



Preclinical Evaluation of Hematinic Potential of the Siddha Formulation *Sarakondrai Chooranam* using Phenylhydrazine Induced Anaemia in rats

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Abstract

Anaemia is a clinical condition characterized by decrease in the total amount of Haemoglobin or the number of Red Blood Cells. Approximately 25% of people worldwide have Anaemia. The most common cause is Iron deficiency, responsible for 50% of all Anaemia. Current treatment methodology for treating Anaemia has several limitations including Gastro- intestinal disturbances, delayed absorption etc. Hence it is a right time to explore alternate therapy for Clinical Management of Anaemia. In the recent years, researches on Siddha System of Medicine have attracted a lot of attention globally. Evidences have been accumulated to demonstrate the promising potential of Medicinal Herbs used in Siddha formulations has various traditional, Complementary and Alternative therapy of treatment for Human diseases. Haematinic property of most of the Siddha preparations has not explored much in recent times. The main aim of the present Investigation is to evaluate the possible Haematinic property of the Siddha Formulation *Sarakondrai Chooranam* using Phenylhydrazine induced Anaemia in rats. The results of the present investigation shown that Mean Haemoglobin, RBC, WBC and HCT level of rats belongs to Phenylhydrazine treated rats were decreased significantly when compare to that of the Saline control group, which signifies the induction of Anaemia in Experimental animals. There was significant increase in Hb, RBC, WBC and HCT content were observed in animals treated with 200 and 400mg/kg of SKC respectively. This observation reflects the promising Haematinic property of the trial drug SKC in treated rats. In conclusion of the observed data's it was clear that the formulation SKC at both the dose level possess significant Haematinic activity and may be used for Clinical Management of Anaemia. Anaemia is a clinical condition characterized by decrease in the total amount of Haemoglobin or the number of Red Blood Cells. Approximately 25% of people worldwide have Anaemia. The most common cause is Iron deficiency, responsible for 50% of all Anaemia. Current treatment methodology for treating Anaemia has several limitations including Gastro- intestinal disturbances, delayed absorption etc. Hence it is a right time to explore alternate therapy for Clinical Management of Anaemia. In the recent years, researches on Siddha System of Medicine have attracted

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Keywords: Siddha system, Haematinic property, *Sarakondrai Chooranam*, Phenylhydrazine, Haemoglobin, RBC, WBC, HCT.

1. Introduction

Anaemia is one of the most widespread disorders of blood which affect the populations of all ages throughout the world. It is a public health problem that affects populations in both rich and poor countries [1]. However, the incidence of this disorder is higher in the developing countries than in the developed countries [2]. Due to poverty and lack of hygiene. The situation is aggravated by factors such as nutritional deficiencies and high prevalence of Parasitic Gastro-Intestinal Infections which cause heavy loss of blood. Other conditions, such as Malaria and Haemoglobinopathies are also responsible [3]. In the Tropics, due to Endemicity of Malaria, between 10 to 20% of the population presents less than 10g/dl of Haemoglobin [4] against Reference values of 12g/dl in Women and Children, 13 g/dl in Men [5].

Indian System of Traditional Medicines serves to the Mankind since several centuries though its innumerable formulations comprises of Herbs and Minerals. Siddha System of Medicine is one of the oldest among all. Therapeutic Efficacy of many Indigenous Plants for several disorders have been described by Practitioners of Traditional Medicine [6]. Plants are rich in a wide variety of Secondary Metabolites such as Tannins, Terpenoids, Alkaloids, Flavonoids, Glycosides etc, which have been found *in vitro* to have Anti-Microbial properties [7,8]. Traditional Medicine continues to be a valuable source of remedies that

have been used by millions of people around the world to secure their health [9].

Cassia fistula Linn is a Semi-Wild Indian Labernum also known as the Golden Shower, is distributed in various countries including Asia, Mauritius, South-Africa, Mexico, China, West Indies, East Africa, and Brazil as an ornamental tree for its beautiful branches of yellow flowers. Recognize by the British pharmacopoeia [10]. It is widely used for its Medicinal properties, its main property being that of a Mild Laxative suitable for Children and Pregnant women. It is also a Purgative due to the Wax Aloin and a Tonic [11]. It has been reported to treat many other Intestinal disorders like Healing ulcers [12, 13]. The plant has a high Therapeutic value and it exerts an Anti-pyretic and Analgesic effect [14, 15].

Iron Deficiency (ID) Control Programs have not been successful in reducing the number of Iron deficient people in developing countries due to unsuccessful supplementation programs, the low absorption of some Fortification Iron compounds and to the presence of other complicating health factors such as Vitamin-A deficiency [16]. This prompted us to pursue the present Investigation. Numerous Formulations indigenous to Indian System of Medicine have been reported to be helpful in successfully managing the Anaemia one among them is *Sarakondrai Chooranam*.

The main aim of the present Investigation is to evaluate the possible Haematinic property of the Siddha Formulation *Sarakondrai Chooranam* using Phenylhydrazine induced Anaemia in Rats.

2. Materials and Methods

2.1. Collection of plant materials

The fresh leaf and flower of *C. fistula* (Sarakondrai) were collected from Southern Zone of Tamil Nadu, India. Plant specimen were identified and authenticated by the Pharmacognosist, SCRI Chennai, Tamil Nadu, India.

2.2. Formulation of Sarakondrai Chooranam (SKC) [17].

Leaves and flowers of *Cassia fistula* commonly known by its Tamil name Sarakondrai. Fresh samples are collected and they are dried in shade. After that they are finely powdered and formulated as per the procedure described by Athmaratchamirtham Ennum Vaithya Sara Sangraham.

2.3. Animals

Healthy adult Wistar Albino Female rats weighing between 220-240 g were used for the study. The animals were housed in poly propylene cages and were kept in well ventilated with 100% fresh air by air handling unit. A 12 light/dark cycle were maintained. Room temperature was maintained between $22 \pm 2^{\circ}$ C and relative humidity 50–65%. They were provided with food (Sai feeds, Bangalore, India) and water *ad libitum*. All the animals were acclimatized to the laboratory for 7 days prior to the start of the study. The experimental protocol was approved by The Institutional Animal Ethics Committee of Sathyabama University, Chennai, Tamil Nadu, India. IAEC: SU/CLATR/IEAC/VII/052/2016

2.4. Induction of Anaemia [18,19]

Induction of Anaemia in rats was performed by intra-peritoneal administration of 40mg/kg of

phenylhydrazine (PHZ) for two days (Day 1 and Day 2).

2.5. Experimental Methodology

Animal belongs to group I received normal saline 5ml/kg. Group II rats were treated with Phenyl hydrazine (PHZ) 40mg/kg (i.p) for two days (Day1 and Day2) and were served as disease control. Animal belongs to group III received PHZ injection 40mg/kg (i.p) and treated with 200 mg/kg *Sarakondrai Chooranam* from 3rd to 16th Day. Whereas animal belongs to group IV treated with 400 mg/kg *Sarakondrai Chooranam* from 3rd to 16th Day and served as Treatment group.

2.6. Blood Collection [20]

At the end of the study, before sacrifice, the animals were fasted for overnight with free access to water. Animals were sacrificed with excess anesthesia. Blood samples were collected from Retro-Orbital Sinus puncture and stored in EDTA (ethylenediamine - tetra acetate) test tubes for Haematological analysis. Bone Marrow of Control and Treatment group animals were collected using Fine Needle Aspiration Technique for further processing.

2.7. Bio-chemical Parameter

At the end of the study blood will be collected by ocular puncture after overnight fasting animals. The blood parameters such as Red Blood Cell Count (RBC), White Blood Cell Count (WBC), Haemoglobin Concentration (Hb) and Haematocrit was determined using Mindray BC 2800 Haematology Analyzer [21].

2.8. Bone Marrow Smear

About 0.2 ml of smear aspirated from the Femur thigh bone of the Experimental animal was dropped onto the glass slide and made into thin smear allow the smear to dry. Dried smear was stained with Leishman stain and washed. Followed by this Cedar wood oil was placed on to the smear and was observed Microscopically [22, 23].

2.9. Statistical analysis

Results are presented as the average \pm Standard deviation and the differences among Test groups were assessed by one-way analysis of variance followed by Dunnett's multiple comparison test using Graphpad prism 5.0 software (Graph pad, La Jolla, CA, USA). A $P < 0.05$ was considered significant.

3. Results

3.1. Effect of *Sarakondrai Chooranam* on Haematology Profile of Phenyl hydrazine induced Anaemic rats

The Mean Haemoglobin (Hb) content (g/dl) of rats belongs to disease Control group was decreased significantly 6.13 ± 0.13 when compare to that of the Saline control group 12.67 ± 0.49 , which signifies the induction of Anaemia in Experimental animals. There was significant increase in Hb content were observed in animals treated with 200 and 400mg/kg of SKC with 7.27 ± 0.13 and 7.7 ± 0.14 respectively. This observation reflects the promising Haematinic property of the trial drug SKC in treated rats.

There was a significant decrease in the level of RBC ($\times 10^6 \mu\text{l}$) were observed in animals belongs to group II 4.57 ± 0.23 when compare to that of the normal control rats with 7.47 ± 0.20 . Treatment with SKC at both the dose level shown marked increase in RBC level with 5.57 ± 0.09 for SKC 200mg/kg and 6 ± 0.1 for SKC 400mg/kg. Similar results were observed with respect to WBC count.

The Haematocrit (HCT) test indicates the percentage of blood by volume that is composed of red blood cells. Treatment with PHZ shown significant decrease in HCT with the level of 19 ± 0.57 lower when compare to control rats with HCT 48 ± 1.23 . Animals treated with 200 and 400mg/kg of SKC has shown increased HCT value of 24 ± 0.85 and 29.33 ± 0.84 respectively. The results were tabulated in Table 1.

Table 1: Haematology Profile of Phenyl hydrazine induced Anaemic rats

Group	Treatment	RBC ($\times 10^6 \mu\text{l}$)	WBC ($\times 10^3 \mu\text{l}$)	HGB (g/dl)	HCT (%)
I	Normal saline (5ml/kg), p.o	7.47 ± 0.20	8.05 ± 0.20	12.67 ± 0.49	48 ± 1.23
II	Phenylhydrazine (PHZ) 40 mg / kg ,i.p	4.57 ± 0.23	6.13 ± 0.13	7 ± 0.36	19 ± 0.57
III	PHZ+ SKC 200mg/kg, p.o	5.57 ± 0.09	7.27 ± 0.13	8.67 ± 0.33	24 ± 0.85
IV	PHZ+ SKC 400mg/kg, p.o	6 ± 0.1	7.7 ± 0.14	10.67 ± 0.42	29.33 ± 0.84

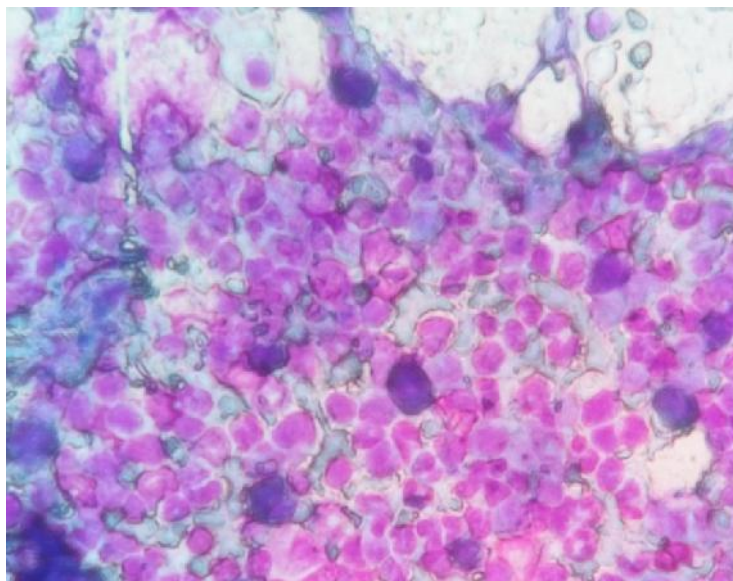
3.2. Effect of *Sarakondrai Chooranam* on Bone Marrow Rejuvenation on Phenyl hydrazine induced Anaemic rats

Bulky marrow with dense cellular portions were observed and active zones of erythropoiesis were observed in sample belongs to group I. Bone marrow smear of group I reveals normal Eosinophilic Myelocyte and Basophilic Myelocyte. Hyperplastic condition of marrow with increased adipocyte cells replaced the marrow

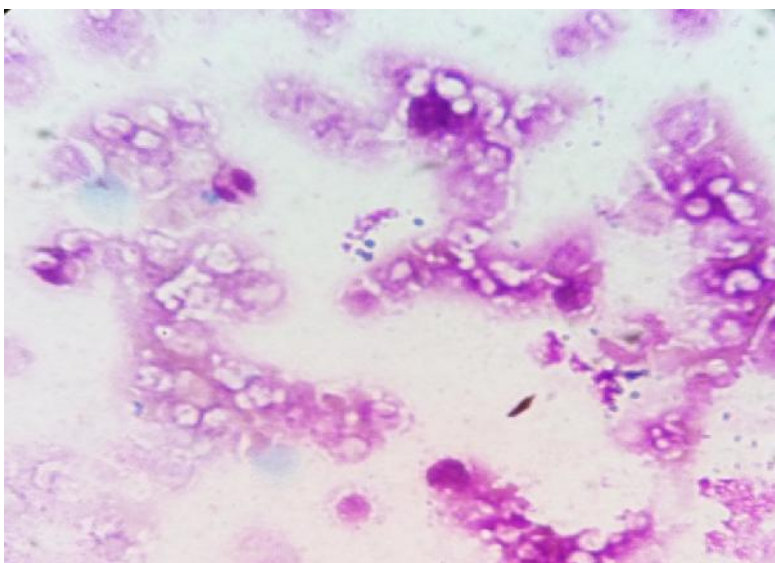
space was observed. Megakaryocyte appears very minimal in number with Erythroid precursors and Granulocytic precursors was observed in sample belongs to group II. Increased network of Erythroblastic Islets were observed, Central reticular cells appears normal long and slender in nature. Increased number of Erythroblastic islets with wavy zone of Erythropoiesis was observed in sample belongs to group III and IV as shown in Figure 1.

Figure 1: Microscopic View of Bone Marrow Smear of Control and Treatment group rats

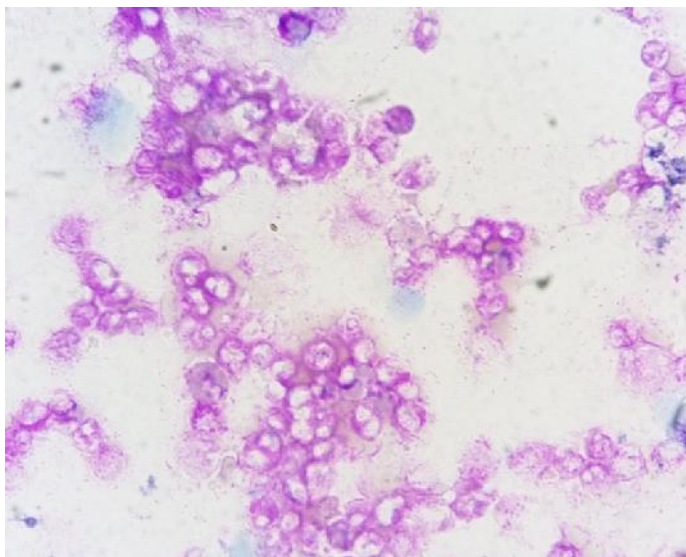
Group I- Control Sample



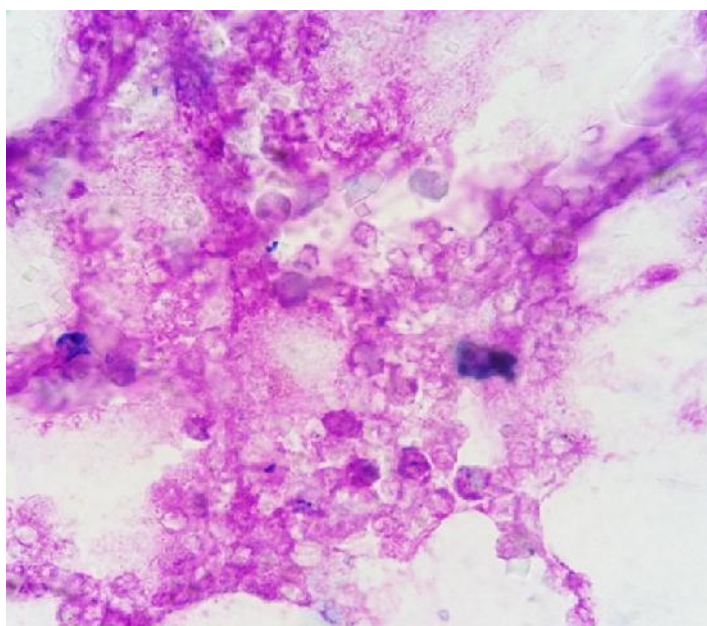
Group II- Phenyl hydrazine induced Group



Group III- Phenyl hydrazine + 200mg/kg of SKC



Group IV- Phenyl hydrazine + 400mg/kg of SKC



4. Discussion

About two billion people suffer from Anaemia worldwide and most of them have Iron deficiency and Haemolytic anaemia due to Toxicants and Oxidants. About 70–80% of the world population, particularly in the developing countries, relies on non-conventional medicine like dietary supplements and herbal remedies in their primary healthcare as reported by the WHO [24]. In developing countries, Low Iron Bio-availability of the diet is the primary cause of Iron deficiency

anaemia. However, in developed countries, decreased Iron absorption and blood loss account for the more likely Etiologies of Iron deficiency. Decreased Iron absorption may also be the result of Atrophic Gastritis or Malabsorption syndromes especially Cealiac disease [25]. Post-Surgical Gastrectomy (partial or total) and Intestinal Resection or By-Pass may also produce Iron deficiency anemia secondary to decreased Iron absorption. Chronic blood loss from Genito-urinary, Gynaecological or Gastro-intestinal tracts

accounts for the majority of causes for Iron deficiency anaemia. The most common Etiology of Iron-deficiency anaemia in Pre-menopausal women is excessive Menstruation [26].

The rate of Iron deficiency is higher in developing countries compared to the United States where the prevalence of Iron-deficiency anaemia in men under 50 is 1%. In Child-bearing age Women in the US, the rate is 10% due to loss from Menstruation while 9% of children ages 12-36 months are Iron deficient and one-third of these children develop Anaemia. While the rate of Iron-deficiency anaemia is low in the US, low-income families, particularly are at risk [27,28].

Plants have been an important source of Medicine with qualities for thousands of years. Mainly on Traditional remedies such as Herbs for their history, they have been used as popular Folk Medicines [29]. Siddha System of Medicine pioneering in emphasize the biological activity of the various phytochemicals with respect to the etiology and Patho-physiology of various dread full disease emerging in humans and animals. It is evident that there are some Medicinal plants used in Siddha has potency of acting as an Anaesthetics, Analgesics, Anti-microbial, Immune modulators, Hepato, Neuro and Nephron protectant. But the most pathetic scenario is most of these potential herbs are extinct and not be used currently.

PHZ produces both Aryl and Hydroxyl radicals when incubated with rat liver Microsomes [30] and oxidized by Hydrogen peroxide at pH 7.4 and 37°C [31]. The radicals induced oxidative stress on the Red cell membrane resulting in Haemolysis by Lipid Peroxidation [32]. The Sub-chronic intoxication of Rats with PHZ (10 mg/kg/day for 8 days) resulted in a marked Haemolytic Anaemia characterized by decreased RBC, Hb and HCT [33]. Similar results were obtained in the present study when Experimental rats were administered PHZ in order to induce Anaemia. The Mean Haemoglobin (Hb) content (g/dl) of rats belongs to disease Control group was decreased significantly 6.13 ± 0.13 when compare to that of the Saline Control group 12.67 ± 0.49 , which signifies the induction of

Anaemia in Experimental animals. There was significant increase in Hb content were observed in animals treated with 200 and 400mg/kg of SKC with 7.27 ± 0.13 and 7.7 ± 0.14 respectively. This observation reflects the promising Haematonic property of the trial drug SKC in treated rats.

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It was evident that intravenously administered Iron is one approach to replacing Iron losses in patients with chronic Gastro-intestinal bleeding in which blood loss exceeds 10 ml/day (around 5 mg iron). With the use of Intravenous Iron the desired Serum Iron levels, in which the marrow production can increase by fourfold to eightfold can be achieved [34]. Bulky marrow with dense cellular portions were observed and active zones of Erythropoiesis were observed in sample belongs to Group I. Bone marrow smear of Group I reveals normal Eosinophilic Myelocyte and Basophilic Myelocyte. Hyperplastic condition of marrow with increased Adipocyte cells replaced the marrow space was observed. Megakaryocyte appears very minimal in number with Erythroid precursors and Granulocytic precursors was observed in sample belongs to Group II. Increased network of Erythroblastic Islets were observed, Central reticular cells appears normal long and slender in nature. Increased number of Erythroblastic Islets with wavy zone of Erythropoiesis was observed in sample belongs to Group III and IV.

5. Conclusion

Siddha formulations offer tremendous advantage to the people of developing countries like India. Now day Anaemia is become a social threat as it progress more in people of lower Economic Zone. Traditional healing therapy like Siddha System of Medicine has novel formulations which resumes the level of Haemoglobin in Anaemic patients. This data's obtained from the present investigation clearly reflects the promising Haematinic property of the trial drug SKC in treated rat's dose dependently. In conclusion of the observed data's it was clear that the formulation SKC at both the dose level possess significant Haematinic activity and may be used for Clinical Management of Anaemia.

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