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Research Article

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Hematinic activity of poly herbal siddha formulation *Narasinga Rasayanam* in rat models

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Abstract

Anaemia constitutes serious health problem in many countries. It is widespread public health problem, and the fourth leading cause of hospital admissions and the second factor contributing to death. Anaemia is characterized by low count of haemoglobin. The empirical use of herbal preparations in the treatment of anaemia dates from ancient times. Despite the obvious effectiveness and efficacy of iron supplementation, there are certain limitations which include gastrointestinal side effects like nausea, constipation, vomiting, and stained teeth. The present study is aimed to evaluate the haematinic effect of aqueous extract of poly herbal traditional Siddha medicine *Narasinga Rasayanam* against phenyl hydrazine induced anemic rat model. Preliminary phytochemical screening was also performed by various tests. Anaemia was induced by an oral administration of phenyl hydrazine for a period of 8 days. Red blood cell count, haemoglobin concentration, and packed cell volume were analyzed as indices of anaemia. The Mean Cell Haemoglobin, Mean Cell Volume and Mean Cell Haemoglobin concentration were calculated accordingly. The results of Phytochemical screening shows that the presence of carbohydrates, glycosides, saponins, phytosterols, phenols, flavonoids, proteins & amino acids, diterpenes, gum & mucilage and quinine and the haematinic activity result reveals that the significant ($P<0.05$) increase in the red blood cell count, haemoglobin concentration, and pack cell volume which had been originally decreased by phenyl hydrazine administration within one week of treatment. The results explored that the drug *Narasinga Rasayanam* has haematinic properties.

Keywords: Anemia; *Narasinga Rasayanam* ; Siddha medicine ; Haemoglobin ; Red blood cell.

Introduction

Anaemia is characterized by low haemoglobin count. WHO defines anaemia as Haemoglobin levels less than 13 g/dl in males & less than 12 g/dl in females. In adults, the lower extreme of the normal haemoglobin is taken as 14.0 -16 g/dl for males and 12 -14 g/dl for females. Newborn infants have higher haemoglobin level and, therefore 15 g/dl is taken as the lower limit at birth, whereas at 3 months the lower level is 9.5 g/dl. Although haemoglobin value is employed as the major parameter for determination of anaemia. The low Hb levels results in a corresponding decrease in the oxygen carrying capacity of blood[1] and other parameters such as total no. of RBCs, PCV, MCV, and MCHC. Anaemia is a widespread public health problem; it is the fourth leading cause of hospital admissions and the second factor contributing to death [1, 2].

Anemia is a condition commonly seen in developing countries because of lack of nutrition and frequent use of drugs to treat diseases. Despite the obvious effectiveness and efficacy of iron supplementation, there are certain limitations. The main limitation is the lack of compliance, especially when long-term daily administration is required [3, 1]. Gastrointestinal side effects associated with oral iron therapy included nausea, constipation, anorexia, heartburn, vomiting,

and diarrhea. These effects are generally dose-related. In addition, stools may appear darker in color in patients taking products containing iron. Other side effects associated with oral iron products included stained teeth and iron overload (hemosiderosis). Secondary hemochromatosis due to prolonged iron ingestion has been reported rarely. Stained teeth have primarily occurred following ingestion of iron liquid preparation.

Iron overload (i.e., hemosiderosis) has been reported in patients genetically predisposed, or have underlying disorders, that augment the absorption of iron [2, 4]

From ancient time, medicinal plants in the form of *Rasayanam* formulations are believed to be useful in strengthening the hematopoietic and immune system of an individual. Various researchers successfully evaluated the potential of several medicinal plants in the treatment of anemia using various experimental animal models. Siddha physicians suggested various formulations for the treatment of hematological disorders as a source of iron and other minerals.

Narasinga Rasayanam [5] is a polyherbal traditional Siddha formulation mentioned in Siddha literature which is being used for the treatment of *Paandu* (Anaemia) *Gunmam* (Peptic ulcer), 18 types of *Kuttam* (Skin diseases), *Magotharam* (Ascites) and *VaiyitruKatti* (Abdominal tumour). But the above trial drug has not so far been scientifically evaluated for its Hematinic activity. Hence an attempt has been made to evaluate the Hematinic activity of *Narasinga Rasayanam* in phenyl hydrazine induced Anemia model.

Materials and Methods

Narasinga Rasayanam [5] Preparation:

Ingredients:

Kodiveli Ver (Plumbago zeylanica)	-	560g
Serankottai (Semecarpus anacardium)	-	560g
Thannervittan Kizhangu (*Asparagus racemosus)*	-	2240g
Nerunjil (Tribulus terrestris)	-	280g
Nilapanai Kizhangu (Curculigo orchioides)	-	700g
Palm jiggery	-	875g
Honey	-	560g
Cow's Ghee	-	280g

Procurement of Raw Drugs:

The raw drugs were procured from a well reputed country shop in Parrys corner, Chennai and authenticated by Botanist, National Institute of Siddha. All the herbal ingredients were purified (detoxification) by the suitable method specified in the Siddha literature.

Method of Preparation:

Kodiveli, Serankottai, Thanneervittan, Nerunjil, Nilappanai Kizhangu were purified and dried in sun shade and then it was made into fine powder separately and finally mixed together. After that in the mixture of all herbal powder, the ghee, honey and palm jiggery were added little by little and ground in a *Kalvam* (stone mortar) until it attained waxy consistency. Then it was stored in an air tight container.

Preliminary Phytochemical Analysis:

Preliminary Phytochemical screening to find out the presence of alkaloids, carbohydrates, glycosides, Phytosterols, Saponins, Phenols, Tannins, Flavonoids, Diterpenes, Proteins and Amino acids, gum & mucilage and quinone were analyzed by using standard procedures.

Experimental Animals:

Male Albino rats (250±25 g body weight) were used for this study. The animals were obtained from animal house, TANUVAS, Madhavaram, Chennai. 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 cycles. Animals had free access to commercial brand rat pellet diet and water given *ad libitum*. The protocol of the experiment was approved by the Institutional Animal Ethical Committee(IAEC) Ethical Committee approved Number: IAEC/XLIX/14/CLBMCP/2016 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. The studies were conducted according to the guidelines of CPCSEA.

Acute toxicity studies:

Acute toxicity studies were conducted to determine the safe dose by staircase method. The overnight fasted rats were orally administered with *Narasinga Rasayanam* suspended in 0.5% lukewarm water 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 [6,7].

Haematinic activity:

Animal treatment and experimental design:

A total of 30 male albino rats were used for this experiment. Six rats were kept as normal control group (Group1), while 24 rats were made anaemic by oral administration of Phenyl hydrazine (10mg/kg body weight) daily for 8 days. Rats that developed anaemia with haemoglobin concentration <14 g/dl were recruited for the study. Anaemic rats randomly divided into 4 groups (Group II to Group V) and treated as follows.

Group I : Vehicle control : Received only lukewarm water

Group II: Anaemia Control : Received CMC (1ml) daily

Group III: Test Group I :Received *Narasinga Rasayanam* orally 200mg/kg/day

Group IV: Test Group II :Received *Narasinga Rasayanam* orally 400mg/kg/day.

Group V: Test drug control :Received Standard haematinic syrup orally 2ml/kg/day for the entire treatment period.

The treatment was continued for 2 weeks. Blood collected from the retro orbital vein of experimental animals after an overnight fast and after 1 and 2 weeks of treatment with *Narasinga Rasayanam* was used for the determination of red blood cell count (RBC), Haemoglobin (Hb) concentration, and Packed cell volume (PCV). The Mean cell Volume (MCV), Mean cell haemoglobin (MCH) and Mean cell Haemoglobin Concentration were calculated.

Statistical analysis

Experimental data was analyzed using analysis of variance (ANOVA) and Dunnet"s "t" test to determine significant difference between means. The statistical analysis system (INSTAT-V3) package was used for this analysis.

Results

Preliminary phytochemical screening

The preliminary phytochemical analysis of aqueous extracts of *Narasinga Rasayanam* indicated the presence of carbohydrates, glycosides, saponins, phytosterols, phenols, flavonoids, proteins & amino acids, diterpenes, gum & mucilage and quinone. (Table. No: 1).

Table. No: 1: Phytochemical Screening of *Narasinga Rasayanam:*

S.No	Phytochemicals	Test Name	H_2O ext.
1	Alkaloids	Mayer's test	Absent
		Wagner's test	Absent
2	Carbohydrates	Molisch's test	Absent
		Benedict"s test	Present
3	Glycosides	Libermann Burchard's test	Present
4	Saponins	Froth test	Present
		Foam test	Present
5	Phytosterols	Salkowski's test	Present
6	Phenols	Ferric chloride test	Present
7	Tannins	Gelatin test	Absent
8	Flavonoids	Alkaline Reagent test	Present
		Lead acetate test	Present
9	Proteins and Amino acids	Xantho proteic test	Present
10	Diterpenes	Copper acetate test	Present
11	Gum & mucilage	Extract + alcohol	Present
12	Quinone	NAOH + Extract	Present

Acute toxicity studies

Studies revealed the aqueous extracts of *Narasinga Rasayanam* to be safe at doses of 2000mg/kg body weight.

Animal studies for anti-anemic activity:

Hematinic activity was evaluated by using phenyl hydrazine induced anemia in an animal model. Hematological data on 9th day 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. No: 2 present the hematological data of blood sample withdrawn on day 9th. Comparison of Groups II III, IV, and V with Group I indicated a significant reduction in Hb content below 14g/dl. In Group V animals received only hematinic syrup 2ml/kg/day for the entire treatment period. Further, the test groups, Groups III and IV were subjected to treatment of *Narasinga Rasayanam* at the doses of 200 mg/kg and 400 mg/kg body weight orally for 14 days respectively, 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. Therefore, data obtained as a result of this investigative study on day 7th and 14th showed that all the hematological parameters were restored to normal as shown in Table. No: 3 and Table .No: 4. This indicates that *Narasinga Rasayanam* is an effective drug and possesses good haematinic activity.

Table. No: 2. Effect of *Narasinga Rasayanam* on Hematological parameters of Hb concentration, RBC count and PCV, MCV, MCH, MCHC after 8 days treatment with Phenyl hydrazine

Parameters	Group I	Group II	Group III	Group IV	Group V
Hb	17.21±0.45	11.2±0.25**	12.65±0.65**	12.15±0.35**	12.04±0.31**
PCV	50.10±1.45	38.76±2.32**	40.11±1.82*	42.10±2.21	39.17±2.72**
RBC	5.85±0.15	4.52±0.22**	4.53±0.24**	4.73±0.20*	4.51±0.32**
MCV	69.11±2.65	81.34±3.3	85.45±0.11*	84.04±3.14	88.40±3.60*
MCH	21.74±1.42	25.28±1.30	28.18±1.64*	30.11±1.11**	30.23±2.42**
MCHC	35.62±0.34	32.10±0.4	34.01±0.24	33.61±1.45	30.08±2.20

Values are mean ± S.E.M. (Dunnet"s test).* P <0.05; **P <0.01 Vs Control N=6

Table.No: 3. Hematological findings of Hb concentration, RBC count and PCV, MCV, MCH, MCHC after 7 days treatment with *Narasinga Rasayanam*

Parameters	Group I	Group II	Group III	Group IV	Group V
Hb	17.10±1.25**	11.3± 1.1	13.63±0.47	14.45±1.23	15.01±1.40**
PCV	51.01±1.33**	40.12±2.5	43.20±2.21	45.24±2.1	54.17±2.32**
RBC	5.15±0.25	4.13±0.51	5.15±0.22	5.25±0.12	6.45±1.01**
MCV	75.15±2.35	74.23±2.42	78.15±1.15	75.42±2.50	75.33±2.51
MCH	22.23±1.46*	28.12±1.40	22.22±1.4*	23.01±1.3	22.25±1.32*
MCHC	34.22±2.87	31.14±1.14	28.62±1.7	29.76±1.4	33.15±1.3

Values are mean ± S.E.M. (Dunnet"s test).* P <0.05; **P <0.01 Vs Control N=6

Table. No: 4. Hematological findings of Hb concentration, RBC count and PCV, MCV, MCH, MCHC after 14 days treatment with *Narasinga Rasayanam.*

Parameters	Group I	Group II	Group III	Group IV	Group V
Hb	16.1±1.47**	9.78± 1.15	14.10±0.65*	15.14±0.56**	16.05±1.35**
PCV	44.35±1.30	40.01±1.86	41.14±3.03	42.65±2.1	52.17±1.82**
RBC	5.23±0.25**	3.26±0.51	4.01±0.36	4.82±0.35*	5.23±0.51**
MCV	72.11±2.07	87.55±2.84	80.15±2.41	75.10±1.60**	73.21±2.55**
MCH	29.23±2.84	32.18±2.25	30.01±1.47	28.54±1.73	29.64±2.16
MCHC	34.25±2.87	31.04±1.33	32.01±1.33	32.89±0.65	34.11±3.01

Values are mean ± S.E.M. (Dunnet"s test).* P <0.05; **P <0.01Vs Control N=6

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 [8]. 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 [9, 10]. 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 [11-14].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 *Narasinga Rasayanam* resulted in increased values of these parameters. The Hb concentration was found to be higher than the positive control animals. This indicates that this polyherbal formulation *Narasinga Rasayanam* has 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. The hematinic property of *Narasinga Rasayanam* may be attributed to the presence of the above mentioned phytochemicals as they are known to exert anti-oxidant activity as reported in the literature. From this study, it can be established that the hematinic potential of *Narasinga Rasayanam* can be explored for further research in developing a novel herbal delivery system.

Conclusion

The collective data of this study revealed that *Narasinga Rasayanam* has considerable hematinic activity as shown in PHZ-induced anemia in experimental rat model indicating the use of this *Siddha* formulation for the treatment of *Paandu* (Anaemia). Further studies are required to precisely ensure maximum bioavailability and therapeutic efficacy.

References

1. Ross and Wilson: Anatomy and Physiology in health and illnes Elsevier Churchill Livingstone London New York Oxford, 9th edition: 60-75.
2. Gerard J. Tortora: Principles of Anatomy and Physiology Sandra Reynolds Grabowski Purdue University, 10th edition: 634-641.
3. Roger & walker: Clinical Pharmacy & therapeutics. 3rd edition: 725-740.
4. Joseph T. Dipiro: Pharmacotherapy A pathophysiologic Approach 6th edition :1799-1800 & 1805-1829.
5. Vasudeva Sasthiri.K, Dr.Venkada Rajan.S. Sarabenthira Vaithiya Muraigal-Gunma RogaSigithchai, Third edition, Saraswathi Mahal,Pg.64,65.
6. Ghosh MN. Toxicity Studies, Fundamentals of Experimental Pharmacology. 2nd ed. Calcutta: SC and RC Book Agencies; 1984. p. 154.
7. Mir AH, Sexena M, Malla MY. An acute oral toxicity study of methanolic extract from *Tridex procumbens* in Sprague Dawley's rats as per OECD guidelines 423. Asian J Plant Sci Res 2013;3(1):16-20.

8. Rifkind RA, Danon D. Heinz body anemia – An ultrastructural study. I. heinz body formation. Blood 1965;25:885-96.
9. Suzuki Y. The development of a sensitive micronucleus test: An *in vitro* method using cultured bone marrow cells. Jikeikai Med J 1985;100:709-19.
10. Adamson JW, Longo DL. Hematologic alterations. In: Braumwald E, Fauci AS, Kasper DL, Hauser SL, Longo DL, Jameson JL, editors. Harrison's Principles of Internal Medicine. New York: McGraw Hills; 2001.
11. Biswas S, Bhattacharyya J, Dutta AG. Oxidant induced injury of erythrocyte-role of green tea leaf and ascorbic acid. Mol Cell Biochem 2005;276 (1-2):205-10.
12. Turashkar A, More S, Sheikh R, Gadhpayle J, Bhongde SI. Inhibitory potential of *Picrorhiza kurroa* royle ex. benth extracts on phenylhydrazine induced reticulocytosis in rats. Asian J Pharm Clin Res 2013;6(2):215-6.
13. Ndem JI, Otitoju O, Akpanaiabiatu MI, Uboh FE, Uwah AF, Edet OA. Haematoprotective property of *Eremomastax speciosa* (Hochst.) on experimentally induced anaemic wistar rats. Ann Biol Res 2013;4(6):356-60.
14. Waghmare AN, Tembhurne SV, Sakarkar DM. Anti-anaemic potential of *Murraya koenigii* fruit extracts in phenylhydrazine induced anaemic rats. IJAPR 2015; 6(05):124-7.

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