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PROTECTIVE EFFECTS OF ETHANOLIC LEAF EXTRACTS OF *CORCHORUS FASCICULARIS* IN PHENYLHYDRAZINE INDUCED ANEMIC RATS

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ABSTRACT: The aim of the present study is to evaluate the anti anemic activity of ethanolic leaf extract of *Corchorus fascicularis* against phenylhydrazine induced hemolytic anemia in rats. Phenylhydrazine (40mg/kg) was administered intraperitoneally for 2 days to induce anemia in rats. The animals were divided in to five groups of 6 animals each. Group I served as normal control, group II as anemic control, group III as reference control administered with Vitamin B_{12} and group IV and V animals were treated with 100 mg/kg and 200mg/kg of ethanolic leaf extract of *Corchorus fascicularis* Linn. (CFE). All the test drugs were administered once daily for 28 days through oral route. On 1st, 14th and 28th day blood was withdrawn, through sinus puncture under phenobarbitone anesthesia and subjected to the estimation of RBC, Hb and percentage Hematocrit. Both the ethanolic leaf extracts of *Corchorus fascicularis* Linn. and Vitamin B12 significantly increased the RBC, Hb and Hematocrit levels which conclude that, *Corchorus fascicularis* Linn. leaf extract exhibits anti-anemic activity.

INTRODUCTION: Anaemia is a common blood disorder that affects people of all ages, although the people at greater risk are the elderly, young women of child-bearing age and the infants. This condition is not a disease but could develop as a result of various diseases. There are over 400 types of anaemia, many of which are rare but in all cases there is lower than normal number of circulating red blood cells. Presently, more than half of the world's population will experience some forms of anaemia in their life time [1]. The great loss in terms of clinical diagnosis and treatment and even depletion in human resources as a result of anaemia could be prevented with adequate knowledge.

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The incidence of anaemia is higher in the third world than in developed countries due to the presence of many aggravating factors such as poor nutrition, high prevalence of blood parasites example, plasmodium, trypanosomes and helminthes infestation. It is also known that women are susceptible to anaemia during pregnancy due to high demand from the developing fetus [2, 3].

Although there are various drugs for the treatment of anaemia, they are not affordable to many poor people especially those in the developing countries such as India. In addition, the rural populations in various parts of the world do not have adequate access to high quality drugs for the treatment of anaemia, so they depend heavily on plants and herbal products for the treatment of diseases and anaemia. As a result of the fact that anaemia is very common and the incidence is likely to increase in future [1]. There is need to prevent it or seek for more cost-effective and better treatment strategies.

Anaemia is one of the numerous ailments claimed to have been successfully treated with plant materials by traditional medicine practitioners. In China for instance, blood diseases such as malformation of blood circulatory system, anaemia, varicose veins and hemorrhages have been treated with plant materials [4]. The crude extract of *Fagara zanthoxylum* was reported to be effective in the treatment of sickle cell anaemia [5]. It was also reported that the aqueous crude extract of *Telfairia occidentalis* leaves has haematinic activity [6]. It is well established that man consumes a wide variety of local crops and vegetables, which are believed to contribute significantly to the improvement of human health in terms of disease prevention and therapy [5, 6].

Among the different species of *Corchorus* reported in India, *Corchorus fascicularis* (Tiliaceae) is considered the most important for its medicinal properties. The plant extract have been reported to possess anti-inflammatory antipyretic, antiviral, anthelmintic, anticarcinogenic, hepatoprotectative activities. Early studies documented the presence of flavonoids, xanthones, terpenoids, iridoid and secoiridoid glycosides in the *Corchorus fascicularis* plant [7 - 12]. Traditionally, the plant was used as Antianemic medicines; therefore, it developed interest for its evaluation for their anti anaemic property, whether it is having or not.

MATERIALS AND METHODS:

Plant Collection: The leaves of plant *Corchorus fascicularis* were collected from the campus of Satara College of Pharmacy Degaon, Satara. Plant was authenticated in Yashawantrao Chavan Institute of Science, Satara, Department of Botany, Maharashtra, India. The dried uniform leaves powder was used for the extraction of constituents of the plant.

Preparation of Extract: Freshly collected leaves of the plant *Corchorus fascicularis* were washed, shade dried under room temperature for a period of three weeks. The dried plant material was made to a coarse powder and weighed quantity of the powder (500gm) was subjected to hot extraction in a Soxhlet apparatus, using alcohol at a temperature range of 60 - 70 °C. After extraction, the extract was completely dried and weighed. The extract was concentrated by evaporation of solvent at room temperature [13].

Experimental Animals: Wistar rats (30) of both sexes, weighing between 150 - 250 gm were used for this study. The animals were housed in plastic cages and acclimatized for two weeks in the animal house. They had been maintained under standard conditions (room temperature 25 ± 3 °C, humidity 35 - 60%, light and dark period 12/12 hours). All animals had regular supply of clean drinking water and food. All the procedures and protocols were reviewed and approved by the Institutional Animal Ethics Committee of Satara College of Pharmacy, Satara.

Preliminary Phytochemical Test: The defatting is done by petroleum ether. Alcoholic extract obtained by the above methods from *Corchorus fascicularis* were subjected to qualitative test for the identification of various plant constituents by the standard procedures [14].

Induction of Anaemia: Anaemia was induced in rats by intra peritoneal administration of 40 mg/kg/day of phenyl hydrazine (PHZ) for two days (D_0 and D_1). The treated rats with phenyl hydrazine whose, haemoglobin concentration was < 13 g / dl were considered as anaemic and included for the study. Phenyl hydrazine induces haemolysis of RBCs by inducing formation of toxin, free radicals that can attack cellular macromolecules like haemoglobin, resulting in oxidative damage within RBCs, resulting in their destruction [15, 16].

Treatment of the Animals: Five groups of six rats were formed and treated daily for 4 weeks as follows:

- **Group I (G_1):** Normal control received 10 ml / kg of 0.5% CMC (Carboxy Methyl Cellulose) from day D_2 to D_{28}.
- **Group II (G_2):** Anaemic control received 0.5% Phenyl hydrazine from day D_0 to D_1.
- **Group III (G_3):** Treated with Vitamin B_{12} Syrup (1 ml/day) from day D_2 to D_{28}.
- **Group IV (G_4):** Treated with ethanolic extract of *C. fascicularis* (CFE) (100 mg/kg) from day D_2 to D_{28}.

- **Group V (G_5):** Treated with ethanolic extract of *C. fascicularis* (CFE) (200mg/kg) from day D_2 to D_{28}.

All administration was done orally using oropharyngeal cannula once per day for 28 days (4 weeks).

Analysis of Haematological Parameters: Blood samples were collected from the rats by tail incision before induction of anaemia (D_0), after induction of anaemia with PHZ (D_2) and at 2nd and 4th weeks of treatment [17, 18]. The Red Blood Cell number (RBC), Haemoglobin concentration (Hb) and Haematocrit were determined at days D_2, D_{14}, D_{28}, using an Automatic Blood Cell Counter and the variations of average values of haematological parameters were calculated, relative to the mean values of D_0 and D_2.

Statistical Analysis: Graph Pad Prism 5.0 software (Microsoft, USA) was used for the analysis of the results obtained. The mean value is accompanied by the Standard Error of Mean (mean ± SEM). It was taken to the ANOVA test to verify the normality of variables. The comparisons of mean values of the different parameters were performed. The significance level was set at $p < 0.05$.

RESULTS: Ethanol, extract *C. fascicularis* of was prepared by continuous hot extraction method using soxhlet apparatus and the yield was 2.1% w/w. The colour of the ethanol extract was dark green, Phytochemical screening test were performed on CFE. CFE phytoconstituents present saponins, tannins, phenols, coumarins, alkaloids and anthraquinones plant constituents present.

Effect of Alcoholic Extracts of *C. fascicularis* Leaves on Body Weight: Table 1 shows the different body weights of the rats before induction of anaemia (D_0), after induction of anaemia with PHZ (D_2) and at 4th weeks of treatment. There was weight loss in rats of the five treated groups with phenyl hydrazine at day D_2 (8.75 %). A gradual increase in weight was observed after treatment in the following days.

TABLE 1: THE EFFECT OF *CORCHORUS FASCICULARIS* EXTRACT (CFE) ON BODY WEIGHT

	Body Weight (gm)		
Drug Treatment	**Day2**	**Day14**	**Day28**
Normal Control	238.33±0.99	240.66±0.81	242.33±1.25
Anaemic Control	235±0.25	227±0.18	218±0.76
STD (Vit.B_{12} Syrup)	230±0.98	232±0.45	235±0.23
CFE (100 mg/kg)	231±0.95	220±0.53	225±0.46
CFE (200 mg/kg)	231±1.40	234±0.41	241±0.58

Effect of Alcoholic Extracts of *C. fascicularis* Leaves on Haematological Parameters:

Red Blood Cells: After injection of phenyl hydrazine to rats of the four groups, except, the normal group, there was a decrease in red blood cells (48.03 %) at day D_2.

TABLE 2: THE EFFECT OF *CORCHORUS FASCICULARIS* EXTRACT (CFE) ON HEMOGLOBIN, RBC AND HTC IN PHENYLHYDRAZINE INDUCED ANEMIC RATS

Drug treatment	Haemoglobin (gm/dl)			RBCs (million cells/cu mm)			HCT (%)		
	DAY_2	DAY_{14}	DAY_{28}	DAY_2	DAY_{14}	DAY_{28}	DAY_2	DAY_{14}	DAY_{28}
Group 1 (Normal)	17.18 ± 1.22	18.25 ± 1.29	18.38 ± 1.40	4.58 ± 0.64	4.62 ± 0.69	4.72 ± 0.81	42.55 ± 0.97	43.29 ± 0.79	43.68 ± 0.98
Group 2 (Negative Control)	10.9 ± 0.11 a** 40.68[c]	11.7 ±1.24 35.89[c]	12.2 ± 1.42 33.62[c]	2.367 ± 0.12a** 48.47[c]	2.8 ± 0.54 39.39[c]	2.9 ± 0.59 38.55[c]	23.80 ± 0.86 a** 44.06[c]	28.10 ± 0.92 35.53[c]	29.20 ± 0.99 33.15[c]
Group 3 (STD.)	10.2 ± 0.13 a** 40.62[c]	14.9 ± 0.18** 18.35[c]	17.31 ± 0.24*** 5.82[c]	2.56 ± 0.48a** 44.10[c]	3.7 ± 0.59** 19.19[c]	4.5 ± 0.70*** 04.60[c]	22.45 ± 0.80a** 46.41[c]	38.20 ± 0.89** 10.95[c]	41.86 ± 0.97*** 04.16[c]
Group 4 (CFE 100 (mg/kg)	11.6 ± 0.16 a** 32.48[c]	13.6 ± 0.20* 25.43[c]	14.57 ± 0.25* 20.72[c]	2.2 ± 0.42 a** 51.96[c]	3.1± 0.48* 32.90[c]	3.9 ± 0.50** 17.37[c]	23.30 ± 0.77 a** 45.55[c]	32.40 ±0.87* 25.29[c]	36.86 ± 0.95 ** 16.30[c]
Group 5 (CFE 200 (mg/kg)	10.8 ± 0.19 a** 37.13[c]	13.9 ± 0.29* 24.25[c]	16.18 ± 0.31*** 11.4[c]	2.4 ± 0.42 a** 47.59[c]	3.4 ± 0.52* 26.40[c]	4.39 ± 0.64*** 06.99[c]	23.29 ± 0.79 a** 45.26[c]	35.58 ± 0.88* 17.81[c]	39.25 ± 0.99*** 10.12[c]

Values are in mean ± SEM. n = 6. Superscript a*, a**, a*** denote statically significant at $p < 0.05$ and $p < 0.01$ $p < 0.001$ when compared to normal group. Superscript * and ** and *** donate statically significant at $p < 0.05$ and $p < 0.01$ $p < 0.001$ when comparison to Negative Control group. Superscript c denoted percentage decrease compare with Normal Control group.

An increased number of red blood cells were observed after treatment in the 24 days. The result shows that, the activity of rats of the groups G_3, G_4 and G_5 have almost completely inhibited at the 2nd week (82.57%, 74.70% and 75.45% inhibition, respectively.) By the 4th week, (94.74% in rats of group G_3, 80.95% in rats of group G_4 and 89.61% in rats of group G_5. inhibition respectively) **Table 2**.

Haemoglobin: The administration of phenyl hydrazine at day D_2 caused a significant decrease ($p < 0.01$) in haemoglobin rate of rats of groups G_2, G_3, G_4, G_5 of 37.03%. After treatment, a progressive inhibition is obtained on the following days (**Table 2**). The results shows on one hand that, the rats those, received Vitamin B_{12} and those, which received alcoholic extract of *C. fascicularis* have almost completely recovered at the 4th week ($p < 0.05$).

Haematocrit: The administration of phenyl hydrazine also decreased haematocrit at day D_2. This decrease is 45.23% at D_2. After treatment, the increased rate of haematocrit at day D_{14} (2nd week) was 89.75% in untreated anaemic rats, 89.87%, 74.87%, 83.39 respectively in the rats of groups G_3, G_4, G_5. By the 4th week (D_{28}), 80.76%, in rats of group G_3, 80.28% in rats of group G_4, and 78.98% in rats of group G_5 **(Table 2)**.

DISCUSSION: PHZ is a non-immunogenic drug that induces changes in the red cell membrane, which result in oxidative denaturation of Hb. The effect of the denaturation is the formation of an altered Hb called "Heinz bodies" which reduces the life span of the erythrocytes [19]. This is often characterized by a significant increase in the incidence of micro- nucleated polychromated and hypochromic erythrocytes resulting in increased mean cell volume and decreased mean cell Hb concentration values [20, 21]. Altered erythrocytes are removed by the spleen and liver of the reticuloendothelial system resulting in compensated hemolytic anemia. PHZ-induced anemia is a model for the study of hematinic effects.

In this study, significant decrease in Hb, RBC count and hematocrit was observed following PHZ injection to the experimental animals ($p < 0.05$). The results of this study indicated that the whole alcoholic extract of *Corchorus fascicularis* increased significantly the concentration of haemoglobin, red blood cell count, and the packed cell volume mainly one week after the treatment. The increase in the blood indices was progressive giving the highest effect on the second week of treatment. Under normal condition, the body can generate new RBCs to replace the lost red cells; this will take much longer time as shown in this study. The increases in the haematological indices exhibited by *Corchorus fascicularis* extract might be with the Vitamin and mineral contents of the leaves of *Corchorus fascicularis*. These constituents are well known haemopoietic factors that have direct influence on the production of blood in the bone marrow.

CONCLUSION: Today, wide range of people are found to be anaemic, specially, females, and thus, they go for allopathic iron supplements, which comes up with large number of adverse effects like constipation, irritation, stomach upset, pain, diarrheoa, nausea and vomiting. So, this plant extract may prove helpful in anaemic patient, as it contains inorganic substance like iron. In the pharmacological evaluation of selected plant *i.e. Corchorus fascicularis* for the anti anaemic activity by using Wistar rats, from the results, it is concluded that, as the concentrations of the alcoholic extract increases, the anti anaemic property of the plant also increases. And so, the alcoholic extracts in moderate concentrations are useful in treatment of anaemia.

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REFERENCES:

1. Duff S: Types of Anaemia. www.innvista.com 2008.
2. Ong HC: Anaemia in pregnancy in an Aborigine population. Med. J. Malays 1973; 28: 22-28.
3. Orna NU: Iron deficiency anaemia. Clin. Pharm. Herb. Med 1991; 7: 12-16.
4. Richard H: Chinese Herbal Medicine. Ancient art and modern Science. Shocker Brook, New York 1978; 49-52.
5. Sofowora A: African Medicinal plants. Proceeding of a conference. University of Ife Press, Ife Nigeria 1979; 3.
6. Dina OA, Adedapo AA, Oyinloye OP and Saba AB: Effect of *T. occidentalis* extract on experimentally induced anaemia in domestic rabbits. Afr. J. Biomed. Res 2000; 3: 181-183.
7. Choudhary SB, Sharma HK, Karmakar PG, Kumar AA, Saha AR, Pranab Hazra and Mahapatra BS: Nutritional

profile of cultivated and wild jute (*Corchorus*) species. AJCS 2013; 7(13): 1973-1982.
8. Robert MN, Alphonse E, Gilbert CM and Christiane EN: Qualitative and Quantitative Assessment of Mineral Elements In the leaves of *Corchorus fascicularis* and *Corchorus olitorius* Harvested In Cameroon. J. Curr. Chem. Pharm. Sc. 2012; 2(1): 17-23.
9. Janbaz KH, Zia U, Mehmood MH, Bashir S, Choudhary MI and Gilani AH: Anti-Inflammatory, Free Radical Scavenging and Calcium Antagonist Activities of *Corchorus fascicularis* Lam. Crude Extractthe. Journal of Animal and Plant Sciences 2012; 22(4): 954-959
10. Rajput P and Rajput TA: Antibacterial and Antifungal activity from leaves extract of *Corchorus fascicularis* Lam. Int. J. PharmTech Res 2011; 3(4).
11. Rajput P and Rajput TA: Pharmacognostic and Preliminary Phytochemical Investigation of *Corchorus fascicularis Lam.* Leaves. Int J Pharm Pharm Sci 2011; 3(4): 365-366.
12. Rajput P and Rajput TA: Isolation of two phenolic compounds from Ethanol extract of leaves of *Corchorus fascicularis Lam.* Biotecnology 2012; 11(5): 292-29.
13. Khandewal KR: Practical pharmacognosy. Nirali prakashan pune. 14th Ed. 2005; 146–157.
14. Mukherjee PK: Extraction of herbal drug and quality control of herbal drug. Business horizon publication New Delhi 2nd Ed, 2005; 381–412.
15. Ashish T, Sachin M, Rizwan S, Gadhpayle J, Bhongade SL and Vikas S: Antianaemic Potential of *Swertia chirata* on Phenylhydrazine Induced Reticulocytosis In Rats. Ajpct 2013; 1(1): 037-041.
16. Peter A, Christian EO and Adaobi CE: The haematinic activity of the methanol leaf extract of *Brillantasia nitens* Lindau (Acanthaceae) in rats. African Journal of Biotechnology 2009; 8(10): 2389-2393.
17. Diallo A, Gbeassor M, Vovor A, Eklu-Gadegbeku K, Aklikokou K, Agbonon A, Abena AA, de Souza C and Akpagana K: Effect of *Tectona grandis* on phenylhydrazine induced anaemia in rats. Fitoterapia, 2008; 79: 332-336.
18. Berger J and Yenon AA: Anti-anaemic activity of aqueous and ethanolic extracts of *Entandrophragma angolense* bark on Phenylhydrazine- induced anaemic rats, J Appl Biomed 1985; 5: 1-4.
19. Rifkind RA and Danon DH: Body anemia – An ultrastructural study. I. heinz body formation. Blood 1965; 25: 885-96.
20. Suzuki Y: The development of a sensitive micronucleus test: An *in vitro* method using cultured bone marrow cells. Jikeikai Med J 1985; 100: 709-19.
21. Adamson JW and Longo DL: Hematologic alterations. In: Braumwald E, Fauci AS, Kasper DL, Hauser SL, Longo DL, Jameson JL: editors. Harrison's Principles of Internal Medicine. New York: McGraw Hills 2001.

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