

STUDY OF ANTI-ANAEMIC EFFECT OF *SCHREBERA SWIETENIOIDES* ROXB. IN RAT MODELSPRASUNA SUNDARI PINGALI^{1*}, PRATHIMA SRINIVAS¹, MADHAVA REDDY B²¹Department of Pharmacognosy & Phytochemistry, Sri Venkateshwara College of Pharmacy, Madhapur, Hyderabad, Telangana, India.²Department of Pharmacognosy & Phytochemistry, G. Pulla Reddy College of Pharmacy, Mehidipatnam, Hyderabad, Telangana, India.

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ABSTRACT

Objective: Ethnobotanical survey of *Schrebera swietenoides* Roxb revealed the bark of the tree to be useful in anemia. The objective of this study was to study the ant-anemic effect of methanolic extract of root bark of *S. swietenoides* Roxb. against phenylhydrazine induced anemic rat model.

Methods: The methanolic extracts of Leaf, Stem bark, and Root bark were prepared by soxhlation. Phytochemical analysis of the extracts was performed using standard testing procedures. The total phenolic content (TPC) of *Schrebera* leaf extract, *Schrebera* stem bark extract and *Schrebera* root bark extract (SRE) was determined by Folin–Ciocalteu method. Hemolytic anemia was induced in male Wistar rats by intraperitoneal administration of phenylhydrazine HCl (PHZ) at doses of 40 mg/kg body weight/day for 3 consecutive days. Anemic rats were orally treated with SRE at doses of 200 and 350 mg/kg body wt/day. The rats were analyzed for hematological parameters such as hemoglobin (Hb), red blood cell count (RBC) and hematocrit or packed cell volume (PCV) on day 4 and 14.

Results: Phytochemical screening of the extracts indicated the presence of carbohydrates, saponins, sterols, polyphenols, tannins, and flavonoids. Folin–Ciocalteu method of testing for TPC demonstrated SRE to be rich in total phenols with a value of 266 mg GAE/g of dry extract. Anemia was induced successfully in Groups II, III, IV, and V, which was indicated by a mean reduction of 51.6% in RBC count; 53.85% in Hb content, and 54.9% in PCV. Analysis of hematological parameters on day 14 showed that SRE significantly ($p < 0.05$) improved Hb, RBC count, and PCV at a dose of 350 mg/kg body weight.

Conclusion: This study, not only substantiates the folklore use of the root bark of *S. swietenoides*, but also suggests its inclusion in the treatment of anemia as it exhibited significant anti-anemic activity.

Keywords: *Schrebera swietenoides*, Anemia, Hematological parameters, Phenyl hydrazine, Hematocrit.

INTRODUCTION

Certain diseases such as malaria, malnutrition, protozoa infections and pregnancy are among various conditions that may lead to anemia in both adults and children. Anemia is a condition, wherein the quantity of circulating hemoglobin (Hb) in the blood is < 13 g/dl for male and < 12 g/dl for female adult [1]. There are several types of anemia, and all are characterized by a reduction in a number of circulating red blood cells (RBC) and Hb [2]. The consequences of anemia included general body weakness, frequent tiredness and lowered resistance to disease. Anemic condition if not treated, may lead to serious problems in pregnant women such as premature delivery and low birth weight. It is of concern in children in whom anemia is associated with impaired mental and physical development. Anemia is a condition commonly seen in developing countries because of lack of nutrition and frequent use of drugs to treat diseases. Hemolytic anemia is a form of inherited or acquired anemia resulting from either intravascular or extravascular RBC destruction. It has numerous external and internal causes which are either relatively harmless or are life-threatening in nature [3]. The exposure to many chemicals has also been associated with RBC destruction and hemolytic anemia [4]. The hemolytic activity of aryl hydrazines, such as phenyl hydrazine, dapson, hydroxylamine, divicine, may lead to acute hemolytic anemia in vertebrates [5]. Yeshoda induced anemia in rats following a single intraperitoneal administration of phenylhydrazine hydrochloride (PHZ) at a dose of 20 mg/kg b.w. (aqueous solution). Erythrocyte concentration lowered to about 50% and Hb level to about 60% of normal values in the course of 4 days [6].

From ancient time, medicinal plants classified as Rasayana in Ayurveda are believed to be useful in strengthening the hematopoietic and immune system of an individual. Ayurvedic physicians suggested

various herbs for the treatment of hematological disorders as a source of iron and other minerals. Silja *et al.* 2008, included *Ageratum conyzoides*, *Boerhavia diffusa*, *Centella asiatica*, *Hemidesmus indicus*, *Ichnocarpus frutescens*, *Momordica charantia*, *Moringa oleifera*, *Phyllanthus amarus*, *Phyllanthus emblica*, *Punica granatum*, *Ocimum tenuiflorum*, *Solanum americanum* as useful plants in the treatment of anemia [7]. *Adenia gummifera*, *Allophylus rubifolius*, *Albizia versicolor*, *Brackenridgea zanguebarica*, *Bridelia cathartica*, *Commiphora africana*, *Hibiscus sabdariffa*, *Lannea stuhlmanni*, *Sorgum bicolor*, *Theobroma cacao*, *Triumfetta rhomboidea* were also reported to be used in conditions of anemia [8-10]. Plants useful in iron deficiency anemia are *Commiphora mukul*, *Emblica officinalis*, *Acorus calamus*, *Spirulina*, *B. diffusa*, *Asphaltum punibium* (*shilajit*), *Terminalia chebula*, *Terminalia bellerica*.

Various researchers successfully evaluated the potential of several medicinal plants in the treatment of anemia using various experimental animal models. The hematinic activity of an orally administered aqueous extract of *Hibiscus cannabinus* leaves was evaluated in phenylhydrazine (10 mg/kg, p.o. for 8 days) induced anemic rats [11]. *Tectona grandis* leaves were evaluated on anemia model of rat induced by intraperitoneal injection of phenyl hydrazine at 40 mg/kg for 2 days [12].

Schrebera swietenoides Roxb. (Oleaceae) is a deciduous tree found in India at Karnataka, Tamil Nadu and restricted to Cannanore in Kerala. It has a folklore history of being used to treat various diseases. Roots, bark, and leaves are useful in treating indigestion, skin diseases, leprosy, anemia, boils and burns and rectal disorders. The bark is used to treat diseases of the throat, anemia, bleeding piles and diabetes [13]. Scientific investigations have proved the anti-oxidant, anti-inflammatory and

antipyretic activities of the root [14] and antidiabetic and antioxidant effect of the fruit [15].

The objective of this study aims at investigating the therapeutic benefit of the plant in the treatment of anemia.

METHODS

Materials

Plant material

Semi-dried plant material of *S. swietenoides* Roxb. was procured from Tirupathi. Plant material was authenticated by Dr. K. Madhava Chetty, Associate Professor, Department of Botany, Sri Venkateswara University, Tirupathi, Andhra Pradesh. Specimen sample has been deposited in the Pharmacognosy Department of Sri Venkateshwara College of Pharmacy, Madhapur, Hyderabad for future reference.

Chemical reagents

All the solvents used were of UV grade. Standard Gallic Acid was a gift sample given by G. Pulla Reddy College of Pharmacy, Mehidipatnam, Hyderabad. Phenyl hydrazine HCl was purchased from SD Fine Chemicals Ltd, Mumbai, Folin-Ciocalteu was purchased from Merck Company, Hyderabad. All other chemicals used were of analytical grade.

Animals

Albino rats of either sex (250±25 g body weight) were used for this study. They were housed in groups of three in polypropylene cage at ambient temperature (25±2°C), relative humidity (55±5%) and 12 hrs/12 hrs light-dark cycle. Animals had free access to commercial brand rat pellet diet supplied by Sri Krishna Enterprises, Warangal, Hyderabad and water given *ad libitum*. The protocol of the experiment was approved by the Institutional Animal Ethical Committee (IAEC) as per the guidance of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India, vide certificate no. IAEC/SVCP/2009/003. The studies were conducted according to the guidelines of CPCSEA.

Preparation of the extract

The received plant material was segregated and weighed. Leaves, root bark, stem bark were dried in the shade. On complete drying, leaves 4.25 kg, stem bark 2.25 kg and root bark 0.7 kg were obtained. The dried parts were packed in separate gunny bags and stored under optimum conditions for future use. 500 g of each plant part was extracted by soxhlation using 80% methanol as a solvent. Recycling of solvent was allowed to continue for 7 cycles to effect complete extraction. The extracts were concentrated under vacuum using a rotavacuum evaporator and placed in a desiccator until further use. Percentage yield of *Schrebera* leaf extract (SLE), *Schrebera* stem bark extract (SSE) and *Schrebera* root bark extract (SRE) were 28, 7.5 and 7.5, respectively.

Phytochemical screening

SLE, SSE and SRE were subjected to phytochemical analysis using standard tests and procedures [16].

Determination of total phenolic content (TPC)

The TPC of the methanolic extracts LE, SSE, and SRE was determined by using Folin-Ciocalteu reagent as phytochemical tests strongly indicated the presence of tannins and phenols. To 0.5 ml of sample (in triplicate) diluted with 10 ml water, 1.5 ml of Folin-Ciocalteu reagent was added followed by 4 ml of 20% Na₂CO₃ solution made up to 25 ml with distilled water. The mixture was incubated for 30 minutes, and the absorbance of the blue color developed was measured at 760 nm using Double beam UV-Visible spectrophotometer. Percentage of total phenolics was calculated from the calibration curve of gallic acid plotted by using the above procedure and the total phenolics were expressed as milligrams of gallic acid equivalent per gram of dry weight of the extract (mg GAE/g dw). The results are presented as the average of three measurements [17].

Acute toxicity studies

Acute toxicity studies were conducted to determine the safe dose by staircase method. The overnight fasted rats were orally administered with SRE extract suspended in 0.5% carboxy methyl cellulose at limit test dose of 2000 mg/kg body weight. They were later on observed closely for 1 hr, frequently for the next 4 hrs, periodically once in 4 hrs and then on a daily basis, i.e. once 24 hrs. Animals surviving the first 24 hrs were observed for the next 14 days [18,19].

Animal treatment and experimental design

A total of 30 male albino rats were used for this experiment. All the test animals were randomly divided into five groups (6 rats per group); Group I: Vehicle control - received only normal saline orally once in a day during the entire study period

Group II: PHZ control - received PHZ i.p. 40 mg/kg body wt., once daily for 3 consecutive days.

Group III: Test Group I - received PHZ i.p 40 mg/kg body wt/day and SRE orally 200 mg/kg/day for three consecutive days.

Group IV: Test Group II - received PHZ i.p 40 mg/Kg body wt/day and SRE orally 350 mg/kg/day for 3 consecutive days.

Group V: Plant extract control - received SRE orally 350 mg/kg/day for the entire treatment period.

On day 4, blood samples were withdrawn from the orbital plexus of rat eye in EDTA vials and evaluated for blood parameters using Sahli's hemoglobinometer for Hb and Neubars chamber for RBC count. Treatment with extracts continued for all groups except Group I and II, Group III receiving 200 mg/kg, Group IV with 350 mg/kg and Group V receiving 350 mg/kg for a further period of 10 days. Blood samples were collected on day 14 from all the rats used in this experiment and evaluated for hematological parameters such as RBC, Hb, and packed cell volume (PCV).

Statistical analysis

The results were expressed as the Mean ± standard error of mean and statistical analysis was carried out using GraphPad InStat software, (version 3.00, GraphPad Software, San Diego, Californis, USA). Differences among the groups were investigated using one-way analysis of variance, followed by student's *t*-test. *p*<0.05 was considered as statistically significant.

RESULTS AND DISCUSSION

Preliminary phytochemical screening

Preliminary phytochemical investigations revealed the presence of a tannins, polyphenolics, saponins, flavonoids, and sterols (Table 1).

TPC

Gallic acid being the most important polyphenol in natural products was used to determine the phenolics of the test extracts. It was found to be in the range of 57.71, 54.14 and 266.0 mg GAE/g of extract in

Table 1: Preliminary phytochemical analysis of SLE, SSE and SRE

Phytochemical	Test	SLE	SSE	SRE
Tannins	FeCl ₃ test	+	+	++
Steroids	Salkowski test	+	+	+
Saponins	Frothing test	+	+	++
Flavonoids	Shinoda test	+	+	+
Carbohydrates	Molisch test	+	+	++
Proteins and amino acids	Ninhydrin test	-	-	-
Alkaloids	Mayer's Drgendoff's, Wagner's and Hager's test	-	-	-
Anthraquinone glycosides	Modified Borntrager's test	-	-	-
Cardiac glycosides	Keller-Kiliani test	-	-	-
Terpenoids	Modifies Salkowski test	-	-	-

+: Present, -: Absent, ++: Indicates strong presence, SLE: *Schrebera* leaf extract, SSE: *Schrebera* stem bark extract, SRE: *Schrebera* root bark extract

Table 2: Hematological findings of Hb concentration (g/dl), RBC count (million cells/c.mm) and PCV (hematocrit or PCV) on day 4 post-PHZ treatment

Blood parameter	Group-I (control)	Group-II (PHZ control)	Group-III (PHZ+SRE) 200 mg/kg	Group-IV (PHZ+SRE) 350 mg/kg	Group-V (SRE) 350 mg/kg
Mean RBC's (10 ⁶ /μl)	4.583±0.166	2.367±0.049	2.063±0.072	2.22±0.0581	4.62±0.19
Mean Hb (g/dl)	14.183±0.439	6.983±0.095	6.217±0.154	6.432±0.194	14.23±0.56
Mean HCT%	42.55±1.318	20.950±0.284	18.15±0.229	18.2±0.058	42.69±0.196

n=6, tabular value represents mean±SEM, SEM: Standard error of mean, PCV: Packed cell volume, PHZ: SRE: *Schrebera* root bark extract, RBC: Red blood cell, SEM: Standard error of the mean

Table 3: Hematological findings of Hb concentration (g/dl), RBC count (million cells/c.mm) and PCV (hematocrit) on day 14 of treatment

Blood parameter	Group-I (control)	Group-II (PHZ Control)	Group-III (PHZ+SRE) 200 mg/kg	Group-IV (PHZ+SRE) 350 mg/kg	Group-V (SRE) 350 mg/kg
Mean RBC'S (10 ⁶ /μl)	4.71±0.23	3.883±0.130	4.336±0.1085	4.5±0.1344	4.73±0.336
Mean Hb (gm/dl)	14.64±0.412	12.133±0.334	13.33±0.131	14.317±0.142	14.62±0.36
Mean HCT%	43.92±0.433	36.4±1.003	40.0±0.392	42.95±0.427	43.86±0.423

n=6, tabular value represents mean±SEM, p<0.05 (comparison of Groups III and IV with Group II), SRE: *Schrebera* root bark extract, SEM: Standard error of the mean

SLE, SSE, and SRE, respectively. As root bark extract showed a strong presence of polyphenolics as indicated by GAE it was selected for anti-anemic study.

Acute toxicity studies

Studies revealed the plant extract to be safe at doses of 2000mg/kg body weight.

Animal studies for anti-anemic activity

Anti-anemic activity was evaluated by using phenyl hydrazine HCl-induced anemia in an animal model. Hematological data on day 4 showed a significant drop in Hb concentration, RBC count and Hematocrit values in rats post-injection of PHZ as compared to control Group I. Table 2 presents the hematological data of blood sample withdrawn on day 4. Comparison of Groups II, III, IV, and V with Group I indicated a significant average reduction of 51.6% in RBC count; 53.85% in Hb content and 54.9% in PCV. Simultaneous administration of SRE in different doses has not shown any hemato-protection. In Group V animals received only SRE 350 mg/kg body wt. and the data obtained indicated that the extract did not stimulate erythropoietic activity in healthy rats. Further, the test groups, Groups III and IV were subjected to treatment using methanolic extract of root bark at the doses of 200 mg/kg and 350 mg/kg body weight orally for 10 days, which resulted in considerable increase of all blood parameters. Comparison of Groups III, IV with Group II showed significantly (p<0.05) increase in the hematological parameters. Group V hematological data showed no significant changes over control. Therefore, data obtained as a result of this investigative study on day 14 showed that all the hematological parameters were restored to normal as shown in Table 3. This indicates that SRE is effective and possesses good hematoprotective activity.

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 [20]. 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 [21,22]. 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 [23-26].

In this study, significant decrease in Hb, RBC count and hematocrit was observed following PHZ injection to the experimental animals (p<0.05).

Treatment with different doses of *S. swietenoides* root bark extract resulted in increased values of these parameters. The Hb concentration was found to be higher than the positive control animals. This indicates that the root bark contains some bioactive agents that are powerful antioxidants, which prevent or repair the damage done to the cells by free radicals or highly reactive oxygen species. A significant observation was that the content of polyphenols, flavonoids and tannins was much higher in SRE compared to SLE and SSE. The anti-anemic property of *S. swietenoides* may be attributed to the presence of the above-mentioned bioactive as they are known to exert anti-oxidant activity as reported in the literature. From our study, it can be established that the anti-anemic potential of SRE can be explored for further research in developing a novel herbal delivery system.

CONCLUSION

The collective data of this study revealed that root bark has considerable anti-anemic activity as shown in PHZ-induced anemia in experimental rat model indicating the use of this plant for the treatment of anemia. Further studies are required to precisely define the bioactive and to develop suitable formulations to ensure maximum bioavailability and therapeutic efficacy.

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