Research article

Pharmacological evaluation for haematinic activity of *Siddha* formulation *Lavana Dravagam* in rat model

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ABSTRACT

Introduction and Aim: Anaemia is the most common deficiency disorder among people of all age groups, known as Paandu in the Siddha system of medicine. The test drug siddha poly mineral formulation Lavana Dravagam mentioned in Siddha literature kannukamiyam ennum vaithiya segaram, has been used for pandu (anaemia). Dravagam is a form of internal medicine, processed by distillation method and is also referred as Pugai neer, Shakthi neer, Dravaga neer. The study aimed to evaluate the haematinic activity and efficacy of poly mineral Siddha formulation Lavana Dravagam against phenylhydrazine induced anaemic albino Wistar rat model.

Materials and Methods: The animals were selected and divided into four groups (I, II, III, and IV) of six rats (n=6) each. Anaemia was induced by an oral administration of phenylhydrazine (single dose of 10 mg/kg per oral for 8 days). Group I served as normal control and Group II received standard drug hematinic syrup in suspension form at dose 2 mL/kg. Groups III, IV received the formulated oral indiffusible mixture of Lavana Dravagam at a dose of 0.02ml to 0.03ml/kg respectively. RBC, Hb, PCV, MCV, MCH, were analyzed as indices of anaemia. The mean corpuscular volume, mean corpuscular Hb, and mean corpuscular Hb concentration were calculated.

Results: From the literature evidence, acute toxicity evaluation and pharmacological studies, the drug Lavana Dravagam is found out to have hematinic activity. This study reveals that there is significant ($P<0.05$) increase in RBC count, Hb level, and PCV by administering phenylhydrazine within one week of treatment.

Conclusion: It could be concluded that the drug Lavana dravagam will have promising effects in the management of anaemia (Paandu).

Keywords: Distillation; Lavana Dravagam; Siddha formulation; Paandu (anaemia).

INTRODUCTION

Siddha medicine is essentially one of the most ancient types of treatment branch wholly based on biotic medium; natural, herbal (*Thavaram*), inorganic (*Thathu*), and animal products (*Jeevam*) as innovative medicinal resources (1). In the usage of metals, minerals, and other chemicals, this system was far more advanced than other systems. The *Thathu* drugs are categorized into 1. *Uppu* (Salts) (water-soluble inorganic substances or drugs produce vapor when it is exposed to fire), 2. *Pashanam* (Arsenicals) are drugs that should not be dissolved in water but produce the vapor when fired, 3. *Ulogam* (Heavy metals). Among these, *Dravagam* type is one of the internal medicines, which is processed by distillation method. The word *Dravagam* means "that dissolves, liquefies". It is also used in the field of medical alchemy. It is known by various names which include *Pugai Neer*, *Shakthi Neer*, *Dravaga Neer*, etc., This *Dravagam* does not deteriorate with the lapse of time (2).

Anemia is the most common deficiency, and it is described as decreasing in haemoglobin level and oxygen carrying capacity below the ordinary range. It can be determined by the reduction in hemoglobin level to less than 13 g/dl in men and 12 g/dl in women (3). The rate of maturation of red blood cells entering the blood from the red bone marrow does not keep pace with the rate of haemolysis in anaemia (4). Iron is the major component of haemoglobin, which transports oxygen, and myoglobin in muscles as well as a part of different enzymes involved in cellular functions, respiration, and cell division (5). Low hemoglobin (Hb) levels reduce the oxygen-carrying capacity of blood (6) and other parameters such as total red blood cell (RBC) count, mean corpuscular volume (MCV), packed cell volume (PCV), mean corpuscular hemoglobin (MCH), and MCH concentration (7).

Anaemia is a common nutritional deficiency disorder in the world. WHO defines anaemia is the condition in which the HB content of blood is lower than normal because of a deficiency of one or more essential nutrients (8). WHO has estimated that more than 2 billion people worldwide suffer from anaemia with 50% attributed to iron deficiency (9). 44% of adolescent girls are affected by anaemia in the rural areas in Tamil Nadu. Among these 2.1% are severe

and 6.3% are moderate and 36.5% are mild (10). Identifying a novel drug of choice for anaemia is a challenge to the researchers. Rather than selecting the drug of choice, the factors such as the nature of the formulation, its palatability and quick therapeutic action must be considered. Many formulations that are more beneficial and clinically effective are described in traditional siddha literature.

Among those formulations, *Lavana Dravagam* is one of the mineral-based siddha formulation which is mentioned in the Siddha literature *Kannusamiyam ennum vaithiya segaram*, page no. 158 and the drug is indicated for *Paandu* (Anaemia), *Gunmam* (ulcer), *Surakkatty* (enlargement of the spleen) *Soothagakkatty* (Polypus uteri, internal abscess; 11). The ingredients of this drug possess hematinic action. But the above trial drug has not so far been evaluated for the said activity. From the literature, it is evident that most of the ingredients of this drug possess hematinic action which is responsible for the therapeutic claim mentioned in the literature. Hence the drug has been chosen for this study to validate its pharmacological (hematinic) and analytical studies.

MATERIALS AND METHODS

Lavana Dravagam preparation

Lavana dravagam was prepared using the ingredients *Vediuppu* (potassium nitrate) - 6 *edai; padigaram* (Alum) - 3 *edai; navacharam* (ammonium chloride) - 1 ½ *edai; kariuppu* sodium chloride - 1½ *edai*. The raw materials were purchased from a local raw drug store in Parris, Chennai and authenticated by Dr. S. Sivakumar, Head of the Department, National Institute of Siddha, Chennai (Certificate no: Gun/Aut/012/21). The raw drugs were purified as mentioned in Siddha literatures, purified salts were ground well and transferred to the *Valaiyanthiram* made of earthen distillation set up and intensely heated. On heating the salts decomposed completely releasing the acidic fumes and got condensed at the condenser submerged in cold water and was collected in the receiver vessel kept adjacently, after which it was stored in a glass container (12).

Biochemical analysis

The investigational drug was subjected to biochemical screening for the presence of various minerals by using standard procedure (Table 1).

Experimental animals

Albino rats (180- 220 g body weight) of both sexes were procured from the animal house of The Tamil Nadu Veterinary and Animal Sciences University, Madhavaram Milk Colony, Chennai, and the study was conducted at National Institute of Siddha, Chennai. They were divided in groups of three in a polypropylene cage at a suitable temperature (25±2°C), relative humidity (55±5%) and 12 hrs/12 hrs light-dark cycles. The animals are provided with standard rodent pellets and water *ad libitum*. Animals were acclimated to laboratory settings. Prior to starting the experiments. The protocol of the test was accepted by the Institutional Animal Ethical Committee (IAEC) Ethical Committee approved Number: NIS/IAEC-1/03/30092020/03. The studies were conducted based on the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India.

Acute toxicity studies

Acute oral toxicity studies were evaluated to analyse the safety and efficacy by using the staircase method. Fasted rats were administered orally with *Lavana dravagam* at a test dose of 2000 mg/kg body weight. We observed the rats after one hour and then the next four hours, regularly once in four hours and then daily. Again, we observed animals surviving for the next 14 days. (13, 14).

Haematinic activity

Totally 24 female and male albino rats were used for this study. Wistar albino rats were grouped into four (n=6). Anaemia was induced by phenylhydrazine (single dose of 10 mg/kg per oral for 8 days) in the animals. Group I served as normal control and Group II was given the standard drug for haematinic activity in suspension form at dose 2 ml/kg. Groups III, IV administered the formulation of an oral undiffuse mixture of *Lavana dravagam* at a dose of 0.02 ml to 0.03 ml/kg respectively.

Group I: Anaemia control: received water
Group II: Standard drug: received hematinic syrup orally 2 ml /kg/day
Group III: Test drug (dose I): received *Lavana dravagam* 0.02 ml/kg
Group IV: Test drug (dose II): received *Lavana dravagam* 0.03 ml/kg

After that rats that developed anaemia with haemoglobin concentration <14 g/dl were selected for this study. The treatment was given for 2 weeks. Blood is collected from the retro-orbital veins of experimental animals after 1 and 2 weeks of *Lavana dravagam* administration was used to determine RBC, Hb, PCV, MCV, and MCH levels, and the results were tabulated.

Statistical analysis

Statistical analysis of the data was done using Dunnett Multiple Comparisons Test.

RESULTS

Biochemical analysis of *Lavana dravagam* showed the presence of sulphate, chloride, aluminium, iron, zinc, ammonium, potassium and sodium (Table 1).

Haematological parameters of the animals before the treatment of Lavana Dravagam, Hb content varies

from 09.7 to 11.49 g/dl, PCV value showed highest in Group IV animals (42.11) and lowest in Group I animals (37.74). In RBC the value of animals showed a variation from 4.48 to 4.72×10^6/ml. MCV value was the highest in Group II animals (87.40 fl) and the lowest value in Group IV animals (80.14 fl). MCH values varied between 25.26 to 31.22 pg. The MCHC value in Group IV animals was the highest (36.60 g/dl) and lowest in Group II animals (31.08 g/dl) (Table 2).

Table 1: Biochemical components of *Lavana dravagam*

S.No.	Experiment	Biochemical components	Observation
1	Action of heat	Carbonate, nitrate	Absence
2	Flame test	Copper	Absence
3	Ash test	Ash test	Absence
4	Test for sulphate	Sulphate	Presence
5	Test for chloride	Chloride	Presence
6	Test for phosphate	Phosphate	Absence
7	Test for carbonate	Carbonate	Absence
8	Test for nitrate	Nitrate	Absence
9	Test for sulphide	Sulphide	Absence
10	Test for fluoride and oxalate	Fluoride and oxalate	Absence
11	Test for nitrite	Nitrite	Absence
12	Test for borate	Borate	Absence
13	Test for lead	Lead	Absence
14	Test for copper	Copper	Absence
15	Test for aluminium	Aluminium	Presence
16	Test for iron	Iron	Presence
17	Test for zinc	Zinc	Presence
18	Test for calcium	Calcium	Absence
19	Test for magnesium	Magnesium	Absence
20	Test for ammonium	Ammonium	Presence
21	Test for potassium	Potassium	Presence
22	Test for sodium	Sodium	Presence
23	Test for mercury	Mercury	Absence
24	Test for arsenic	Arsenic	Absence

Table 2: Haematological parameters of the animals before treatment of *Lavana Dravagam*

Blood parameters	(Group-I) (Anaemia control)	(Group-II) (Standard)	(Group-III) (0.2 ml/kg/p.o)	(Group-IV) (0.3 ml/kg/p.o)
Hb (g/dl)	09.7±0.24**	10.26±0.31**	10.77±0.64**	11.49±0.35**
PCV	37.74±2.31**	38.16±2.75**	40.12±1.83*	42.11±2.24
RBC(×10^6/ml)	4.48±0.21**	4.59±0.37**	4.50±0.23**	4.72±0.19*
MCV(fl)	83.34±3.2	87.40±3.58*	81.45±0.10**	80.14±3.14
MCH (pg)	25.26±1.28	31.22±2.48**	28.16±1.61*	30.20±1.10**
MCHC (g/dl)	33.10±0.3	31.08±2.21	35.01±0.23	36.60±1.45

Values are expressed as mean ± SEM; n=6; followed by the Dunnett test. * P<0.05; ** P< 0.01 vs control

Table 3: Haematological values after one week of treatment with *Lavana dravagam*

Blood parameters	(Group-I) (Anaemia control)	(Group-II) (Standard)	(Group-III) (LD-0.2ml/kg)	(Group-IV) (LD-0.3 ml/kg)
Hb (g/dl)	11.2±1.5	12.45±1.41**	11.98±0.46	12.47±1.24
PCV	39.41±2.5	48.25±2.31**	45.23±2.21	46.34±2.3
RBC(×10^6/ml)	4.24±0.51	6.43±1.01**	5.15±0.27	5.33±0.12
MCV(fl)	72.45±2.41	75.37±2.51	76.12±1.5	74.42±2.50
MCH (pg)	28.11±1.45	22.60±1.32*	22.23±1.4*	23.01±1.3
MCHC (g/dl)	32.11±1.14	32.14±1.3	28.67±1.7	29.66±1.3

Values are expressed as mean ± SEM; n=6; followed by the Dunnett test. * P<0.05; ** P< 0.01 vs control

Table 4: Haematological changes after two weeks of treatment with *Lavana dravagam*

Blood parameters	(Group-I) (Anaemia control)	(Group-II) (Standard)	(Group-III) (LD-0.2ml/kg)	(Group-IV) (LD-0.3 ml/kg)
Hb (g/dl)	10.54±1.14	12.54±1.25**	14.11±0.65*	15.21±0.57**
PCV	41.01±1.76	50.17±1.82**	46.14±2.03	48.53±2.1
RBC($\times10^6$/ml)	3.16±0.52	5.22±0.53**	5.04±0.37	6.85±0.33*
MCV(fl)	71.65±2.83	78.21±2.47**	81.18±2.41	80.12±1.62**
MCH (pg)	32.28±2.27	29.61±2.26**	30.10±1.47	28.38±1.73
MCHC (g/dl)	31.15±1.37	34.1±3.03	33.01±1.35	33.89±0.65

Values are expressed as mean ± SEM; n=4; followed by the Dunnett test. * P<0.05; ** P< 0.01 vs control

According to Table 3, the haematological value after one week of treatment with Lavana Dravagam, compared to the control, Group III and Group IV animals showed the Hb content increased. The average total red blood cell counting of rats in every concentration of the *Lavana dravagam* test groups (5.18±0.25, 5.15±0.27×10^6 µl) were observed and it is compared with the control group (4.24±0.51× 10^6 µl), but these differences were not appreciable (P>0.05). Packed Cell Volume showed an increase in rats in all *Lavana Dravagam* treatment groups compared to the control group. The increase was appreciable (P<0.05) for rats in the large dose group when it is compared with the control group but the Mean corpuscular volume (MCV) was a slight increase observed in all drug-treated rats. The Mean Corpuscular Haemoglobin (MCH) value decreased in all low dose treated rat groups.

After the treatment of 14 days with *Lavana Dravagam*, all abnormal parameters significantly reduced normal. Haemoglobin level was 14.11±0.65 and 15.21±0.57 g/dl for female and male rats respectively in 0.03 ml/kg *Lavana Dravagam* treated group and it is tabulated in Table 4.

DISCUSSION

According to the siddha system, all medicinal preparations are categorized into 64 types. Thirty-two types of dosage forms are mentioned as internal medicines and thirty-two are laid down for external therapies, which are provided based on the form of medicine, method of preparation, or application (15,16). *Dravagam*, an internal medicine, means an acid. They are all acidic liquid preparation obtained by a process of destructive distillation of salts and alkalis of mineral origin with or without any addition of fluid in a peculiar distillation set up called *Valaai Iyanthiram* (17-19).

Dravagam is highly concentrated. so minimal dosage is required for producing more effect, easy administrable route, more potent (20). Among the ingredients,*Vediuppu* (Potassium nitrate-KNO3) is a source of potassium nitrate that occurs extensively in Bengal, Punjab, naturally as an efflorescence on the soil; but the nitre obtained in the bazaars is generally impure. *Kariuppu* (Sodium chloride-NaCl) - source of sodium chloride is found in nature forming 2.5 p.c of the water in the ocean. Common salt is being cultivated in the eastern coast of Tamil Nadu in the places like Cheyyur, Choonambedu, Marakkanam, Athirampattinam, Arumuganeri and Tuticorin (21-23).

The test drug *Lavana dravagam* selected from siddha text *Kannusamy ennum vaithiya sagaram*, was subjected to various studies based on the evidence like literature collection, biochemical analysis, toxicological studies, and pharmacological activity. Acute oral toxicity studies were conducted, no toxic signs, changes in general and functional behaviour and mortality during the study and that confirmed the safety of the test drug. The drug constituents are found to have hematinic activity. But this activity has not so far been evaluated for the above drug in the treatment of anaemia (*Paandu)* by assessing its therapeutic efficacy in phenyl hydrazine (10 mg/kg given as single dose orally for 8 days) induced anaemia in Wistar albino rats.

CONCLUSION

From the literature evidence, biochemical analysis, and acute toxicity evaluation it is evident that the drug *Lavana Dravagam* is safe. The pharmacological study of the drug *Lavana Drvagam* has proven that it possesses hematinic activity. The study result indicates that the test drug has potent activity against *Paandu* even in a short duration of about two weeks. Since the toxicity profile stands support to the safety of the drug it could bring out more significant improvement in the haematological parameters and clinical trials must be carried out to prove the therapeutic efficacy of this formulation.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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