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Evaluation of the haematinic activity of *Opuntia elatior* Mill. fruit

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Opuntia elatior, known as *Nagaphani* or *Hathalo-thore* belongs to the family Cactaceae. It is one of the *Opuntia* species used as medicine for various ailments due to its beneficial health-promoting properties. Fruits of *Opuntia elatior* have been advocated in anaemia, asthma, cough, inflammation, and gonorrhoea in Gujarat. The present study was planned to evaluate the hematinic effect of *Opuntia elatior* Mill. fruit on mercuric chloride ($HgCl_2$) induced anaemia in rats. *Opuntia elatior* fruit *Swarasa* was administered to Charle's foster albino rats for 30 consecutive days at the doses of 1.8 mL/kg and 3.6 mL/kg. The effects of both drugs were assessed on ponderal changes, haematological, serum biochemical, and histopathology of various organs. The fruit *Swarasa* showed significant increase in the haemoglobin content, serum ferritin level and serum TIBC level. The test drug at both dose levels produced adverse changes of mild intensity in liver, kidney and heart and reverted the disturbance in the cytoarchitecture of the spleen, thymus and lymph node. Test drug *Opuntia elatior* fruit *Swarasa* reversed anaemia induced by $HgCl_2$ in a dose-dependent manner. The results support the traditional use of fruits in the treatment of anaemia.

Keywords: Anemia, Haematinic activity, Mercuric chloride*, Nagaphani*, *Opuntia elatior* Mill.
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Introduction

The WHO report shows that 35 to 40 % of women, 43% of children below 5 years of age and 27 % of adolescents are anaemic in developing countries[1]. As per National Family Health Survey (NFHS-3), in India, the prevalence of anaemia was 70 % in children (6–59 months), 55 % in females (15–49 years), and 24 % in males (15–49 years) during 2005-2006[2]. Iron is responsible for the transport of molecular oxygen in higher organisms[3]. Lack of iron shows a specific deficiency syndrome namely iron deficiency anaemia. This disease, though described many years ago in ancient classics by the name of *Panduroga*, has even today got its place among other diseases.

Among some of the folklore claims, the presence of iron has been reported in the *Swarasa* of the fruit of *Opuntia elatior* Mill. belonging to the family Cactaceae[4]. This supports its ethnomedicinal claim in the management of anaemia and general debility. It is being used by the people of Gujarat as *Nagaphani* or *Hathalo-thore*. The Pharmacognostic evaluation of its stem has been reported earlier[5]. Its fruit is also a rich source of nutrients and vitamins[6,7] and is eaten fresh, dried or preserved in jams, syrups or processed into candy-like products[8,9]. *O. elatior* is reported to possess anti-oxidant[10], anti-asthmatic[11], anti-ulcer[12], anti-leukemic[13], anti-inflammatory activity[14] etc. In the previous study, haematinic evaluation *O. elatior* fruit juice was done at the higher dose level of (5, 10 and 15 mL/kg) on mercuric chloride ($HgCl_2$) anaemia in rats[15]. Till date, reports of the hematinic effect of this medicinal plant were not available at the dose prescribed in folklore practices. During extensive literature review, it was thought worthwhile to undertake detailed experimental study in modulating the extent and severity of anaemia in rats to substantiate its folklore claim at actual prescribed dose in practice.

Materials and Methods

Collection and preparation of test formulations

The fresh fruits were daily collected around the place of Jamnagar, Gujarat. They were authenticated by the taxonomist of Pharmacognosy Department, Gujarat Ayurved University, Jamnagar and deposited in the museum (Specimen: phm/6138/1/1/2014) for future references. Freshly collected fruits were thoroughly washed with compressor nasal with an adequate amount of water and the bunch of thorns

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over the fruits was neatly plucked using forceps. Following this, the outer skin of fruits was removed and the remaining part of fruits was macerated and the resultant juice obtained was passed through a sieve and filtered. The residue consisted of the sludge and seeds. The filtered juice was used for this experimental study.

Animals

Adult Charle's foster albino rats of either sex, weighing between 200±20 g were obtained from the Animal house attached to Pharmacology laboratory. They were maintained under standard conditions of temperature (23±2 °C, relative humidity (50-60 %) and 12 h light and dark cycles. They were fed with diet Amrut brand rat pellet feed (Pranav Agro Industries) and drinking water *ad libitum*. The experimental protocol was approved by the Institutional Animal Ethical Committee (IAEC/16/2014/08) as per CPCSEA, India.

Dose fixation

In Ayurveda, the usual dose of *Swarasa* i.e. expressed juice is quoted to be 20 mL. The test dose of the drugs for the experimental study was calculated by extrapolating this human dose (20 mL per day) to animal dose based on the body surface area ratio by referring to the standard table of Paget and Barnes[16].

Haematinic activity

In the present study, mercuric chloride ($HgCl_2$) was used to induce anaemia in rats[17]. A solution of mercuric chloride was administered in 9 mg/kg dose through oral route for 30 days. In the treated group, suspension of test drugs was given along with mercuric chloride solution. Total 24 Charles Foster rats of either sex weighing between 180 to 250 g were taken and divided randomly into 4 groups, each containing 6 animals (3 male and 3 female). Group (I) received distilled water (5 mL/kg, p.o.), Group (II) received $HgCl_2$ control group, Group (III) received juice of *O. elatior* Mill. therapeutically effective dose (TED) (1.8 mL/kg, p.o. equivalent to the dose of *Swarasa* i.e 20 mL per day, and Group (IV) received Juice of *O. elatior* Mill. TED*2 (3.6 mL/kg, p.o.) equivalent to double the dose i.e 40 mL per day.

The test drugs and vehicle were administered for 30 consecutive days. Mercuric chloride solution was administered orally to the group (III) and (IV) in a dose of 9 mg/kg after one of test drug administration daily. The same schedule was continued for 30 days with daily doses of test drugs and vehicle.

Body weight was noted down before the commencement of the study and afterwards every 7th day along with general behaviour pattern by exposing each animal to open arena. Haematological and serum biochemical parameters were estimated on the day of sacrifice i.e. 31st day. On the 31st day, all animals were kept for overnight fasting. Next day blood was collected by supra-orbital puncture with the help of microcapillary tubes under mild ether anaesthesia for estimation of serum biochemical and haematological parameters and animals were sacrificed by an overdose of ether anaesthesia. The abdomen was opened through a midline incision.

The haematological analysis was performed by using an automatic haematological analyzer (Swelab, Sweden). Total red blood cell (RBC), hemoglobin (Hb), hematocrit, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), white blood cell (WBC), neutrophils, lymphocyte percentage, eosinophils percentage, monocyte percentage, packed cell volume (PCV), and platelet count were measured from the blood samples.

Serum biochemical parameters were carried out by using fully automated biochemical random access analyzer (BS-200, Lilac Medicare Pvt. Ltd., Mumbai). The studied parameters were blood glucose[18], urea[19], creatinine[20], total cholesterol[21], HDL-cholesterol[22], triglyceride[23], VLDL-cholesterol, LDL-cholesterol, total protein[24], albumin[25], alkaline phosphatase[26], SGOT[27], SGPT[28], uric acid[29], direct bilirubin, total bilirubin[30], serum calcium[31], serum iron[32], serum ferritin and TIBC[33].

All the important internal organs were carefully dissected namely liver, heart, spleen and kidney. After noting signs of the gross lesion and ponderal changes of major organs, all were transferred to 10 % phosphate-buffered formalin solution for fixation and later on, subjected to dehydrating, wax embedding, sectioning and staining with haematoxylin and eosin for histological evaluation. The slides were viewed under trinocular research Carl-Zeiss's microscope at various magnifications to note down the changes in the microscopic features of the tissues.

Statistical analysis

The data are expressed as mean±standard error of the mean for six rats per experimental group. Student

't' test and one-way analysis of variance (ANOVA) was used to compare the mean values of quantitative variables among the groups followed by Dunnet's multiple comparison test for unpaired data to determine the significant difference between groups at $p < 0.05$.

Results

Effect on body weight

Control group showed a significant increase in body weight during experimental period in comparison to initial values. $HgCl_2$ treatment and drug-treated groups did not show any changes in body weight during the experimental period of 30 days (Table 1). TED and TED*2 group showed insignificant increase in the relative weight of thymus, TED*2 group showed insignificant increase in the relative weight of spleen, TED and TED*2 group showed non-significant effect in the relative weight of liver, TED and TED*2 group insignificant decrease was observed in kidney weight and TED group showed significant increase in heart weight as compared to $HgCl_2$ group (Table 2).

Effect on haematological parameters

Test drug did not affect the WBC count. At the higher dose level, the test drug reverted the lymphocyte count and neutrophil count at non-significant level in comparison to $HgCl_2$ control group. Both dose level test drug produced non-significant increase in monocyte count and significant increase in haemoglobin content in comparison to $HgCl_2$ control group (Table 3).

Biochemical parameters

Test drug at both dose levels produced non-significant decrease in blood sugar, blood urea, cholesterol and not affected the creatinine level in comparison to $HgCl_2$ control group. Test drug at both dose levels did not affect the SGPT level. The TED dose level produced non-significant decrease while higher dose level did not affect the SGOT level in comparison to $HgCl_2$ control group. Test drug at both dose levels did not affect the total protein level while TED dose level produced non-significant decrease in alkaline phosphatase in comparison to $HgCl_2$ control group. Test drug at both dose levels produced significant increase in serum ferritin and marked but non-significant increase in serum iron content in comparison to $HgCl_2$ control group. Test drug at the TED*2 dose level produced non-significant decrease in triglyceride level in comparison to $HgCl_2$ control group. Test drug at the TED dose level produced non-significant increase while higher level did not affect the HDL-cholesterol level in comparison to $HgCl_2$ control group. Test drug at TED dose level produced significant decrease in serum TIBC while TED*2 dose level produced the same magnitude of effect but failed to reach a significant level in comparison to $HgCl_2$ control group (Table 4 & 5).

Table 1 — Effect of different samples of on body weight

Treatment	Dose	Initial body weight (g)	Final body weight (g)	Actual change (g)	% change
Control	--	181.67±9.80	205±5.00	23.33±9.19	12.84↑
$HgCl_2$	9.0 mg/kg	201.67±8.72	201.67±11.08	0.0±7.30	0.49↑
TED	1.8 mL/kg	203.33±9.88	200.00±13.03	0.0±18.71	0.49↑
TED*2	3.6 mL/kg	186.67±7.14	196.00±9.27	8.00±9.69	4.28↓

Data presented as Mean±SEM, ↑= Increase, ↓= Decrease

Table 2 — Effect of different samples on the relative weight of organs

Organs	Groups							
	Control(-)	Change %	$HgCl_2$ (9.0 mg/kg)	Change %	TED (1.8 mL/kg)	Change %	TED*2 (3.6 mL/kg)	Change %
Spleen (mg/100 g)	189.73±14.20	-	186.80±14.79	1.54↓	179.89±3.10	--	202.28±14.60	8.28↑
Thymus (mg/100 g)	145.77±3.71	--	150.42±6.28	3.19↑	179.29±13.82	19.19↑	159.59±4.79	6.09↑
Liver (g/100 g)	2.86±0.08	--	2.81±0.12	1.677↓	2.65±0.09	5.579↓	2.80±0.10	0.42↓
Kidney (mg/100 g)	778.15±17.11	--	856.96±27.45*	10.13↑	838.56±37.39	2.15↓	852.01±31.15	0.57↓
Heart (mg/100 g)	284.37±18.87	--	272.76±9.01	4.08↓	304.82±10.89*	11.75↑	259.29±5.34	4.93↓

Data presented as Mean±SEM, ↑ = Increase, ↓ = Decrease

Table 3 — Effect of *Opuntia elatior* fruit *Swarasa* on haematological parameters

Parameters	Groups							
	Control (-)	Change %	$HgCl_2$ (9.0 mg/kg)	Change %	TED (1.8 mL/kg)	Change %	TED*2 (3.6 mL/kg)	Change %
TWBC (10^3/mL)	8.70±0.635	--	7.60±0.718	12.64↓	6.70±0.363	11.84↓	7.56±1.242	0.52↓
Lymphocyte (%)	78.67±5.41	--	64.00±3.61[a]	18.64↓	65.60±3.85	2.5↑	69.60±5.44	8.75↑
Neutrophil (%)	17.50±5.37	--	32.17±3.74[a]	83.81↑	30.00±3.77	6.74↓	26.20±5.20	18.55↓
Eosinophil (%)	2.16±0.167	--	2.16±0.307	0	2.20±0.20	1.5↑	2.40±0.245	10.75↑
Monocyte (%)	1.66±0.211	--	1.66±0.333	0	2.20±0.200	31.97↑	1.80±0.200	7.98↑
TRBC (10^6/mL)	8.78±0.208	--	8.40±0.419	4.29 ↓	8.51±0.203	1.34↑	8.46±0.211	0.74↑
Hb (g/dL)	15.62± 0.29	--	13.67±0.25[a]	12.48↓	15.02±0.48*	9.87↑	14.62±0.31*	6.95↑
PC (%)	49.07±1.29	--	47.00±2.08	4.21↓	46.22±0.80	1.66 ↓	46.18±1.03	1.74↓
MCV (fl)	55.68±0.41	--	56.03±0.97	0.63↑	54.28±0.43	3.13↓	54.54±0.18	2.66↓
MCH (pg/red cell)	17.77±0.27	--	17.85±0.49	0.47↑	17.20±0.26	3.64↓	17.16±0.08	3.86↓
MCHC (g/dL)	31.90±0.42	--	31.55±0.30	1.09↓	31.64±0.24	0.28↑	31.44±0.11	0.35↓

Data presented as Mean±SEM, ↑ = Increase, ↓ = Decrease

[a]$p < 0.01$ when compared to control group *$p < 0.05$ when compared to $HgCl_2$ control group

Table 4 — Effect of *Opuntia elatior* fruit *Swarasa* on biochemical parameters

Parameters	Groups							
	Control (-)	Change %	$HgCl_2$ (9.0 mg/kg)	Change %	TED (1.8 mL/kg)	Change %	TED*2 (3.6 mL/kg)	Change %
Blood Sugar (mg/dL)	65.33±2.18	--	65.83±7.33	0.765↑	54.60±4.83	17.06↓	50.80±7.43	22.83↓
Serum Cholesterol (mg/dL)	62.83±3.68	--	62.83±4.65	0.0	59.00±1.38	6.10↓	50.00±3.22	20.42↓
Triglycerides (mg/dL)	44.33±6.37	--	45.17±4.12	1.88↑	47.75±7.61	2.58↑	39.00±2.66	13.65↓
HDL (mg/dL)	33.00±1.57	--	29.50±1.19	10.60↓	34.40±1.43	16.61↑	28.20±1.40	4.40↓
Blood urea (mg/dL)	65.17±5.90	--	77.17±4.53	18.41↑	57.80±12.10	25.09↓	66.40±7.82	77.17↓
Creatinin (mg/dL)	0.52±0.017	--	0.48±0.017	10.60↓	0.46±0.040	16.61↑	0.50±0.032	4.40↓
SGPT (IU/L)	72.25±6.79	--	83.67±6.93	15.80↓	84.60±6.61	1.11↑	80.80±4.46	3.42↓
SGOT (IU/L)	198.17±12.07	--	228.17±21.06	15.14↑	196.40±5.69	13.92↓	219.40±22.83	3.84↓
Protein (g/dL)	7.15±0.178	--	7.00±0.163	15.80↓	6.76±0.103	1.11↑	7.32±0.354	3.42↓
Albumin(g/dL)	157.25±31.36	--	213.83±30.41	35.98↑	127.40±14.62*	40.42↓	219.66±45.94	--

Data presented as Mean±SEM, ↑ = Increase, ↓ = Decrease

HDL: high-density lipoprotein; SGPT- Serum glutamic pyruvic transaminase; SGOT- Serum glutamic oxaloacetic transaminase

Effect on cytoarchitecture of different organs

O. elatior fruit *Swarasa* at both dose levels to some extent reverted the changes induced in heart, kidney and liver. It was observed that there were minimal decrease in cellularity of white pulp in the spleen & thymus in drug treated groups as compared to changes induced by $HgCl_2$ in disease control (Fig. 1-4).

Discussion

The mean body weight (g) of the albino rats in different treatment groups was recorded initially and after 30 days. Loss of body weight is a common clinical feature of anaemia. Test drug at both dose levels did not produce any significant effect on body weight parameters compared to $HgCl_2$ group. $HgCl_2$ group produced non-significant decrease in heart weight and significant increase in relative weight of kidney. The adverse changes manifested in histopathological findings in heart and kidney resembles the results found in a study by Chauhan *et al*[15]. The adverse changes were protected by test drug to a certain extent which was evident in heart, kidney and liver.

Mercuric chloride altered the function of RBC by hemolysis characterized by decreased levels of RBC and haemoglobin in comparison to control group[34]. Haemoglobin estimation is considered as the marker for evaluating the correction of anemia[15]. In a previous study, the haemoglobin level showed a significant decrease in $HgCl_2$ Control group[35]. The decrease may be the result of diminution in the number of circulating red cells, in the size of red cells, their concentration of haemoglobin or any combination of these[36]. In iron deficiency anaemia, the reduction of red cells size MCV and MCH is the reason but reduction in cell number eventually

Table 5 — Effect of *Opuntia elatior* fruit *Swarasa* on specific biochemical parameters

Parameters	Groups							
	Control (-)	Change %	$HgCl_2$ (9.0 mg/kg)	Change %	TED (1.8 mL/kg)	Change %	TED*2 (3.6 mL/kg)	Change %
S. Iron (µg/dL)	177.33±12.73	--	123.00±9.32[a]	30.63↓	139.40±5.20	13.33↓	155.20±20.296	26.18↑
T.I.B.C (mg/dL)	275.17±7.04	--	313.20±6.16	13.82↑	293.00±2.65*	6.45↓	291.20±14.73	7.02↓

Data presented as Mean±SEM, ↑ = Increase, ↓ = Decrease

[a]p <0.01, [b]p <0.001 when compared to control group

*p <0.05 when compared to $HgCl_2$ control group

S.Iron- Serum iron, T.I.B.C- Total Iron Binding Capacity

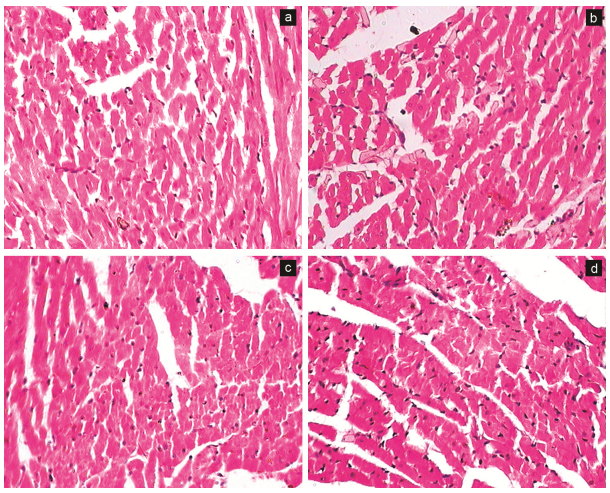

Fig. 1 — Photomicrographs of sections of the heart taken at X400 magnification. a) Normal cytoarchitecture (Control group), b) Fatty degenerative changes ($HgCl_2$), c) Mild fatty degenerative changes (TED), and d) Mild fatty degenerative changes (TED*2)

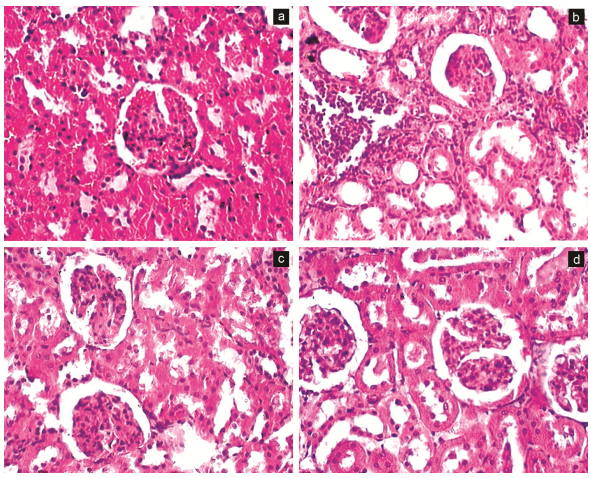

Fig. 2 — Photomicrographs of sections of kidney taken at X400 magnification. a) Normal cytoarchitecture (Control group), b) Fatty degenerative changes, intense cell infiltration and edematous changes ($HgCl_2$), c) Mild fatty and oedematous changes (TED), and d) Mild fatty and oedematous changes (TED*2)

contribute to the anaemia. However, in the $HgCl_2$ Control group decrease observed in MCV and MCH was not significant. Hence, it may be suggested that the decrease in haemoglobin content is due to the decrease observed in the production of erythrocytes in the $HgCl_2$ control group. The drug at both dose level significantly increases the haemoglobin content in comparison to $HgCl_2$ Control group. The mean total and differential WBC count give the information regarding defence system of the body. Fruit juice of *O. elatior* improved the differential WBC count except for neutrophil in $HgCl_2$ induced anaemia. The results were dose-dependent and protective against deleterious effect of $HgCl_2$ in rats. Ferritin is a major iron-storage protein found principally in the liver, spleen, and bone marrow. For plasma (or serum) ferritin concentration to be a valid measure of iron status in rats, plasma ferritin concentration must respond to iron deficiency as well as iron repletion. Prolonged iron deficiency (3 to 10 weeks) tends to decrease mean plasma or serum ferritin concentration. In the present study, $HgCl_2$ control group produced a significant decrease in serum ferritin, which may be due to significant loss/deficiency of iron content in the $HgCl_2$ control group. Test drug at both dose level produced a significant increase in serum ferritin level which may be due to increased/restored iron content in comparison to $HgCl_2$ control group.

Total iron-binding capacity (TIBC) is a medical laboratory test that measures the blood's capacity to bind iron with transferrin. Taken together with serum iron and percent transferrin saturation clinicians usually perform this test when they are concerned about anaemia, iron deficiency or iron deficiency anaemia. However, because the liver produces transferrin, alterations in function must be considered when performing this test. It can also be an indirect test of liver function. In iron deficient anaemia the TIBC level increase may be due to liver producing

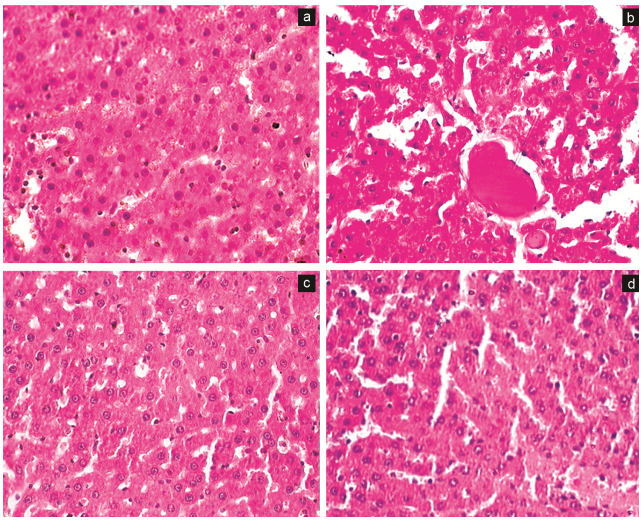

Fig. 3 — Photomicrographs of sections of liver taken at X400 magnification. a) Normal cytoarchitecture (Control group), b) Fatty degenerative changes and oedema ($HgCl_2$), c) Mild fatty changes (TED), and d) Mild fatty changes (TED*2)

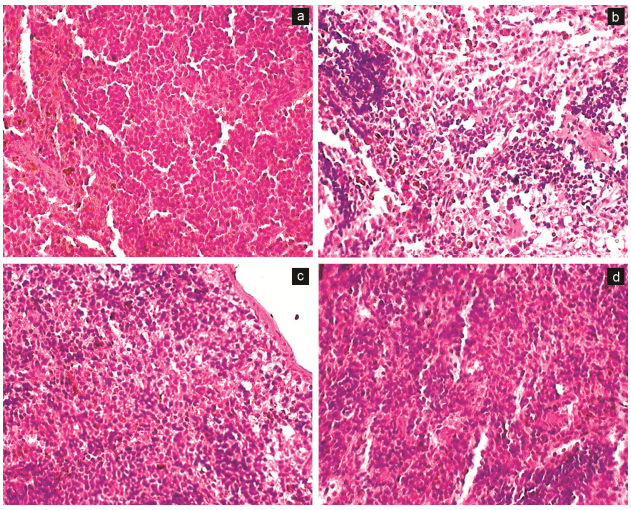

Fig. 4 — Photomicrographs of sections of spleen taken at X400 magnification. a) Normal cytoarchitecture (Control group), b) Severe lymphocytosis, decreased in white pulp and fibrosis ($HgCl_2$), c) mild decrease in cellularity and white pulp (TED), and d) Mild decrease in cellularity and white pulp (TED*2)

more transferrin, presumably attempting to maximize use of the little iron that is available. In the present study, $HgCl_2$ control group produced a non-significant increase in TIBC, which may be due to significant loss/deficiency of iron content in the $HgCl_2$ control group. Test drug at lower dose produced significant while higher dose level produced non-significant decreases in serum TIBC level, which may be due to increased/restored iron content in comparison to $HgCl_2$ control group.

The kidney and liver get badly damaged by $HgCl_2$ exposure[37]. Among human beings, inorganic Hg salt ingestion results in anuria and uremia from acute tubular necrosis[38]. Administration of $HgCl_2$ leads to fatty degenerative changes, intense cell infiltration and oedematous changes in kidney and fatty degenerative changes and oedema in liver and heart. The test drug at both dose level produced adverse changes of mild intensity hence revert the $HgCl_2$ induced changes in liver, kidney and heart.

The spleen is the storehouse of dead RBC and it is where the breakdown of haemoglobin occurs. Hemolytic anaemia leads to the accelerated breakdown of haemoglobin causing larger iron deposition in spleen[39]. This is likely to be the cause of fibrosis and lymphocytosis observed in the spleen in $HgCl_2$ treated groups. $HgCl_2$ treated also produced decreased in cellularity in thymus and lymph node. The disturbance in the cytoarchitecture of spleen, thymus and lymph node was significantly reversed by 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 in above organs (Fig. 1-4).

Conclusion

O. elatior fruit *Swarasa* at both dose levels significantly increase the haemoglobin content, serum ferritin level in comparison to $HgCl_2$ Control group and protected the damage caused by $HgCl_2$ in rats. Fruit juice was comparatively better because, in addition to attenuating the fibrosis, it restored cellularity to moderate level thereby inhibiting the toxicant-induced cell depletion in kidney, liver, heart and spleen. These results support the traditional use of fruits in the treatment of anaemia. Though human physiology differs from the lower animals like rats, it would be prudent to watch efficacy of test drugs in clinical settings especially when administered for a longer duration.

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