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Research Article

HAEMATINIC ACTIVITY OF ECHURAMOOLI LEAF CHOORANAM IN PHENYLHYDRAZINE INDUCED ANAEMIC RATS

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ABSTRACT
Iron deficiency is the most common and widespread nutritional disorder in the world. India is among the countries with highest prevalence of anaemia in the world. It has serious consequences for the health and well-being as well as social and economic impacts of India. Untreated iron deficiency anaemia can become severe enough to interfere with daily life. Knowledge of iron deficiency and its treatment in Siddha System of medicine dates back from time immortal. Many plants in Siddha system of medicine are known to have remarkable effects in treating anaemia. One such medicine is "Echuramooli Leaf Chooranam" *Aristolochia indica* Linn. The haematinic activity of Echuramooli Leaf Chooranam was studied in Phenylhydrazine induced anaemic rats. Anaemia was induced by an oral administration of Phenylhydrazine for a period of 7 days. The Echuramooli Leaf Chooranam was administered at the various dose levels of 50 mg/kg, 100 mg/kg and 200 mg/kg orally to the animals for 14 days. Hb concentration, RBC count, PCV, MCH, MCHC were analysed as indices of anaemia. Phenylhydrazine significantly decrease the haematological parameters. After 14 days of treatment with trial drug Echuramooli Leaf Chooranam at the dose level of 200 mg significantly reverse the above parameters and turn towards the normal value. This result supports the traditional use of Echuramooli Leaf Chooranam in the treatment of anaemia.
Keywords: Haematinic activity, *Aristolochia indica*, iron deficiency anaemia, phenylhydrazine, Echuramooli Leaf Chooranam.

INTRODUCTION

Iron is the main component of Haemoglobin which is responsible for transporting oxygen, myoglobin in muscles and part of many enzymes which are involved in cellular processes, respiration and cell division. Anaemia is defined as the reduction of haemoglobin below the normal limit and is the most common disorder of the blood. They are usually due to iron deficiency. Recently, the World Health Organization estimated that anaemia affects one-quarter of the world's population and is concentrated within preschool age children and women[1]. Iron deficiency affects a significant part, and often a majority, of the population in nearly every country in the world[2]. Iron deficiency is estimated to be the most common cause of anaemia worldwide and is particularly prevalent in developing nations in Africa and Asia[3]. Prevalence of anaemia in all the groups is higher in India as compared to other developing countries[4]. It has serious consequences for the health and well-being as well as social and economic impacts of India. Untreated iron deficiency anaemia can become severe enough to interfere with daily life. From the above scenario, we are in a critical need to control anaemia. In this connection, a search for cheap, easily available and efficacious haematinic drugs in the modern world is going on. Drugs from the plant material are generally believed to be more effective and having fewer side effects compared to modern synthetic medicines. Knowledge of iron deficiency and its treatment in Siddha System of medicine dates back from time immortal. Many plants in Siddha system of medicine are known to have remarkable effects in treating anaemia. One such medicine is "Echuramooli Leaf Chooranam" (leaf of *Aristolochia indica* Linn.) belonging to the family Aristolochiaceae commonly known as birthwort and snakeroot. It is a perennial climber shrub with a long slightly tuberous or stout root that penetrates deep into the soil. The plant is distributed in all the provinces of India, Nepal, Bangladesh and in Srilanka[5-6]. All parts of the plant have a bitter taste and emit when crushed a characteristic sharp nauseous odour. The main constituents are aristolochic acid and aristolactams[7]. Aristoloside was reported to inhibit carcinogenesis[8]. Aristolochic acid was reported to possess various biological activities such as antibacterial, anti- inflammatory, anti-adenocarcinoma, antineoplastic[9], antitumor activities[10] and antiviral[11]. In Siddha system of medicine the plant is believed to have the power to neutralize or resist snake poison, cardio vascular disorder, intermittent fever, diarrhoea, anaemia etc[12]. The roots are extensively used to reduce dry cough and inflammation. The leaves of the plant are used as an antidote for snake poisoning. The plant is also used to treat leprosy. In view of the many health benefits of *Aristolochia indica*, we propose to study the haematinic activity of the trial drug Echuramooli Leaf Chooranam in phenylhydrazine induced anaemic rats which may serve as a beacon light in treating anaemia.

MATERIALS AND METHODS

The leaves of *Aristolochia indica* were procured from Idappadi, Salem District, Tamil Nadu, India during the month of May 2012. Then the plant was identified and authenticated by the Pharmacology experts of Post graduate department of Gunapadam, Government Siddha Medical College, Arumbakkam Chennai and botanist from Plant Anatomy

Research Centre (PARC), Tambaram, Chennai, India based on organoleptic characters, taxonomy and pharmacognostical aspect. The specimen sample was kept in the department for future reference. The collected leaves were cleaned well in water and dried in a sunshade. Then the dried leaves were kept in the iorn mortar and grounded well until the fine powder form obtained. The powder was sieved through a clean white cloth and further purified by pittaviyal method (steam boiling with milk) based on Siddha classical literature[13]. The Choornam was moistened with cow milk. One mud pot was taken and half filled with milk and water. The mouth of pot was covered and tied with white cotton cloth. Then the moistened Choornam was kept above the tied cloth. The mouth of the pot was tightly closed with another mud pot. The gap between the two pots was sealed with a wet cloth to prevent evaporation. Then the pot was put on fire and allowed to boil until the water level gets reduced in the lower pot. Then the powder was taken out from the pot and dried well and once again grounded well and stored in an airtight container. This chooranam was labelled as ELC and used for the present study.

Preparation of stock solution

The suspension of ELC in 2 % CMC (suspending agent) was prepared and this was adjusted to achieve the stock concentration of 200 mg/ml stock solution. This stock solution was administered orally by gastric intubation in animals

Animals

Albino rats of either sex weighing about 150-180 g were obtained from animal house of department of pharmacology, Vel's University, Pallavaram, Chennai, India. The rats were acclimated to standard laboratory conditions (temperature: 25 ± 2ºC) and maintained on 12 h light/dark cycle. All the rats were provided with standard food and free access to water *ad libitum*. This present study was approved by the Institutional animal ethical committee (IAEC) with approval number: XIII/VELS/PCOL/66/2000/CPCSEA/IAEC/08.08.2012).

Evaluation of Haematinic Activity

Phenylhydrazine (PHZ) induced anaemia model was used to evaluate the haematinic effects of ELC in rats[14-15]. Animals were divided in to six groups of 5 each. The first group considered as a normal control group and received distilled water. Except normal control group (Group 1), all the other groups were administered phenylhydrazine (10 mg/kg b.w) by oral administration daily for seven days to reduce the concentration of haemoglobin. Rats were considered as anaemic model if haemoglobin concentration was less than 14 g/dl[16]. Anaemic rats were then randomly grouped in to five. The second group was kept as an anaemic control received 2 % of CMC only. The third, fourth and fifth groups were administered with test drug ELC at single oral doses of 50 mg, 100 mg and 200 mg respectively. The sixth group was kept as reference group received Standard haematinic syrup (2 ml/kg p.o). All the drugs, distilled water and vehicle were administered up to 2 weeks.

Haematological investigation

Blood was collected from the animals from initial phase (pre-treatment), after one week and two weeks (during and post-treatment) by puncture of retro-orbital vein. To analyse the haematinic potential of ELC with different doses and standard drug, the haematological parameters were assessed which include Hb concentration, Packed Cell Volume (PCV), Total Red Blood Cells (TRBC), MCV (Mean corpuscle volume), MCH (Mean Cell Haemoglobin) and MCHC (mean corpuscular haemoglobin concentration) and compared with normal control and anaemic control[17].

Statistical analysis

Results of the present study were statistically analysed and expressed as mean ± SEM by using One-Way ANOVA followed by Dunnett's multiple comparison test. *P < 0.05; **P < 0.01 when compared to normal and anaemic control groups.

Table 1: Effect of phenylhydrazine (10 mg/kg, p.o. daily for 7 days) alone on hematological parameters

Para meters	Group 1 (Normal)	Group 2 (Anaemic)	Group 3 (Anaemic)	Group 4 (Anaemic)	Group 5 (Anaemic)	Group 6 (Anaemic)
Hb (g/dl)	18.64 ± 0.46	13.85 ± 0.32**	13.76 ± 0.30**	13.64 ± 0.34**	11.75 ± 0.32**	12.56 ± 0.30**
PCV (%)	52.32 ± 1.44	40.41 ± 2.21**	41.52 ± 2.11**	41.20 ± 2.79**	40.31 ± 2.02**	40.34 ± 2.55**
RBC ($x10^6$/ml)	6.88 ± 0.14	4.42 ± 0.30**	4.28 ± 0.20**	4.46 ± 0.22**	4.57 ± 0.28**	4.39 ± 0.31**
MCV (fl)	74.62 ± 2.74	85.23 ± 4.18	86.99 ± 4.36	85.20 ± 3.12	85.48 ± 4.19	88.14 ± 3.12
MCH (pg)	25.10 ± 1.56	27.18 ± 1.31	27.40 ± 1.51	29.30 ± 1.32	30.40 ± 1.20	30.18 ± 2.34
MCHC (g/dl)	32.55 ± 0.40	34.12 ± 0.6	34.22 ± 0.92	34.11 ± 1.18	34.45 ± 3.24	30.42 ± 1.74

Values are mean ± S.E.M. (Dunnet't' test). **P<0.01Vs Control N = 6

Table 2: Hematological Parameters of Rats after 14 Days Treatment with ELC

Para meters	Group 1 (Normal)	Group 2 (Anaemic Control)	Group 3 (50 mg/kg)	Group 4 (100 mg/kg)	Group 5 (200 mg/kg)	Group 6 (Heamatinic syrup)
Hb (g/dl)	18.45 ± 1.32**	9.00 ± 1.12	20.01 ± 0.74**	20.18 ± 0.80**	20.45 ± 1.95**	22.10 ± 1.88**
PCV (%)	47.38 ± 1.10	44.11 ± 1.30	44.08 ± 2.4	42.10 ± 2.2	45.00 ± 1.2	52.02 ± 1.46*
RBC ($x10^6$/ml)	4.22 ± 0.24	4.84 ± 0.30	4.86 ± 0.33	4.78 ± 0.28	4.84 ± 0.29	5.10 ± 0.28
MCV (fl)	78.38 ± 2.12**	90.56 ± 2.42	82.26 ± 2.21*	78.31 ± 1.72**	81.46 ± 2.23*	74.52 ± 2.21**
MCH (pg)	25.78 ± 2.64	33.12 ± 2.17	31.24 ± 1.96	29.86 ± 1.82	28.14 ± 1.70	28.10 ± 2.53
MCHC (g/dl)	32.26 ± 1.11	33.15 ± 1.20	33.19 ± 1.23	33.52 ± 0.65	33.28 ± 2.30	34.17 ± 2.20

Values are mean ± S.E.M. (Dunnet't' test). *P<0.05; **P<0.01Vs Control N = 6

RESULTS AND DISCUSSION

Phenylhydrazine is used for the induction of haemolytic anaemia and the study of its mechanism in many species including rats[18-19]. Phenyl free radical produced via the 2-electron oxidation of phenylhydrazine by oxyhemoglobin. This free radical binds with red cell and hemolyzes it rapidly and converts oxyhemoglobin into methemoglobin. Thus, PHZ-induced haemolytic injury seems to be derived from oxidative alterations to red blood cell proteins rather than to membrane lipids[20]. Haematological parameters of phenylhydrazine (10 mg/kg b.w) administration daily for 7 days are represented in Table 1. The RBC, Hb, and PCV of rats administered Phenylhydrazine decreased significantly ($P < 0.01$) while the MCV and MCH increased giving rise to macrocytic anaemia ($P < 0.05$). ELC at the dose of 50-200 mg/kg showed good percentage of improving in haemoglobin level, which was almost equivalent to standard treated group indicating correction of anaemia induced by Phenyl hydrazine after 14 days treatment. Treatment with ELC at the dose levels 100 and 200 mg/kg for 14 day is represented in Table 2. Significant increase in Hb ($p < 0.01$) was observed when compared to positive control and it was comparable to standard drug used in this study. Phenylhydrazine altered the haematological parameters by haemolysis characterized by decrease in haemoglobin concentration, total RBC counts and PCV on day 7. However, the haematological parameters were restored to normal range after treatment with ELC for 14 days. Effective changes were observed after one week of treatment of anaemic rats with ELC reversed the influence of Phenylhydrazine resulting to a significant ($P < 0.05$) increase in RBC, Hb, and PCV. The Hb, RBC and PCV reached near normal at the second week of the treatment. Rats treated with Phenylhydrazine (10 mg/kg/day for 7 days) resulted in a marked haemolytic anaemia characterised by decreased RBC, Hb and PCV. The main function of the RBC is the transportation of oxygen in to the tissues of the body. At such, any pathological or physiological condition that affects the RBC alters its function and this may be detrimental to the body. In this study Phenylhydrazine altered the function of RBC by haemolysis characterised by decreased levels of RBC, Hb and PCV. However, this effect was restored after one week of ELC treatment. Also the recovery was progressive such that after 1 week of continuous treatment, the Hb concentration and PCV were higher in the treated groups than in the normal control group.

CONCLUSION

In order to provide effective, safe and cheap drug and to prove the traditional claim for the treatment of anaemic conditions, the Echuramooli Leaf Chooranam at a dose of 50, 100 and 200 mg/kg was evaluated and found significantly increased the Hb, haematocrit and RBC count in anaemic rats indicating the haematinic effect. Haematinic effect was more pronounced in ELC 200 which showed its dose-dependent activity. The rapid and progressive recovery of anaemic rats responding to treatment of ELC may be due to increased erythropoiesis. However, the mechanism of action by which ELC produced its effect on increasing RBC, Hb and PCV in experimental animals need to be evaluated in a detailed scientific manner and also conducting clinical trials which are required to understand the exact molecular mechanism of action. Based on the result in can be concluded that the Echuramooli Leaf Chooranam is a good drug of choice for the anaemia.

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