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HAEMATINIC ACTIVITY OF DHASADEEBAAKINI CHOORANAM (A SIDDHA HERBO-MINERAL FORMULATION) IN PHENYL HYDRAZINE INDUCED ANAEMIC RATS

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ABSTRACT

Anaemia is the most widespread nutritional problem in the world and is predominant in children, particularly in developing countries like India. Untreated anaemia may manifest as fatigue, reduced immunity, impaired memory and decreased quality of life. The gross incidence of anaemia in India called for formulation of an effective and safe traditional drugs to treat anaemia. One such medicine mentioned in the Siddha system of medicine is Dhasadeebaakini Chooranam. Hence, the present study was aimed to validate the haematinic activity of Dhasadeebaakini Chooranamin phenyl hydrazine induced anaemic rats. Anaemia was induced by an oral administration of phenyl hydrazine for a period of 5 days. The trial drugwas administered at the various dose levels of 200 mg/kg and 400 mg/kg orally to the animals for 14 days. Hb, RBC, PCV, MCV, MCH were analysed as indices of anaemia. Phenyl hydrazine significantly decreases the haematological parameters. After 14 days of treatment with the trial drug significantly reverse the haematological parameters and come towards the decreased values into normal levels. This result supports the traditional practice of Dhasadeebaakini Chooranam to treat anaemia.

KEYWORDS: Haematinic activity, Dhasadeebaakini Chooranam, anaemia, phenyl hydrazine, Siddha.

INTRODUCTION

Haemoglobin is composed of haem (iron portion), and globin (protein portion). The function of haemoglobin is to carry the respiratory gases (O2, and CO_2). Anaemia is defined as a reduction of the red blood cell volume or haemoglobin concentration below the ranges of values occurring in healthy persons [1].

Anaemia is the most common and widespread nutritional disorder in the world. The world health organization (WHO) classified anaemia as a severe public health problem (prevalence > 40%) for children under five in 69 countries and for pregnant women in 68 countries [2]. According to National Family Health Survey (NFHS-3), the incidence of anaemia in urban population is 71%, in rural areas it is 84% and the overall incidence is 79%[1]. If no proper management is done, it can manifest as tiredness, anorexia, inability to concentrate, somnolence, reduced immunity, poor school performance, and decreased quality of life [3]. Anaemia has been associated with motor and cognitive developmental defects in children and may be irreversible [4]. Anaemia may occur due to increased requirements or impaired absorption or blood loss. Anaemia reduce the work capacity of individuals and entire population bringing a serious economic consequences and obstacles to the national development. Long term oral iron therapy is commonly used as first line therapy but iron salts such as ferrous sulphate are associated with a high incidence of gastrointestinal side effects such as nausea, vomiting, diarrhoea and constipation[5]. Because of their side effects, a safe, effective, cheap, and easily available drug is needed. Many drugs are available in siddha system of medicine which have remarkable effects in treating anaemia. One such medicine is Dhasadeebaakini Chooranam(DDC)indicated for anaemia mentioned in Siddha classical literature [6]. DDC is also indicated for fever, cough, indigestion and diarrhoea.

DDC contains ten ingredients include Perungaayam *(Ferula asafoetida),*Vasambu *(Acorus calamus),*Vaaividangam *(Embelia ribes),* Induppu *(Sodium chloride impura),* Omam *(Tachyspermum ammi),* Kadukaaithol *(Terminalia chebula),*Kodiveli vaerpattai *(Plumbago zeylanica),*Koshtam *(Costus specious),*Tippili *(Pipper longum)* and Seeragam *(Cuminum cyminum*). Most of the ingredients of DDC contain Iron, copper, zinc and vit.B and C. Cuminum cyminum is a rich source of high quality iron.Scientifically it has been proven that, it helped to increase the amount of iron in the body. Most of the ingredients enhance iron intake, improve nutritional status and control infection which favours all aspects of iron deficiency and anaemia. DDC contains very high concentration of natural anti-oxidants, iron

in ferrous form, vitamins C and E which fight against free radicals.Free radicals damage DNA, altering biochemical compounds, corroding cell membranes. In addition to that, for most iron-rich ingredients, it is essential to combine with vitamin C to promote assimilation in the body.The great thing about DDC is that this formulation contains both iron and vitamin C. In the view of many health benefits and iron rich contents leads to this study of haematinic activity of the trial drug DDCin phenyl hydrazine induced anaemic rats which may serve as a beacon light in treating anaemia.

MATERIALS AND METHODS

All the ingredients of the trial drug DDC were procured from indigenous drug shop, Park town, Chennai. The raw drugs were identified and authenticated by the botanist and pharmacology experts of Post Graduate Department of Gunapadam, Govt.Siddha Medical College, Arumbakkam, Chennai. After identification, the specimen samples of each raw drug have been kept in the department for future reference. Then all the raw drugs were purified individually as per the classical text by roasting, until it becomes golden brown in colour. Then these raw materials were ground individually in stone mortar with pestle until the achievement of fine powder form and filtered in a fine cloth, measured separately. Then all the powders in the respective ratio mentioned in the formula were taken and mixed well and made into Chooranam form. This powder was sieved through a clean white cloth and the filtrate was subjected to another purification process named as *Pittaviyal method* (steam boiling with cow's milk) based on siddha classical literature[7]. The DDC was moistened with cow's milk and made into a solid form. Then it is kept in a clean cloth which is tied to the mouth of a mud vessel containing equal amount of cow's milk and water. Then it is finally covered over with a top vessel; sides are covered with a cloth, so that vapour does not escape over by boiling. After complete boiling of liquid, the solid mixture is taken and dried in direct sunlight and ground finally and stored in an air tight container. This Chooranam was labelled as DDC and used for the present study.

Preparation of Stock Solution

The suspension of DDC was prepared by mixing with water at a concentration of 200 mg/ml stock solution. The solution was administered orally by gastric intubation in rats.

Animals

Female Swiss albino rats weighing about 230-250 gm were obtained from the animal house of king institute of preventive medicine, Guindy, Chennai. The animals were acclimated to

standard laboratory condition (temperature – 24 to 28°C and humidity 60- 70%) and maintained on 12 hr light/ dark cycle. The animals were housed in polypropylene cages and fed with standard rodent pellet obtained and water ad libitum. The present study was approved by the institutional animal ethical committee (IAEC) with the approval number: IAEC/XXXIX/14/CLBMCP/2013/ dated 29.6.2013.

Evaluation of the Haematinic Activity

Anaemia was induced in rats by administrating a single intraperioneal injection of phenyl hydrazine at a dose of 20 mg/kg b.w. Drop out period of four days was awaited until the sufficient drop in Hb level was noticed in animals. Rats were considered as anaemic model if haemoglobin concentration was less than 14g/dl [8]. From day 5 to day 19, the test drug was administered orally at two different doses (200mg/kg, 400mg/kg). On day 20 blood was collected by retro orbital puncture for haematological evaluation and the blood parameters were analysed.

Group I – treated as negative control (phenyl hydrazine induced anaemic rats) administered vehicle only.

Group II– Animal injected with phenyl hydrazine 20mg/kg + treated with test drug 200 mg/kg, p.o. (Low dose Group)

Group III– Animal injected with phenyl hydrazine 20mg/kg + treated with test drug 400 mg/kg, p.o. (High dose Group)

Group IV- Considered as positive control administered standard haematinic syrup.

Haematological Investigations

The blood samples form negative control and drug treated rats were collected into heparinized tubes after 14 days treatment (on day 20) and parameters such as haemoglobin count, red blood cells (RBC), packed cell volume (PCV), Mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) were analysed

Statistical Analysis

The statistical analysis was carried by one way ANOVA (GRAPH PAD PRISM 5 computer program). Results are expressed as mean ± standard error. A statistical comparison was carried out using the Dunnets 't' multiple comparison test for comparing control and treatment group.P value less than 0.05 considered as statistically significant.

RESULTS AND DISCUSSION

Phenyl hydrazine is used for the induction of anaemia [9-10]. Phenyl free radical is produced via a 2-electron oxidation of phenyl hydrazine by oxyhaemoglobin. This free radical binds with red cell and haemolysis it rapidly and converts oxyhaemoglobin into methemoglobin. Thus PHZ-induced haemolytic injury seems to be derived from oxidative alterations to RBC proteins rather than to membrane lipids [11]. The haematological parameters after the administration of PHZ decreased significantly ($p<0.05$) while MCV &MCH increased giving rise to macrocytic anaemia (Table No.1, Fig No.1 and Fig No.2) DDC at a dose of 200mg /kg showed good percentage of improving in Hb level which was almost equivalent to the standard drug treated group indicating the correction of anaemia induced by PHZ after 14 days treatment. Treatment with DDC at dose levels of 200 and 400 mg for 14 days showed that significant ($p<0.05$ and $p<0.01$ respectively) increase in Hb when compared to positive control and standard drug. PHZ altered the haematological parameters by haemolysis characterised by decrease in Hb, RBC count, PCV on the day 5. However the haematological parameters were restored to normal range after the treatment with DDC for 14 days. Also the recovery was progressive such that alter one week of continuous treatment, Hb and PCV were high than in normal control group.

The main and the highest part of the drug is seeragam *(Cuminum cyminum*) which has Fe-66mg , vitamin.C- 7.7 mg , Zn-4.8mg and folate 10 mg [12] and *Costus speciosus* contain Fe-46mg, vitamin.C- 216 mg per 100 mg [13]. Acute and chronic administration of cumin oil increased the Hb, haematocrit and platelet counts [14]. *Piper longum*, possess haematinic activity[15].*Plumbago zylanica* (Plumbagin) has been shown to exert anticarcinogenic, antiatherosclerotic, and antimicrobial effects. Their components possess antiatherogenic, cardiotonic, hepatoprotective, and neuroprotective properties [16].Fruits of *Tachyspermum ammi* contain Fe, Ca, P, Zn, and Cu, riboflavin, thiamine, carotene and nicotinic acid which improves iron absorption [17]. *Ferula asafoetida* also contains Fe, Ca, P, carotene, riboflavin and nicotinic acid [18]. Rock salt Contains Fe, Ca, Zn, Mg, Cu and K and it increases the production of RBC[19]. *Terminalia chebula* Shows anti-oxidant, anti-microbial, and improves gastrointestinal motility and dermal healing[20]. *Embelia ribes* shows a number of pharmacological activites such as wound healing, antitumor, cardiovascular, hypoglycemic, antioxidant, antimicrobial, antidiabetic and antifertlity. Many other traditionally uses are also reported such as blood purifier, cosmetic agent, oil pulling and oral contraceptive [21]. *Acorus calamus* root and rhizome shows anti-helminthic activity [22] and anti- Oxidant activity [23].

All the above results and facts confirm the Siddha traditional practice of Dhasadeebaakini Chooranam to treat anaemia successfully with inexpensive and effective way.

Table No.1: Haematological Parameters of Rats after Treatment with DDC.

Groups (n=6)	RBC (x 10^6/ml)	PCV (%)	Hb (g/dl)	MCV(fl)	MCH(pg)	MCHC(g/dl)
Control	4.30± 0.11	38.50±2.80	10.5±0.85	41.00±2.55	12.17±0.98	26.33±4.50
DDC (200mg)	6.54 ± 0.14*	44.00±1.00*	13.0±0.51*	46.00±0.68	13.67±0.66	35.00±0.68*
DDC (400mg)	6.83 ± 0.17**	48.00±0.80*	15.5±0.22**	55.67±0.33*	17.67±0.61*	30.17±1.11**
Standard	7.06±0.16**	52.02±0.46*	22.1±0.88**	74.52±0.21**	28.10±0.53**	34.17±0.20*

*Values are expressed as mean+SEM (Dunnet't' test) *p<0.05; **p<0.01 vs control n=6.*

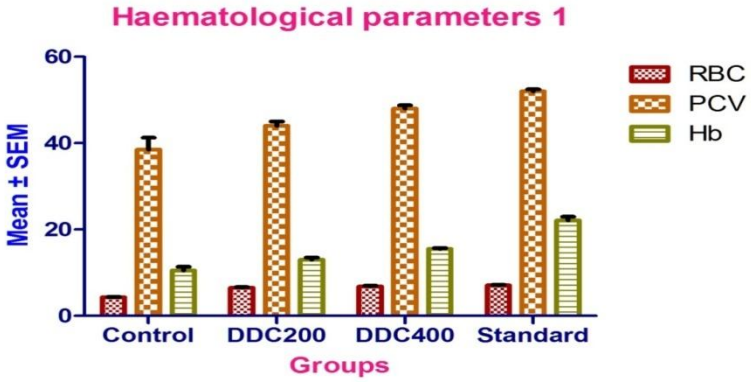

Fig No. 2. Showing Haematological Parameters (RBC, Pcvand Hb) After Treatment with DDC

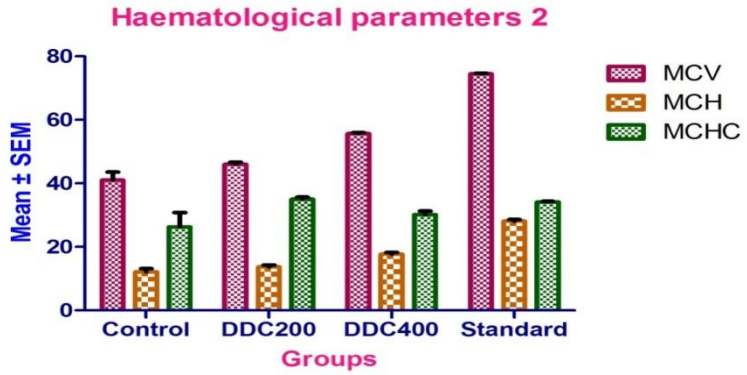

Fig No.3. Showing Haematological Parameters (MCV, MCH and MCHC) After Treatment with DDC

CONCLUSION

Traditional medicines are in great demand in the developed as well as developing countries for primary health care because of their wide biological and medicinal activities and higher safety margins. In order to provide an effective, safer drug for the treatment of iron deficiency anaemia, *DDC* was evaluated at low and high dose levels. This study revealed that

administration of DDC result in increased Hb, RBC, PCV, MCV and MCH in a significant level indicating the haematinic activity. Based on the results it can be concluded that the *DDC* is a good drug of choice for anaemia. Further study should be carried out to confirm this result through clinical trial.

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