

The Study On Acute, Sub-Acute Toxicity And Hematinic Activity Of Nimilai Chenduram (Siddha Formulation) In Wistar Rats.

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Abstract: The Nimilai Chenduram is a siddha formulation used widely as a traditional medicine to treat anemia in Indian system of medicine. However due to increased speculations about the safety profile of siddha formulations and lack of scientific validation, this study has been carried out to assess the effect of Nimilai Chenduram on *in vivo* rat models after carrying out the toxicological tests. After 14 days of drug treatment through oral route to 6 wistar rats of either sex from control and test drug treating groups, the trial drug has been found to increase the hemoglobin (Hgb) count ($p < 0.001$) and red blood cells (RBC) count ($p < 0.05$) indicative of the hematinic activity. Further it did not alter liver and kidney functions assessed by their markers. The values of packed cell volume ($p < 0.01$), mean corpuscular volume ($p < 0.01$) and mean corpuscular hemoglobin ($p < 0.01$) were also significantly increased when compared with control treated animals.

INTRODUCTION

Anemia possess severe health burden in India and is one of world's major public health problems [1]. It is characterized by the hemoglobin count lower than 13 g/dl in male and 12g/dl in female according to World Health Organization. The anemia is endemic among pregnant women in India and its prevalence ranges from 33 % to 89 %, even the iron folic acid tablets doesn't decrease this prevalence due to lack of trained health care staffs, failure to reach target people, etc, [2] and severely affects the pregnancy outcomes [3].

Anemia is disastrous and is an independent risk factor for Cardio vascular disease outcomes [4] in patients with chronic kidney diseases [5] and severe heart failure [6]. It worsens both the systolic and diastolic chronic heart failure (CHF) in which among 46.8 % of anemic people died with CHF [7]. Anemia also occurs in cancer by the disease process itself or by the treatment and it has been considered as a strong predictor and an independent prognostic factor for the survival of

cancer patients [8]. Erythropoiesis stimulating agents which have been used to treat anemia in cancer patients indeed has shown to reduce survival rate of cancer patients according to a meta-analysis conducted on cancer patients by [9]. Keeping in mind with the severity of anemia in different disease process, this study has been conducted on a traditional formulation called Nimilai Chenduram from Siddha system of medicine.

The haematinic activity has been previously reported in *Hibiscus cannabinus* [10], *Sphenocentrum jollyanum* [11], *Brillantasia nitens linadu* [12]. However Nimilai Chenduram has long been used in clinical practice from the traditional medicine for the treatment of anemia for its excellent safety profile and efficacy; it is not scientifically reported elsewhere so far. This study is conducted to scientifically validate not only to prove their effectiveness but also to raise confidence and awareness in wider sections of populations about the gift of traditional medicine. Hence preliminary physical, chemical properties

were analyzed followed by the toxicological, hematological, hematinic examination in wistar rats of 14 days drug treatment.

MATERIALS AND METHODS:

Experimental animals

Colony inbred animals strains of wistar rats of either sex weighing 200 – 250 g were used for the pharmacological and toxicological studies. The animals were kept under standard conditions 12:12 (day/night cycles) at 22 degree Celsius room temperature, in polypropylene cages. The animals were fed on standard pelleted diet (Hindustan Lever Pvt Ltd., Bangalore) and tap water ad libitum. The animals were housed for one week in polypropylene cages prior to the experiments to acclimatize to laboratory conditions. The experimental protocol was approved by the institutional ethical committee (IAEC).

Preparation of the test drug (Nimilai Chenduram)

The purified Nimilai (pyrite) and Gandagam (sulfur) were purchased from the indigenous raw drug store, Chennai. The drug was prepared by sastric preparation described in the siddha text books [13]. Briefly, the Nimilai of 35 gm and Gandagam of 8.5 gm were mixed and grinded into a fine powder in the pestle and mortar and added the few ml of the aqueous extract of *Thespesia populnea* leaves to form into a thick paste. This thick paste was allowed to dry and placed in the centre of the paste prepared by grinding the *Thespesia populnea* leaves alone. Then it was covered with 8 folds of clay cloth and placed it in the cow dung cake which weighs 4 times more than the above product and heated. Finally after completion of the process, end product Nimilai Chenduram was obtained.

Experimental Design

The test drug was evaluated for the physical and chemical properties before the in-vivo evaluation for the hematological, toxicological and hematinic effects.

Physicochemical and phytochemical evaluation:

The analysis of physical properties as loss on drying at 100 degree Celsius, total ash value, water soluble and acid insoluble ash value, pH at 10% aqueous solution values of the test drug Nimilai Chenduram were determined as per WHO guidelines [14].

Chemical properties:

For the chemical evaluation, 5 gm of the test drug is boiled in 50 ml of distilled water for 10 minutes, cooled, filtered and then made up to 100 ml with

distilled water. The resultant extract was used for volumetric analysis for the determination of presence of metals, minerals and toxic chemicals and also analyzed for the presence of phytochemical such as alkaloids, tannins, etc,

Experimental design and procedure for In vivo study:

Acute oral toxicity study

Acute oral toxicity was conducted as per the OECD guidelines (Organization of Economic Cooperation and Development) 423 (Acute Toxic Class Method). Briefly, the Wistar albino rats of either sex weighing 200 – 250 g animals were fasted overnight, but allowed water ad libitum. Since the test drug is relatively non toxic in clinical practice in traditional medicine the highest dose of 2000 mg/kg PO was used in this study as per OECD guidelines. The animals were observed closely for behavioral toxicity, if any by using FOB (Functional observation battery).

Repeated oral toxicity study

The animals were divided into two groups of each with six animals.

Group I: Control animals received 1% Carboxy Methyl Cellulose (CMC) of 10ml/kg PO for 14 days

Group II: Aqueous suspension of Nimilai Chenduram (NC) 28.5 mg/kg PO for 14 days

Preparation of the test drug and controls:

The CMC is used as a vehicle for carrying the test drug for the administration to animals through oral route. The control animals were administered with CMC alone.

The dose level of 28.5 mg/kg PO of the test drug were administered to the group of six animals of either sex weighing 200-250 mg for 14 days to get additional information regarding the toxicity profile of the drug. After 14 days of treatment, blood samples were collected into non heparinized tubes and centrifuged for 10 min at 3000 rpm to separate the serum. The serum was used to evaluate the liver enzyme function such as aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), etc, [15] and kidney parameters such as urea and blood urea nitrogen (BUN), other biochemical parameters such as blood glucose and cholesterol levels were also analyzed.

Hematological studies:

The blood samples from control and drug (28.5 mg/kg PO) treated rats were collected into heparinized tubes after 14 days treatment and parameters such as hemoglobin count, red blood cells (RBC), white blood corpuscles (WBC), differential leucocytes counts, packed cell volume (PCV), Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), etc, were analyzed [16][17].

Statistical analysis:

The statistical significance between the groups was analyzed by means of a student paired t test and the results of the study were expressed as mean \pm SD. A probability level of less than 0.05 was considered significant.

RESULTS AND DISCUSSION

The physical parameters such as loss on drying at 100 degree Celsius, acid and water soluble ash values, pH at 10 % aqueous solution was listed in table 1. Following micronutrients such as copper, zinc, magnesium, iron, etc and phytochemical constituents such as tannic acid, oxy quinolone, epinephrine and pyrocatechol were observed in volumetric analysis. The presence of phytochemical may be from the Thespesia populnea leaves which are used in the preparation of the test drug Nimilai Chenduram. Further as

evidenced from volumetric analysis, the toxic metals such as lead, mercury, aluminum and minerals such as arsenic were absent in the Nimilai Chenduram preparation thus making it safer for animal trials.

The drug did not induce any mortality, abnormalities, etc in rats used for acute toxicity studies with the heavy dose of 2000 mg/kg PO after the initial 24 hr study period, thus making it as a safer drug to continue with sub acute toxicity studies. In sub acute oral toxicity studies, the hematological parameters such as levels of leucocytes were not significantly altered than that of control treated rats (table 2). Both the hemoglobin ($p < 0.001$) and RBC ($p < 0.05$) counts were increased significantly. Further the function of liver and kidney were also not altered (Table 3) because the enzymes of the liver such as SGOT, SGPT, alanine phosphatase levels were not significantly changed than the control treated rats. The urea and blood urea nitrogen levels which were the indicators of kidney function were also remained unchanged than the control. The same is observed in glucose and cholesterol levels (table 3).

As expected, the values of packed cell volume ($p < 0.01$), mean corpuscular volume ($p < 0.01$) and mean corpuscular hemoglobin ($p < 0.01$) were significantly increased when compared to control treated rats as listed in table 4.

Table 1: Physical Properties of Nimilai Chenduram

S. No	Parameters	Results
1	Loss of drying @ 100 Degree Celsius	0.41 %
2	Total ash value	46.47 %
3	Water soluble ash	3.5 %
4	Acid insoluble ash	43.14 %
5	pH at 10 % aqueous solution	3.04 %

Table 2: Effect of Nimilai Chenduram on hematological parameters after 14 days repeated dosing (28.5 mg/kg PO) in wistar rats

Groups	Hb (gm)	% ±	RBC (milli/cu.mm)	WBC (cells/cu.mm)	Differential leukocyte count (%)		
					Lymphocytes	Monocytes	Granulocytes
Control	11.08 ± 0.348		5.20 ± 0.347	5583.33±334.94	77.00±3.89	5.50±1.04	15.66±3.07
NC 28.5 mg/kg PO	14.52±0.20 **		7.00± 0.13 *	5443.±349.23 ns	78.33± 4.32 ns	6.00± 2.28 ns	17.5 ± 4.27 ns

N = 6; values are expressed as mean \pm S. D followed by students paired T test * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, Ns - Non significant as compared to control. NC- Nimilai Chenduram, Control- Treated with carboxy methyl cellulose.

Table 3: Effect of Nimilai Chenduram on biochemical markers of liver and kidney, Glucose and Cholesterol levels after 14 days repeated oral dosing in wistar rats.

Groups	ALP (IU/L)	AST (IU/L) SGOT	ALT (IU/L) SGPT	Urea (mg/100 ml)	BUN (mg/100 ml)	Glucose mg/dl	Cholesterol Mg/dl
Control	29.73 ± 0.3929	43.89 ±1.906	25.48± 2.93	16.38± 2.12	7.52± 0.84	83.57± 6.97	57.64± 4.54
Test drug NC 28.5 mg/kg PO	31.85± 0.2074 ns	48.03 ± 2.4 ns	24.75 ± 0.88 ns	16.93 ± 0.79 ns	7.92 ± 0.37 ns	89.57 ± 12.72 ns	64.07 ± 2.73 ns

N = 6; values are expressed as mean ± S. D followed by students paired T test * p < 0.05, ** p < 0.01, *** p < 0.001. Ns - Non significant as compared to control. NC- Nimilai Chenduram, Control- Treated with carboxy methyl cellulose.

Table 4: Evaluation of Hematinic activity of Nimilai Chenduram

Groups	PCV %	MCV	MCH
Control	40.14 ± 1.16	90.02 ± 0.47	22.83 ± 0.11
NC 28.5 mg/kg PO	44.52 ± 0.49*	96.55 ± 1.19 **	29.50 ± 0.42**

N = 6; values are expressed as mean ± S. D followed by students paired T test * p < 0.05, ** p < 0.01, *** p < 0.001, Ns - Non significant as compared to control.

NC- Nimilai Chenduram, Control- Treated with carboxy methyl cellulose. PCV – Packed cell volume, MCV- Mean Corpuscular Value, MCH- Mean corpuscular Hemoglobin.

The data from this investigation reveals that the hemoglobin contents and RBC were significantly increased which may be due to the presence of iron, copper, etc in the trial drug known to induce erythropoiesis and the iron from the compound has been incorporated into the hemoglobin. The literature reveals that increasing hemoglobin from <12g/dl in patients with class IV heart failure has shown to decrease the hospitalization and increase the functional status of the heart [18]. Further, the iron containing supplementations can be used to increase the MCV values of RBC in anemic conditions caused due to poorer intake of iron containing foods [19].

The higher percentage of total ash value (46.47%) and acid insoluble ash value (43.14%) are due to the presence of metals and minerals but not due to presence of contaminants like silicates, sulphides, etc which were confirmed absent in the volumetric analysis. The presence of micronutrients like iron, copper, zinc has commendable role in the biological activity. These micronutrients compete with each other for absorption if given in 1:1:1 ratio [20], but from the results of our study we can say that these micronutrients in the formulation were indeed present in desired unequal quantity such that the competition is unlikely to take place, otherwise, Hgb, RBC, Hct values would not have increased.

For instance, copper is also essential as Fe for erythropoiesis [21] and its deficiency leads to Anemia due to decreased intestinal absorption of Fe in male and female rats which in turn resulted in reductions in number of blood variables like low blood Hgb, Hct and serum Fe usually seen in Fe deficient rats [22]. The copper supplementation in defined doses corrects the resistance to erythropoietin treatment in hemodialysis patients with anemia [23].

The ability of the drug to increase the hematocrit values from baseline has a good clinical significance. As already reported, the increase in hematocrit values from 26.3 to 34.7 %, 23.6 to 47.2 % decreases the occurrence of Left Ventricular Myocardial Infarction in individuals with Chronic kidney diseases and anemia [24]. However the results were encouraging, this study is a preliminary investigation of the potential of Nimilai Chenduram, it did not evaluate the same in anemia induced rat models which has been planned for future study, though it has not induced toxicity as evidence by liver and kidney marker analysis, the distribution of different micronutrients across the body also has to be elucidated in detail in future studies.

CONCLUSION

The drug evaluated in this study has no toxicity and has significantly increased the levels of hemoglobin, red blood cells, packed cell volume, Mean corpuscular volume, mean corpuscular hemoglobin, etc. Hence after successful evaluation of above parameters, it can be used as safer hematinic for treating anemia in pregnant, cardiovascular and cancer patients.

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