WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 5.045
Volume 3, Issue 9, 686-692. Research Article ISSN 2277– 7105

HEMATINIC ACTIVITY OF HERBO MINERAL DRUG AYAVEERA CHENDURAM (AVC) IN SWISS ALBINO RATS

D. Sangeetha *[1], M. Mohamed Musthafa[2], G.Sekar[3]

*[1]PG Scholar, Dept.of Sirappu Maruthuvam, Govt. Siddha Medical College, Chennai, Tamilnadu.

[2]HOD, Dept.of Sirappu Maruthuvam, Govt. Siddha Medical College, Chennai, Tamilnadu.

[3]ASST. Lecturer, Dept.of Sirappu Maruthuvam, Govt. Siddha Medical College, Chennai, Tamilnadu.

Article Received on
21 August 2014,

Revised on 14 Sept 2014,
Accepted on 09 Oct 2014

***Correspondence for Author**
Dr. D. Sangeetha
PG Scholar, Dept.of Sirappu Maruthuvam, Govt. Siddha Medical College, Chennai, Tamilnadu.

ABSTRACT

Anemia is a serious public health challenge in India and has devastating effect on health physical and mental productivity affecting the quality of life. Ayaveera chenduram, a traditional Siddha herbo mineral formulation has been employed to treat anemia as per SR pharmacopeia. The present study has been carried out to access the hematinic activity of AVC on *invivo* rat models after the toxicological study (as per OECD guidelines 423 & 407) and proven safety up to 400mgs/kg p.o. The clinical trial has been approved by IEC [IEC NO: GSMC-CH/1/2013/014] and preclinical by IAEC [IAEC XXXIX/10/CLBMCP/2013 Dated 29.06.2013]. The hematinic activity was conducted in swiss albino rats by single intra peritoneal injection of phenyl hydrazine at a dose of 20mg/kg b.w. Drug treatment was carried out for 14 days at 2 dose level 200mg/kg p.o and 400mg/kg p.o. the trial drug was found to increase The hemoglobin count (Hb), Red blood count (RBC), packed cell volume (PCV), Mean corpuscular volume (MCV), Mean corpuscular hemoglobin (MCH) significantly ($P < 0.05$).

KEY WORDS: Anemia, Ayaveera Chenduram, Hematinic activity, preclinical study.

INTRODUCTION

Anemia is the most predominant global public health problem affecting both developing and developed countries [1]. It affects about a quarter of the world population especially children

and women of reproductive age [2]. South Asia suffers from some of the highest rates of anemia worldwide. In India more than half are anemic and around 1/3 of women of reproductive age are underweight [3]. Iron deficiency anemia is the most common of malnutrition in the world and 8th leading cause of disease in adolescence girls and women [4]. The risk factors develop anemia are diet, intestinal disorder, blood loss, pregnancy, chronic disease, inherited disorder, infection, toxic chemical and certain drugs. Adverse effects of hematinic drugs used in modern system are constipation, diarrhoea, metallic taste, abscess, anaphylatoid reaction, increased viscosity and clot formation [5]. These disadvantages of allopathic drugs have turned the focus on the safe and effective medication of siddha formulation. All the ingredients of AVC were body improving tonics, reduces tiredness (the important symptom of anemia) and thus adds value and efficacy of the drug. The empirical use of different preparation of iron in the treatment of anemia dates from ancient time [6]. The ingredients of AVC are Ayam, Veeram, Vediyuppu, Padikaram and Majal karaisalai. Ayam improves the quality of the blood, stimulate all the organ of the body and used in treating anemia [7, 8]. Manjal karisalai is a hepatoprotective purifier used in dropsy, enlargement of liver and spleen [9, 10]. Hence the challenge of the present study is to provide scientific proof that the AVC is a potential drug for its anti-anemic property in terms of hematinic action using phenyl hydrazine induced anemic rat model.

MATERIALS AND METHODS

Drug Materials

Ayam and Karisalai were purchased from Puttru Maharishi Trust Vellore. Vediyuppu and Veeram were purchased from indigenous raw drug store Chennai. Materials were authenticated by research officer [Pharmacognosy department] SCRI Chennai -106.

Preparation of Ayaveera Chendurum

Vediyuppu -1 part, Padikaram -$2_{1/2}$ parts are finely powdered, mixed together and subjected to distillation apparatus which is heated to get Vediyuppu dravagam. Then the purified Ayam and Veeram are nicely powdered together, rubbed with Vediyuppu dravagam and allowed to dry with stone mortar [kalvam] in sunlight. The same is powdered and rubbed with manjal karisalai juice for 2 days. Villai is made dried and subjected to chatty erippu as Deepagini, Kamalagini and Kadagini respectively for 3 days from 8 Am to 8 Pm each day. On being cooled seelai is removed, Chenduram is carefully secured and administrated with karisalai chooranam [11].

Chemicals and Reagents

All chemical and reagents were obtained from sigma chemical Ltd, USA. All other reagents used in the study of analytical grade were obtained from Qualigen fine chemical pvt. Ltd.

Animals

Healthy Swiss albino female rat weighing 230–250 gm were obtained from animal house department, King Institute of preventive medicine, Guindy. The animals were acclimatized for one week under laboratory condition. Rats were housed in polypropylene cages individually and fed with standard rodent pellet diet. The animals were subjected to12:12hr Light: Dark cycle under standard laboratory condition at temperature of 24-28 Celsius with relative humidity of 60% -70%. The experimental protocol was approved by the Institutional Animal Ethical Committee [IAEC/XXXIX/10/CLBMCP/2013 Dated 29.6.2013] of CL BAID METHA COLLEGE OF PHARMACY, Thuraipakkam, Chennai, Tamilnadu.

Acute Oral Toxicity

Acute oral toxicity was conducted as per OECD guidelines [organization of economic cooperative and development] 423. Three female nulliparous and non-pregnant Swiss rats were fasted overnight, but allowed water ad libitum. Since the trial drug was consider non-toxic according to text, highest dose of 2000 mg/kg p.o was administered to different group of rats and absorbed for toxicity study. The animals were absorbed individually after dosing the first 30 min periodically during the first 24 hr with special attention given during the first 4 hr, and daily thereafter for 14 days [12, 13, 14]. The animals were closely monitored and no abnormal toxic changes were observed in skin, spur, eye, mucous membrane, autonomic and central nervous system, body weight. Table (1)

Table (1): Dose Finding Experiment and Behavioral Signs of Toxicity.

GROUP	DAY
Body weight	Normal
Assessments of posture	Normal
Signs of convulsion(limb paralysis)	Absence of sign(-)
Body tone	Normal
Lacrimation	Absence
Salivation	Absence
Change in skin color	No significant color change
Piloerection	Not observed
Defecation	Normal
Sensitivity response	Normal
Locomotion	Normal

Muscle gripness	Normal
Rearing	Mild
Urination	Normal

Sub-Acute Toxicity Study

Sub-acute toxicity study was carried out by administrating AVC for 28 days as per OECD guidelines 407. Swiss albino rats of either sexes of 200-250gm body weight were divided into 3 groups of 6 rats each [Three male and three female] [12, 13, 14].

GROUP 1 - Control, received 0.025%CMC [carboxy methyl cellulose]
GROUP 2 - Treated with low dose of AVC 200mg/kg of body weight
GROUP 3 - Treated with high dose of AVC 400mg/kg of body weight

In sub-acute toxicity food consumption and body weight gain were found to be comparable throughout the dosing period of 28 days. Hematological parameters show slight increase with high dose than low dose when compared to control group. Serum HDL increased with high dose and decreased with low dose. In serum enzyme SGOT is decreased with high dose. Serum protein and albumin doesn't show significant alteration. opthalmoscopic examination, gross pathology and histopathological examination did not reveal any abnormality. However the increase or decrease in the value obtained was within the normal biological and laboratory limit.

Evalution of Hematinic Activity

Anemia Induction: Animal: Healthy Swiss albino female rat weighing 230–250 gm. Phenyl hydrazine (PHZ) is used for the induction of anaemia. Induction of anaemia in rats carried out by a single intraperitoneal 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. From 5th day to 19 day drug treatment was carried out with test drug at two dose level (200 & 400 mg/kg, p.o).

Animal Grouping

Group I – Negative control Animal injected with phenyl hydrazine 20mg/kg, i.p
Group II– Animal injected with phenyl hydrazine 20mg/kg + treated with test drug 200 mg/kg, p.o
Group III– Animal injected with phenyl hydrazine 20mg/kg + treated with test drug 400 mg/kg, p.o

Hematological Studies

At the end of the study period blood was collected by retro orbital puncture for analysis of haematological parameters. The blood samples from negative control and drug treated rats were collected into heparinized tubes after 14 days treatment and parameters such as Haemoglobin count (Hb), Red blood cells (RBC), packed cell volume (PCV), Mean corpuscular volume (MCV), Mean corpuscular haemoglobin (MCH) were analysed [15].

Table (2): Hematological Parameters in Rats After Treated With 14 Days of AVC at Two Dose Level.

PHZ	Total red cells count (×10 6 µl)	Packed cell volume (%)	MCV (fl)	MCH (pg)	MCHC (g/dl)	HB (g/dl)
Mean	5.733	33	39.67	11.67	24.83	9.667
Std. Deviation	0.5046	3.795	3.266	1.366	3.488	1.633
Std. Error	0.206	1.549	1.333	0.5578	1.424	0.6667
LOW DOSE	**Total red cells count (×10 6 µl)**	**Packed cell volume (%)**	**MCV (fl)**	**MCH (pg)**	**MCHC (g/dl)**	**HB (g/dl)**
Mean	7.167	42.83	45.5	14.5	34.17	13.17
Std. Deviation	0.7528	2.229	1.378	1.378	2.639	1.169
Std. Error	0.3073	0.9098	0.5627	0.5627	1.078	0.4773
HIGH DOSE	**Total red cells count (×10 6 µl)**	**Packed cell volume (%)**	**MCV (fl)**	**MCH (pg)**	**MCHC (g/dl)**	**HB (g/dl)**
Mean	8.283	46.83	53.33	17	39.83	15.17
Std. Deviation	0.4401	1.602	2.338	1.673	1.602	0.7528
Std. Error	0.1797	0.654	0.9545	0.6831	0.654	0.3073

Mean values are expressed as ± S.E.M. $p<0.05$ [dunnet test] n=6.

Statistical Analysis

All the data were expressed as mean± SEM. Statistical significance between more than two groups were using one way ANNOVA followed by DUNNET'S TEST. Calculations were done using graph pad prism software. The significance level was set at P value< 0.05 for all tests.

RESULTS AND DISCUSSION

In Acute oral toxicity AVC didn't induce any abnormality and mortality in rats with heavy dose of 2000mg/kg b.w. Sub-acute oral toxicity reveals the safer dose up to 400mg/kg p.o. as human therapeutic dose. Food consumption and body weight gain were found to be comparable throughout the dosing period of 28 days. The other hematological and biochemical parameters were found to increase with high dose than low dose within the normal limit. The hematinic activity showed significant increase ($P<0.05$) in Haemoglobin count (Hb), Red blood cells (RBC), packed cell volume (PCV), Mean corpuscular volume (MCV), Mean corpuscular hemoglobin (MCH) with high dose than low dose when compared to control group.

CONCLUSION

Acute and sub-acute oral toxicity study proved that the drug was non-toxic and safer up to 400mg/kg p.o. as human therapeutic dose. This safer dose of drug showed significant increase in Hb, RBC, PCV, MCV and MCH indicating the hematinic activity of traditional siddha herbo mineral drug Ayaveera chenduram (AVC).

ACKNOWLEDGEMENT

The Author Grateful thanks to the Principal, GSMC and the HOD Dept. of Sirappu Maruthuvam, Govt.Siddha Medical College, Chennai, Tamil Nadu.

REFERENCE

1. G.A. Koffuor, P.E. Dadzeasah and G.H.Sam, Haemopoitic effect of an ethanolic leaf extract of *Ipomoea in-volucrata* p.beauv in phlebotomized New Zealand White Rabbits. Journal of medical and biomedical sciences, 2012; 1(2):10-16.
2. Maurício S Leite, Andrey M Cardoso, Prevalence of anemia and associated factors among indigenous children in Brazil: results from the First National Survey of Indigenous People's Health and Nutrition. *Nutrition Journal* 2013, 12:69 doi: 10.1186/1475-2891-12-69.
3. Yarlini S Balarajan, Wafaie W Fawzi, S V Subramanian, Changing patterns of social inequalities in anaemia among +women in India: cross-sectional study using nationally representative data. *BMJ Open* 2013; 3:e002233 doi: 10.1136/bmjopen-2012-002233.
4. World Bank: World development report: Investing in health, New York, Oxford university press 1993.
5. K.D. Tripathi, Essentials of Medical Pharmacology, 4th Edition. Jaypee brother's medical publisher's pvt ltd, Pg: 583,586,594 (1999).
6. PK Sarkar[1], PK Prajapati[1], AK Choudhary[1], VJ Shukla[2], B Ravishankar[2] Haematinic evaluation of *Lauha bhasma* and *Mandura bhasma* on $HgCl_2$ -induced anemia in rats. Indian journal of pharmaceutical science. (2007)
7. K.M.Natkarunai Indian Materia Medica, 1982 revised 3rd edition, Vol 2. Pg.57
8. Dr.Anaivaari, R.Anandhan,Ph.D., Dr.Thulasimani, M.D.(Pharmaco), siddha materia medica, Translation and publication wing, Department of Indian medicine and Homeopathy, Chennai-106 . Pg 70 & 241.
9. Siddha Material Medica (Medicinal plants division), 6th edition 2002, Translation and publication wing, Department of Indian medicine and Homeopathy, Chennai-106. Pg.231

10. K.M. Natkarunai Indian Materia Medica, 1982 revised 3rd edition, Vol 1. Pg.1291.
11. The pharmacopoeia of siddha research medicines publisher - GD.Naidu charities, President Hall. Pg.16 (1978).
12. Paris: Organisation for Economic Co-operation and Development; Acute oral toxicity, acute oral toxic class method guideline 423(2002)
13. Chandra P, Sachan N, Ghosh AK, Acute and sub-chronic oral toxicity studies of a mineralo- herbal drug Amlena on expremential rats. Int J Pharm Res Innnov, 1, 15-8 (2010).
14. Rajesekaran et al, The study on Acute and sub- acute toxicity and haematinic activity of nimilai chenduram (siddha formulation) In Wister rats. International journal of pharm tech Research. vol 4, no.4:1493 -1503 (2102).
15. Decie JV and Lewis SM. Practical Haematology 8th ed. ELBS, Churchill, Livingstone. 1994; 49-59.