±0.12@	1.597±0.164**	1.168±0.04**
Glutathione (µmoles/mg)	65.49±14.53	43.11±2.25	87.88±10.69**	76.89±5.87*
GPx (ng of GSH utilized/ mg protein/ min)	59.91±1.09	49.88±0.63@@	57.95±1.87**	62.27±1.77**

All values are expressed in mean±SEM.@$p<0.05$, @@$p<0.01$, when compared to normal control group; *$p<0.05$, **$p<0.01$, when compared to phenylhydrazine control group (Annova followed by Dunnett's multiple '*t*' test).

possesses antioxidant activity in a concentration-dependent manner, which may imply its relevance in attenuating oxidative damage to cellular components and thereby prevent oxidative stress. *Nagaphani* fruit is rich in nutrition, vitamins and phenolic,[20] confirmed by quantitative presence of high phenolic content in the present study, which may be major contributor to for their antioxidant capacity,[21] and presence of sugar content.

Nagaphani fruit has attracted attention due to its nutritional and health-promoting benefits, being rich in bioactive antioxidant compounds (betalains, ascorbic acid and polyphenols) and also as a good source for red and yellow food coloring. *Nagaphani* contains betalains in the fruits, particularly betacyanins in the purple variety and betaxanthins in the orange variety. Betalains have a number of health properties such as anti-cancer, anti-viral activity and natural antioxidant.[22] The most convenient way to quantify betalains is spectrophotometric method. The spectrophotometric analysis suggests that the external colour of prickly pear fruits depends on the relative concentration of betacyanins (red=pigments with maximum absorbance at around 535 nm) and betaxanthins (yellow pigments with maximum absorbance at around 480 nm). Results confirm the, *Nagaphani* fruits, has rich in betacyanins and low level of betaxanthins which may be responsible for its antioxidant effects.

Haemolytic anaemia caused by phenylhydrazine, causes stimulation of erythropoiesis resulting in splenomegaly and hepatomegaly,[23] which is corroborate in the present study with significant increase in relative weight of liver and spleen in Phenylhydrazine treated rats. Administration of *Nagaphani* fruit *swarasa* at both dose levels significantly decreases the liver and spleen weight thus provide protection against inflammatory changes in spleen and liver.

The blood profile usually furnishes vital information on the response of the body to impairment, distress and/or stress. Decrease or increase in cell counts and depletion plasma constituents or their elevation beyond reference range equally demonstrates haematoxicity.[24] Exposure to phenylhydrazine may cause damage to red blood cells by increased oxidative stress, potentially resulting in anaemia and consequential secondary involvement of other tissues, such as the spleen and liver.[25]

Phenylhydrazine-induced toxicity in rats shown by decrease in WBC, significant decrease in neutrophils and increase in lymphocytes count, which was reversed by *Nagaphani* fruit *swarasa* at both dose levels. Phenylhydrazine has been reported to cause the direct lysis of erythrocytes by non-immune mechanisms,[23] generates free radicals that in turn increase the aging process of red blood cells as a result of anaemia in albino rats.[7] In present study, Phenylhydrazine treated rats showed highly significant decrease in total RBC count and haemoglobin levels. Haemolytic condition in rats was revealed by significant decrease in PCV and MCHC, while significant increase in MCV and MCH which may be due to rise in free plasma haemoglobin.[26] *Nagaphani* fruit *swarasa* reversed total RBC count, haemoglobin and RBC related parameters in albino rats. Present result correlated with the results of previous study as increase in total RBC count by the drug which ultimately leads to carry out more amount of haemoglobin to the tissues thereby, normalizing the anaemic condition.

To predict erythroid changes, reticulocyte counts are more sensitive than erythropoietin level. It is reported as a percentage of total RBC count or as absolute numbers.[27] During massive haemolysis, spleen became unable to deal with immature reticulocytes causing delay in reticulocytes maturation. So, it will become a primary task for any drug to promote maturation of reticulocytes. Present study shows positive effects of *Nagaphani* fruit *swarasa* by significant reduction of reticulocytes in Phenylhydrazine treated rats.[28] Here present study proved that, *Nagaphani* fruit *swarasa* have a strong role in the maturation of macrocytic, hyperchromic erythrocytes to macrocytic, normochromic erythrocytes thereby, restoring normal range of haematological parameters along with stimulating erythropoiesis process.

Nagaphani fruit *swarasa* showed significant increase in blood sugar level in albino rats may be due to presence of natural sugar content, however the blood sugar values are still within normal range. The result confirms that *swarasa* is natural source of nutrition and energy. Phenylhydrazine adversely affects the lipid metabolism by non-significant decrease in serum cholesterol, HDL-cholesterol and increase in triglyceride and VLDL cholesterol levels may be due to liver toxicity. *Nagaphani* fruit *swarasa* reversed above conditions showing its anti-hyperlipidaemic and cholesterol lowering activity.

Bilirubin measurement is also a useful index to assess the haemolytic anaemia as well as to detect impairment in excretory

function of liver.[29] *Nagaphani* fruit *swarasa* at both dose levels significantly reduces serum bilirubin level particularly total bilirubin, exhibiting its capacity to improve the liver functions significantly as well as reversed the haemolytic condition in albino rats. Phenylhydrazine treated rats showed significant decrease in blood urea in comparison to control group, which is reversed back to normal range by test drug.

The transaminases (SGOT and SGPT) are popular enzymes used as biomarkers for anticipation of possible liver and heart toxicity. Alkaline phosphatase level in serum rises due to leakage of damaged liver cells and also during biliary tract damage.[30] In present study, phenylhydrazine treated rats showed increase in SGOT and significant increase in alkaline phosphatase levels which may suggest liver toxicity in rats. Protective role of test drug is depicted by reversal in serum transaminases and alkaline phosphatase levels in phenylhydrazine treated rats.

Ferritin is a chief iron-storage protein, present in sera as well as positively correlated with iron stores, where it functions to detoxify and store intracellular iron.[31] Iron although present in trace amount, act as an essential mineral for oxygen transport and energy metabolism. Dietary iron entering the body is carried out throughout the blood stream by the protein called transferrin, produced by liver. TIBC evaluates how well transferrin carries iron through the blood. Mostly deficiency of iron occurs in iron-deficiency anaemia and haemolytic anaemia and liver damage.[32] In present study, phenylhydrazine administration resulted into significant increase in TIBC level, while decrease in total iron content in anaemic rats, which was reversed by *Nagaphani* fruit *swarasa* at both dose levels. *Nagaphani* is good source of iron as revealed from quantitative analysis of *swarasa*.

Phenylhydrazine-induced haemolysis is mediated through active haemolytic agent i.e. most probably phenyl free radical formed by the reaction of PHZ with oxygen.[33] In present study, Phenylhydrazine treated rats showed significant decrease in superoxide dismutase and glutathione peroxidase while non-significant decrease in glutathione suggest the reactive oxygen species generation during anaemic conditions in albino rats. The mode of defensive properties of antioxidants agents against cellular damaging effects of reactive oxygen species includes counteract its formation, interruption negative attack and conversion to stable molecules thus lower reactivity.[34] Superoxide anion was found to be unimportant in phenylhydrazine-induced haemolysis and destruction of oxyhaemoglobin.[35] Glutathione, an excellent antioxidant molecule has redox signalling properties while reduced glutathione frequently targets reactive oxygen species, serves to maintain reduced cellular environment. Antioxidant properties of *Nagaphani* fruit *swarasa* revealed by significant increase in anti-oxidant parameters in serum of rats when compared to phenylhydrazine control group.

Previous *in vitro* and *in vivo* studies confirms the protective role of Vitamin C and Betalains as antioxidants against oxidative stress-related disorders.[36] The betalain pigments have recently emerged as a novel class of antioxidants. It suggests that betalains can be absorbed in their unchanged form and produce biological action. *Nagaphani* species possesses high levels of betalains, taurine, calcium, magnesium and Vitamin C antioxidants thereby exhibiting its traditional uses.[37] The progressive recovery of anaemic rats responding to *Nagaphani* may be due to increased erythropoiesis and/or antioxidant property of betalains.

CONCLUSION

Detail data profiles clearly indicate hematinic activity of *Nagaphani* (*Opuntia elatior*) fruit *swarasa* against phenylhydrazine-induced anaemia in albino rats. The result of study supports the ethanomedicinal claims of *Nagaphani* fruit *Swarasa* as being potent haemitinic drug to treat haemolytic anaemia may be due to presence of betalains as an active constituent and potent antioxidant agent.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

IAEC: Institutional Animal Ethics Committee; **PHZ:** Phenylhydrazine Hydrochloride; **TED:** Therapeutic Equivalent Dose; **GPx:** Glutathione Peroxidase; **TIBC:** Total Iron Binding Capacity; **SGOT:** Serum Glutamic Oxaloacetic Transaminase; **SGPT:** Serum Glutamic Pyruvic Transaminase.

SUMMARY

Ethano-pharmacologically, *Nagaphani* (*Opuntia elatior* Mill.) is a prominent species of Saurashtra region of Gujarat where its fresh fruit juice is primarily reported to possess anti-anaemic efficacy due to presence of bio-active compounds like carbohydrates, flavonoids, phenols, vitamins etc. without causing any toxic effects for long term use. Study design was focused with an aim to evaluate *in vitro* antioxidant activity and *in vivo* haemitinic activity of fresh juice of *Nagaphani* at two dose levels (1.8 mL/kg and 3.6 mL/kg, po) in wistar albino rats against phenylhydrazine-induced haemolytic anaemia. The effects were assessed on haematological and biochemical parameters, reticulocyte count and serum anti-oxidant parameters. Fruit juice of*Nagaphani*, significantly improved the liver functions as well as reversed the haemolytic condition and also increased iron content at both dose levels. From the present study, it is concluded that, data profile

clearly indicates hematinic activity of *O. elatior* fruit juice in Phenylhydrazine-induced haemolytic anaemia in rats may be due to presence of betalains as an active constituent and potent antioxidant agent.

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