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ANTIOXIDANT AND HAEMATOPROTECTIVE ACTIVITY OF THE *SARACA INDICA* STEM BARK

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ABSTRACT: Present study was designed to evaluate the antioxidant and hematoprotective activity of methanolic extract of *saraca indica* bark. Antioxidants and hematoprotective effect of *saraca indica* bark may be due to its polyphenolic compounds. Polyphenols act as reducing agents via scavenging of free radicals and chelation of transition metals. The hematoprotective activity of *Saraca indica* was studied in Phenylhydrazine induced anemic rats. Anemia was induced by an oral administration of Phenylhydrazine for a period of 7 days. Hgb concentration, RBC count, PCV, MCH, MCHC were analyzed as indices of anemia. Phenylhydrazine significantly decrease the Hgb, RBC and increased MCV and WBC. The *Saraca indica was* administered at the dose levels of 200mg/kg, 400mg/kg orally to the animals for 14 days. After 14 days of treatment with *Saraca indica* at the dose level of 400 mg/kg significantly reverse the above parameters and turn towards the normal value. These results support the traditional use of *Saraca indica* in the treatment of anemia.

INTRODUCTION: In India a large number of herbal extracts are used in folk medicine to treat various types of disorders. The discovery of new and novel pharmaceutical products from plants used in traditional system of medicine for the treatment of the incidence of anemia. *Saraca indica* stem bark is renowned for its various medicinal properties, but no scientific information is available regarding its Antioxidant and hematoprotective activity. The earliest chronicled mention is in the Ayurvedic treatise, the Charka Samhita (100 A.D.), in which Asoka is recommended in formulations for the management of pain with relation to uterus (Gynecological) as Anodynes. All the plant parts are considered to contain medicinal properties.

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As a medicinal tree the utility of ashoka seems to have been a reconised first in the Agnivesa Caraka Samhita which is supposed to have been complied somewhere near 1000 B.C. Ayurvedic physicians of the present century are using ashoka in different female diseases specially for uterine affections. Saraca indica used in the Ayurvedic system of medicine as hypothermic and diuretic [1] and as a blood purifier, in stomach ache [2]. It is used also in bleeding piles, bacillary dysentery, Asokarista, asokaghrta, asoka decoction and asoka pills are the famous pharmaceutical preparations. The drug Asoka Aristha is traditionally used in India and Sri Lanka to treat menorrhagia [3].

The bark of Saraca indica contains an estrogenic compound called ergo sterol. Flavonoids, terpenoid, lignin, cardiac glycosides, phenolic compounds, tannins, were the major constituents present in the *Saraca asoca* stem bark extracts [4]. The isolation of flavonoids and leucoanthocyanidins [5] were previously reported

from the bark. These phytoconstituents may be responsible for various pharmacological actions of this plant part, like antiulcer [6] anticancer [7] and chemo protective activities [8]. Petroleum ether extract of *Saraca indica* leaves exhibits potential antitumor and antioxidant activities [9]. The above scientific studies suggested that *saraca indica* ethnobotanically used from ancient time.

MATERIALS AND METHODS:

Chemicals:

1,1-Diphenyl-2-picrylhydrazyl (DPPH), Methanol, Ascorbic acid and Phenylhydrazine were purchased from Sigma Aldrich (USA). All other chemicals used in the study were of Analytical R grade and available locally.

Plant materials:

The fresh plant sample of *Saraca indica* (Caesalpiniaceae) stem bark was obtained from a local commercial source in Lucknow (India). The plant was identified at Division of Botany, CSIR-Central Drug Research Institute Lucknow.

Preparation of plant extracts:

The stem barks was dried at room temperature and ground into powder. Dry powder (2kg) was macerated in 100% Methanol and then kept 48 h at room temperature. The resulting extract was filtered. The filtrate was concentrated in an oven at a temperature of 40◦C for 24 h to a constant weight of 167.1gm giving an 8.357% yield (w/w). The extract was stored at -20 ◦C.

In vitro antioxidant activity:

DPPH radical scavenging assay:

The antioxidant activity of the *Saraca indica* stem bark was assessed on the basis of the radical scavenging effect of the stable DPPH free radical[10]. *Saraca indica* stem bark or reference (10-100 µg/ml) was added to 200 µl of DPPH in methanol solution (100 M) in a 96-well microtitre plate (Tarsons Product (P) Ltd., India). After incubation at 37 ◦C for 30 min, the absorbance of each solution was determined at 490 nm using ELISA micro plate reader (Bio Rad Laboratories Inc., California, USA, Model 550). The corresponding blank readings were also taken and the remaining DPPH was calculated IC_{50} value is the concentration of the sample required to scavenge 50% DPPH free radical.

Superoxide anion (SOD) scavenging activity assay:
Superoxide dismutase activity was measured based on its ability to inhibit the autoxidation of epinephrine to adrenochrome at alkaline *p*H[11]. The absorbance of reaction mixture was followed for 4 min at 480 nm in a spectrophotometer (Model 1201, Shimadzu). Enzymatic activity was expressed as U/mg protein at 30°C. The amount of enzyme that caused 50 percent inhibition of epinephrine autoxidation was defined as one unit (U).

In vivo Hematoprotective activity:

Test animals:

Charles Foster rats (150-175 gm) of both sexes were obtained from National Laboratory Animal Center (NLAC), Central Drug Research Institute, Lucknow (India) and were allowed to acclimate to animal room conditions for 7 days prior to experimentation. They were kept in a controlled environment at 25±2^{o}C and 30–60% relative humidity with a 12 h light and dark cycle. The animals were fed a standard rodent pellet diet and water *ad libitum*. Animal studies were conducted according to the regulations of the Institute Animal Ethics Committee and the protocol was approved by the Committee for the Purpose of Control and Supervision of Experiments on Animals, New Delhi (India), IAEC No.- IAEC/2012/86.

Induction of Anemia:

Basal hematological profile determined for healthy Charles Foster rats using the fully automated MS9 hematology analyser.

Phenylhydrazine (PHZ) induced anemia is a model for the study of hematinic effects [12, 13]. In present study Phenylhydrazine was used to induce anemia (10mg/kg B. Wt. for seven consecutive days). After 7days the hematological profile was determined again and the rats with a ≥30% reduction in red blood cells count and hemoglobin concentration were considered anemic and used for this study.

Experimental procedure:

Rats were randomly divided into five groups, each containing ten (5M+5F) animals of both Sex. The

Rats of group-I(Control) were orally administered with 1%gum acacia. The group-II rats were orally administered with *saraca indica* methanolic extract (400mg/kg in 1% gum acacia) once daily for 7day. The Phenylhydrazine treated anemic rats were divided in three, four and fifth experimental groups. Group three severed as PHZ control group. Animals of group fourth and fifth were orally administrated with *saraca indica* methanolic extract (200mg/kg and 400mg/kg in 1%gum acacia). At the end of the experiment, animals were anesthetized with die-ethyl ether and sacrificed.

Food and water consumption:
Monitoring 24-hour food and water consumption of the animals in a group was done at the beginning and end of the study by giving a measured quantity of water and pellet diet followed by estimation of the amounts remaining at the end of 24 hours. Average food and water consumption per animal was calculated for each group.

Hematological analysis:
Blood samples were collected at 0, 7 and 14 days through tail vein in EDTA coated vials. Hematological parameters were analyses using MS-9 fully automated hematology analyzer. The following parameters were determined of blood samples. Erythrocyte (RBC), total and differential leukocyte(WBC), hematocrit (Hct), hemoglobin (Hb), platelet count, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), mean platelet volume (MPV), platelet distribution width (PDW), and red distribution width (RDW).

Absolute and relative organ weight:
At the end of the study (14days), all organs were collected using standardized surgical procedures. The abdominal cavity of each animal was dissected and organs namely the heart, liver, lungs, spleen, kidney and brain were quickly removed, cleaned with normal saline, weighed and preserved in 10% formalin.

Statistical analysis:
All *in vitro* antioxidant assay were done in triplicate manner and results are expressed as mean ± S.D. Data were analyzed with one-way ANOVA. Statistically significant effects were further analyzed. The statistical significance was determined at $p < 0.05$.

RESULTS:

***In vitro* assays:**
DPPH free radical scavenging activity
Antioxidants can reduce the oxidative stress and as a result ameliorate the progress of stress related diseases. The Saraca indica stem bark exhibited strong antioxidant activity in the DPPH inhibition assay as evidenced by the low IC_{50} values (**Table 1**). The IC_{50} values obtained are 229.437±149.324µg/ml, respectively in the DPPH inhibition assays.

TABLE 1: *IN VITRO* ANTIOXIDANT ACTIVITY OF *SARACA INDICA* STEM BARK ON DPPH ANTIOXIDANT (% INHIBITION)*

Concentration (µg/ml)	scavenging or %inhibitory activity of 1,1-diphenyl 1-2-picryl-hydrazyl (DPPH)
100	19.39±2.50
150	21.06±3.91
200	49.51±2.64
250	56.48±4.58
300	68.32±6.05
350	74.96±8.73
400	97.34±11.24
IC_{50}	229.437±149.324
Reference (Ascorbic acid)IC_{50}	42.06 ± 5.27

*Values are expressed as percentage mean of 3 replicates

Superoxide anion (SOD) scavenging activity assay:
In vivo antioxidant studies of *Saraca indica* stem bark were carried out to find out its antioxidant properties. Superoxide dismutase (SOD) was assayed according to (Misra *et al.*) based on the inhibition of epinephrine auto-oxidation by the enzyme[11]. In the control group the SOD activities were 0.089 ± 0.47(U/mg protein) after the treatment with *Saraca indica* stem bark it increases the SOD activity 0.285 ± 0.27 (U/mg protein) similar to reference antioxidant ascorbic acid treated group's SOD 0.294 ± 0.87 (P <0.01, when compared with control). The antioxidant activity of the *Saraca indica* stem bark has been suggested to play a role in the relief of long-term complications and the oxidative stress.

Induction of anemia:

After administering Phenylhydrazine to experimental animals there very significant reduction (p>0.001) in RBC (percentage reduction- 40%), Hgb (Percentage reduction- 33%) and MCV(percentage increase- 100%). There were no significant differences in Hct, MCH, MCHC, RDW and Pct values between the normal and anemic animals(**Fig. 1&2**).

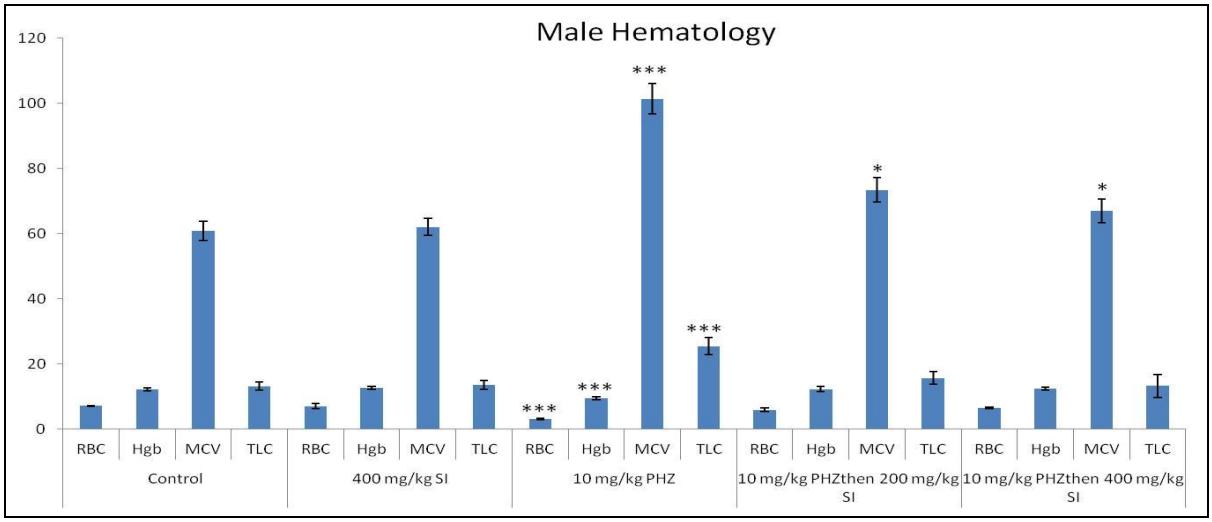

FIG.1: EFFECT OF PHENYLHYDRAZINE 10mg/kg b. Wt. ON DIFFERENT HEMATOLOGICAL PARAMETERS IN CF MALE RATS AS COMPARE TO CONTROL AND OTHER GROUPS.

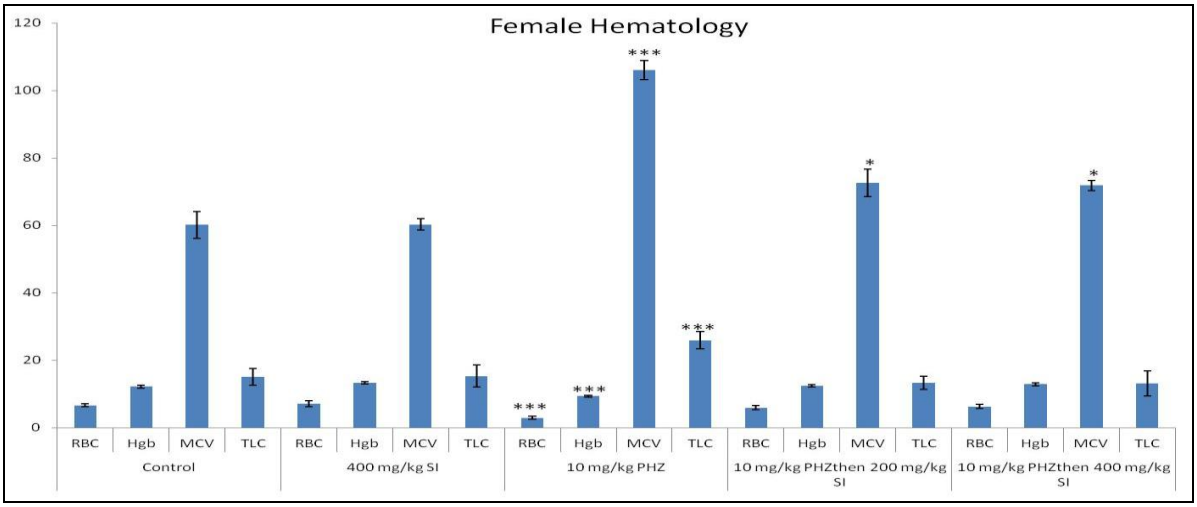

FIG.2: EFFECT OF PHENYLHYDRAZINE 10mg/kg b. Wt. ON DIFFERENT HEMATOLOGICAL IN CF FEMALE RATS AS COMPARE TO CONTROL AND OTHER GROUPS

***In vivo* Hematoprotective activity:**

PHZ showing the Significant decrease in RBC and Hgb after 7 day treatment by altered the function of RBC through hemolysis both male and female (**Table 2&3**). In the Group-I (control) no difference were seen in the at hematological parameters (RBC, Hgb, MCV, MCHC, Hct WBC, Platelet Count.). In the group II (400mg/kg SI) animals all the hematological parameter are significantly not different from the control group animals (**Fig. 3&4**). In the Group-III (PHZ control group) oral administration of Phenylhydrazine decreased hemoglobin rate from 12.52 g/dl at day 0 to 8.52 g/dl at day 7. This variation is statistically significant and corresponds to 31.95% of reduction (**Table 2**) in male rats and 32.08% in Female Rats. The number of RBC were also decreased 40% both male and female in this group. This reduction is reversed naturally and progressively from 8.52 g/dl to 9.52 g/dl, respectively at days 7 to 14 after Phenylhydrazine administration, these values correspond to 11.74% recovery that is not significant. The anemic rats of Group-IV were treated with the *Saraca indica* Me-OH extract at

200mg/kg, the hemoglobin is 9.26 g/dl at day 7 that increased up to 12.36 at day 14th (value that corresponds to 33.48% of recovery) in male rats and same effect were seen in female rats. The rats of group-V treated with *saraca indica* at dose 400 mg/kg increase the concentration of the hemoglobin 12.28, 9.22,12.52g/dl respectively at 0, 7, 14 days ((**Fig. 3**) these value that corresponds to 35.79% of recovery. These results indicate that the Saraca indica bark extract at dose rate 200 mg/kg and 400 mg/kg increasing the concentration of hemoglobin significantly in the rats treated with Phenylhydrazine. Same effect were seen in female rats. The extract induces the same effect on the number of the red blood cells and MCV.

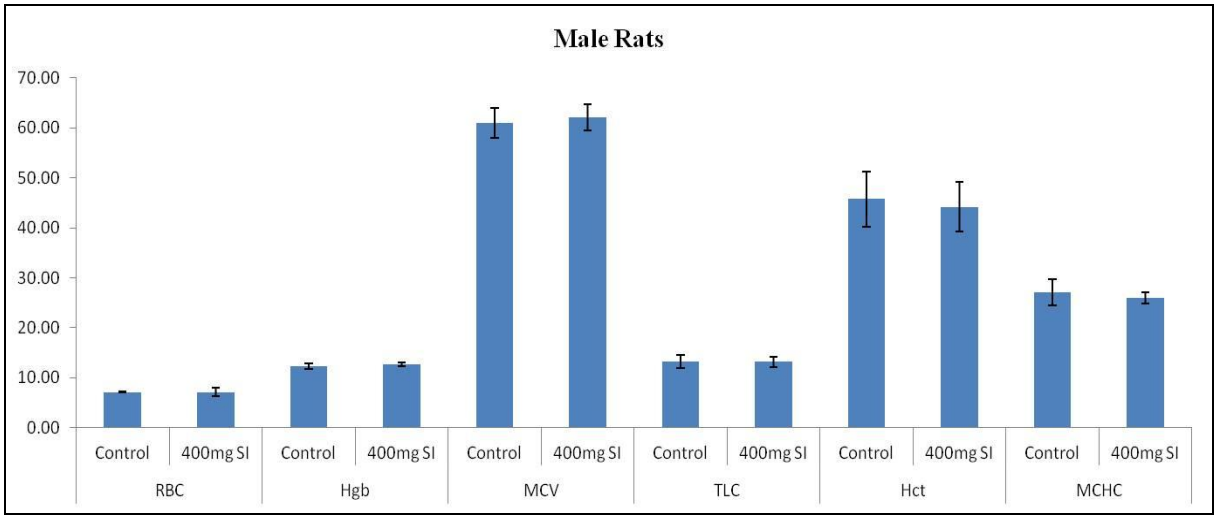

FIG.3: EFFECT OF *SARACA INDICA* 400mg/kg b. Wt. ON DIFFERENT HEMATOLOGICAL IN CF MALE RATS AS COMPARE TO CONTROL.

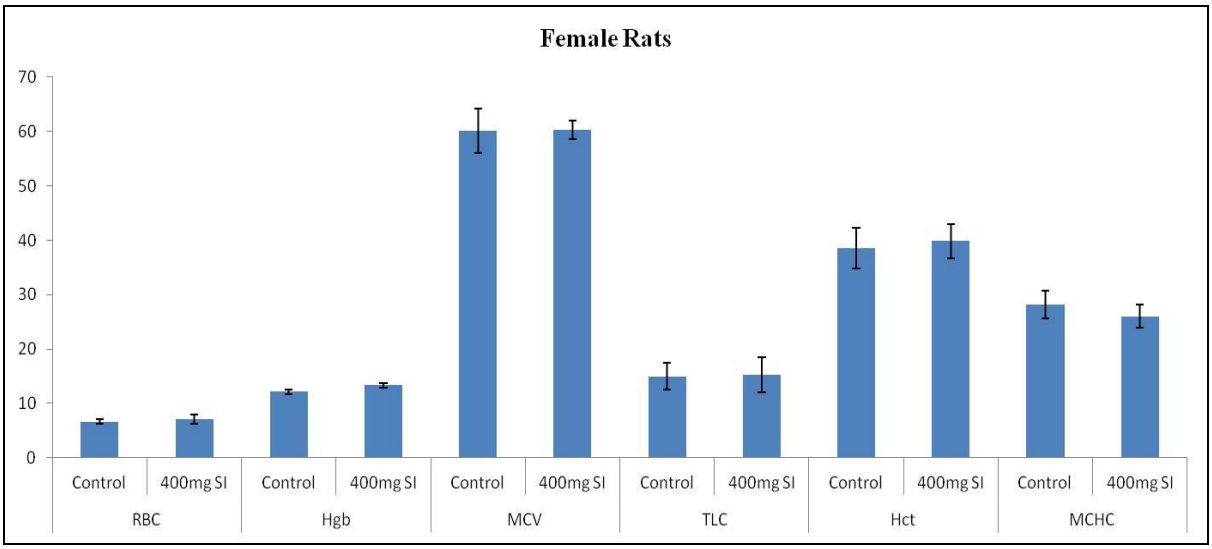

FIG.4: EFFECT OF *SARACA INDICA* 400mg/Kg B. Wt. ON DIFFERENT HEMATOLOGICAL IN CF FEMALE RATS AS COMPARE TO CONTROL.

TABLE 2: HEMATOLOGICAL PARAMETERS IN MALE RATS OF ALL GROUPS.

Parameter		Group I (Control)	Group I I (200mg/kg SI)	Group III (10mg/kg PHZ)	Group IV (PHZ+SI 200)	Group V (PHZ+400SI)
Hgb (g%)	Day 0	12.42±0.22	12.42±0.36	12.52± 0.70	12.66±1.13	12.28±0.19
	Day 7	12.27±0.12	12.58±0.15	8.52± 0.49**	9.26±0.96**	9.22±0.43**
	Day 14	12.30±0.50	12.68±0.41	9.52± 0.48	12.36±0.78	12.52±0.41
T-RBC ($x10^6/mm^3$	Day 0	7.04±0.43	6.87±0.60	7.19± 0.52	7.21±0.76	7.42±0.35
	Day 7	7.27±0.59	6.94±0.68	3.12± 0.44***	3.23±0.37***	3.20±0.61***
	Day 14	7.16±0.16	7.13±0.89	3.18± 0.28***	5.99±0.50	6.55±0.16
Hct (%)	Day 0	41.34±1.73	42.92±2.63	42.46± 5.57	41.64±4.97	43.14±1.16

	Day 7	45.64±3.27	43.82±3.15	35.28± 2.17	41.64±4.97	43.14±1.16
	Day 14	45.76±5.57	44.22±4.90	39.42± 2.04	45.50±4.33	45.50±4.71
	Day 0	60.64±3.53	62.66±2.43	58.90± 3.83	57.74±2.12	58.14±1.67
MCV(micron^3)	Day 7	61.20±1.76	63.24±2.71	104.80± 3.81***	103.82±5.90***	108.94±3.77***
	Day 14	60.92±2.98	62.14±2.66	101.44± 4.65***	73.50±3.78*	67.00±3.71*
	Day 0	28.58±1.81	29.04±1.91	29.82± 2.42	30.56±2.86	28.54±0.66
MCHC(g%)	Day 7	25.28±1.40	26.60±2.05	24.33± 2.29	24.88±2.99	24.06±4.02
	Day 14	27.16±2.63	26.00±1.10	32.28± 2.35	25.86±0.63	27.72±2.94
	Day 0	433.20±22.26	426.20±28.53	406.40±22.59	421.20±23.19	430.80±18.09
Platelets(x10^3/mm^3)	Day 7	485.80±31.70	474.20±34.92	399.40±40.97	442.40±27.79	465.80±21.16
	Day 14	437.00±28.44	401.00±10.04	353.80±35.62	393.00±30.04	389.20±19.28
	Day 0	15.31±1.94	12.89±3.13	12.65± 2.63	14.28±3.63	12.02±1.51
TLC(x10^3/mm^3)	Day 7	13.24±2.20	13.06±2.32	21.15±2.63***	22.53±2.31***	22.73±2.10***
	Day 14	13.25±1.30	13.59±3.38	25.51±2.63**	15.77±1.88	13.31±3.58

0.05= *, 0.01= **, 0.001=*** Data represent mean±S.D. (n = 5).
Effect of *Saraca Indica* Bark extract on PHZ induced anemic model. All Data represent mean±S.D. (n = 5).
Data are significantly different from the control group, $p < 0.05$.

TABLE 3: HEMATOLOGICAL PARAMETERS IN FEMALE RATS OF ALL GROUPS.

Parameter		Group I (Control)	Group I I (200mg/kg SI)	Group III (10mg/kg PHZ)	Group IV (PHZ+SI 200)	Group V (PHZ+400SI)
	Day 0	11.98±0.18	12.36±0.51	12.84± 0.95	12.62±0.40	13.00±0.64
Hgb (g%)	Day 7	12.20±0.32	12.36±0.18	8.72± 0.69***	8.68±0.63***	8.42±0.97***
	Day 14	12.16±0.42	13.30±0.39*	9.38± 0.27***	12.34±0.36	12.86±0.46
	Day 0	6.67±0.44	6.97±0.18	6.57± 0.43	6.39±0.62	6.62±0.9 6.62±0.94
T-RBC (x10^6/mm^3)	Day 7	6.66±0.52	7.02±0.54	3.48± 0.37***	3.73±0.55***	3.23±0.68***
	Day 14	6.62±0.44	7.10±0.83	2.93± 0.38***	5.94±0.61	6.26±0.59
	Day 0	40.50±1.62	42.04±1.44	34.26± 3.67	34.80±3.16	38.46±1.16
Hct (%)	Day 7	37.96±7.52	41.80±3.18	26.20± 3.17	36.95±1.06	39.08±4.56
	Day 14	38.58±3.73	39.82±3.20	31.02± 3.94	33.18±6.13	32.06±4.76
	Day 0	60.76±2.08	58.60±1.11	52.08± 3.32	54.54±3.71	57.84±4.36
MCV(micron^3)	Day 7	62.60±2.11	60.38±1.70	105.86± 3.78***	101.82±7.67***	103.11±10.21***
	Day 14	60.14±4.04	60.26±1.69	105.98± 2.84***	72.60±4.00*	71.82±1.55*
	Day 0	29.70±1.51	32.12±4.12	29.82± 2.42	33.52±2.44	28.54±3.18
MCHC(g%)	Day 7	26.48±1.41	29.46±2.03	33.46± 2.67	24.50±1.88	26.29±7.23
	Day 14	28.16±2.57	26.02±2.10	32.04± 2.04	29.50±2.82	28.78±2.60
	Day 0	402.20±28.35	411.80±44.74	451.40± 64.52	417.60±61.05	527.60±78.98
Platelets(x10^3/mm^3)	Day 7	486.40±45.18	455.20±56.76	465.00± 79.38	535.40±50.40	533.00±37.40
	Day 14	425.20±17.42	421.80±20.30	411.40± 28.71	410.60±23.24	441.40±28.91
	Day 0	12.82±1.37	14.29±4.49	11.39± 6.95	13.07±2.19	12.77±3.88
TLC(x10^3/mm^3)	Day 7	14.10±3.84	14.23±3.49	25.30± 2.23***	23.63±2.24***	25.40±6.22***
	Day 14	14.98±2.48	15.28±3.26	25.94± 2.53***	13.28±2.01	13.17±3.72

0.05= *, 0.01= **, 0.001=*** Data represent mean±S.D. (n = 5).
Effect of *Saraca Indica* Bark extract on PHZ induced anemic model. All Data represent mean±S.D. (n = 5).
Data are significantly different from the control group, $p < 0.05$.

Absolute and relative organ weight:

No significant difference was seen in the absolute and relative organ weight of control groups.

The rats treat with PHZ at 10mg/kg showing splenomegaly[14], there is significant difference were seen in the weight of spleen as compare to control(**Fig. 5&6**).

FIG. 5: EFFECT OF PHZ AND SI ON SPLEEN SIZE AN WEIGHT

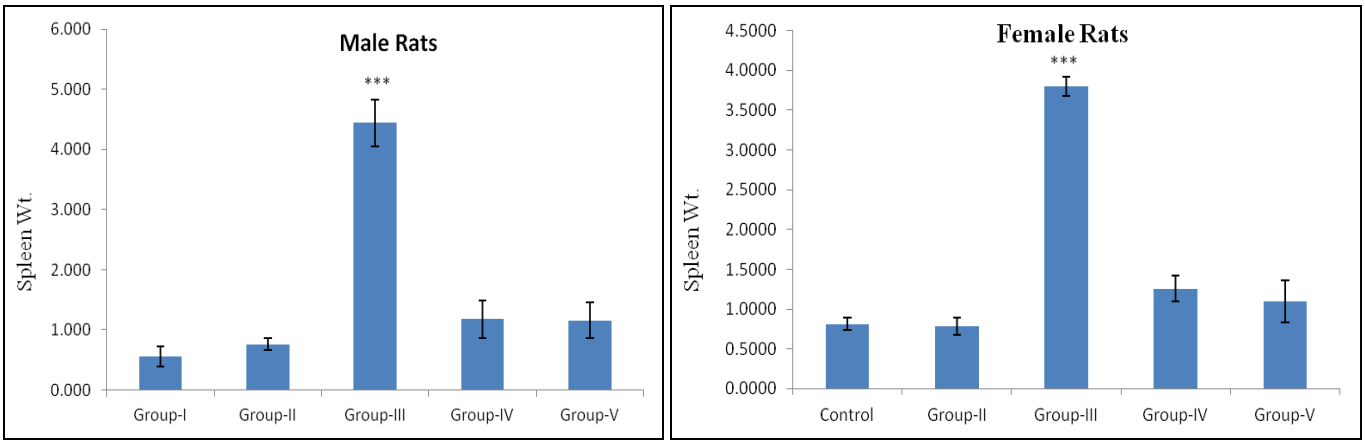

FIG.6: EFFECT OF PHENYLHYDRAZINE 10mg/kg b. Wt. ON SPLEEN IN CF RATS AS COMPARE TO CONTROL AND OTHER GROUPS (MALE AND FEMALE)

DISCUSSION: In present study *saraca indica* plant extract administered at dose 400mg/kg alone, there were no significant differences were seen in Hgb, RBC, Hct, MCH, MCHC, RDW and Pct as compare to control group. These data suggested that *Saraca indica* at dose 400mg/kg b.wt. not show any toxicity on blood profile(**Fig. 5 and 6).**

Saraca indica showing strong antioxidant activity. The radical scavenging activity of *Saraca indica* seems to be correlated with its polyphenolic constituents though active components could play important roles in its antioxidative effect. Polyphenols act as reducing agents and antioxidants via several mechanisms including the scavenging of free radicals, chelation of transition metals, as well as the mediation and inhibition of enzymes[15]. Consequently, it is possible that the total phenolic constituents may contribute to antioxidant activity of *Saraca indica*.

Two different doses of *Saraca indica (*200 mg and 400 mg) were evaluated in phenylhydrazine induced anemic rats. Phenylhydrazine is used for the induction of haemolytic anemia and the study of its mechanism in rats[16]. Phenylhydrazine generates ROS within both human and rat erythrocytes; ROS production was associated with extensive binding of oxidized and denatured hemoglobin to the membrane cytoskeleton. Thus, phenylhydrazine-induced haemolytic injury seems to be derived from oxidative alterations to red blood cell proteins rather than to membrane lipids[17]. Phenylhydrazine-induced anemia is a model for the study of hematinic effects[12]. In this present study, the hematological parameters of Phenylhydrazine (10mg/kg) administration alone after seven days on experimental rats are summarized in **Table 2&3**. Hemoglobin levels, RBC count were significantly reduced in all phenylhydrazine treated anemic rat groups (Group 3 to 5) as compared to the normal control group. MCV level was increased in all anemic rat groups, whereas no significant change in MCH, MCHC level. This result indicated the development of hemolytic anemia after administration of phenylhydrazine for seven days. The result of one week treatment of *Saraca indica* at two different dose level (200 mg and 400 mg) were represented in **Table 2&3**. From the above result, it is indicated that phenylhydrazine induced oxidative alterations were reversed by the administration of *Saraca indica* for one week.

Among the two different doses studied, *Saraca indica* 400mg produced marked effect than the lower dose 200mg. The *Saraca indica* possesses an excellent effect on haemopoietic system on phenylhydrazine induced anemic rats. During the treatment period, the *Saraca indica* significantly increased ($p<0.01$) the hemoglobin concentration and red blood cell counting in the treatment of anemia, the beneficial effects of analyzed *saraca indica* especially from the presence of chemical constituents.

CONCLUSION: In conclusion, our results demonstrated that *saraca indica* inhibits the formation of free radicals in rats showing potential antioxidant effect. It was also exhibiting significant protection on blood parameters. *Saraca indica* might be potent therapeutic agent in treating

hematinic related disorders since they possess both antioxidant and Hematoprotective potentials.

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