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Pharmacological Evaluation of Anti – Anaemic Activity of Siddha Herbal Formulation - Madhulai Manappagu

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Abstract: *Iron deficiency anaemia is the most common health issue among the women and children in India. Women in the fertile age group and children are more susceptible to be affected by this disorder. It is described as "Veluppu Noi" in Siddha literatures. It is classified as a type of 'Azhal' humour disorder wherein pallor of the conjunctiva, nailbed, skin, tongue is the chief clinical feature. Untreated anaemia leads to serious consequences of health issues among them and hence management of this disorder is of paramount importance. "Madhulai Manappagu", a Siddha herbal syrup formulation found in Siddha literatures has been in practice for a quiet long period. Scientific evidences favouring its therapeutic efficacy have to be established.*
The haematinic efficacy of the manappagu was done by inducing iron deficiency anaemia in rats by giving Phenylhydrazine for 8 days. The animals were grouped in to four groups; Control, disease control, standard Iron syrup and trial drug syrup. Treatment with both the trial drug and the standard iron syrup were continued for 28 days. The blood samples collected from these rats before and after treatment were analysed and the changes in the parameters of Hb, PCV, MCV, MCH, MCHC, PCV, and Serum Iron were noted. The results were analysed statistically and the syrup showed 16.41% improvement in HB levels. This proved the efficacy of this Madhulai Manappagu in treating Veluppu noi. (Iron deficiency anaemia).

Key words: ***Iron deficiency anaemia, veluppu noi, pandu noi, Madhulai Manappagu, Siddha.***

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I. Introduction

Anaemia is a most common public health problem; it is the fourth leading cause of hospital admissions and the second factor contributing to death [(1), (2)]. Though science and technology has advanced much during this modern era, anaemia is a major killer of India. Anaemia is defined as a decrease in the total number of circulating red blood corpuscles or blood haemoglobin concentration resulting in concomitant impaired capacity to transport oxygen. According to WHO, anaemia is a condition in which the number of red blood cells or their oxygen-carrying capacity is insufficient to meet physiologic needs, which vary by age, sex, altitude, smoking, and pregnancy status[(3)]. Iron Deficient Anaemia is the most common type of various types of anaemia which is more common among children and women of reproductive age group [(4)].

Iron Deficiency Anaemia (IDA) is the most common deficiency disorder of the children which is known as "Pandu Noi" in Siddha system [(5)]. Specific Siddha drug formulations exclusive for anaemic disorder are given by Siddhars to treat anaemia and to combat its complications [(6)]. One of the drugs is the herbal formulation "Madhulai Manappagu" mentioned in the text "Siddha Vaidya Thirattu". This formulation may have promising results in the disorder of anaemia by improving the Haemoglobin level in the blood [(7)]. Though the formulation is in clinical practice for a period more than 75 years with proven efficacy, its pharmacological activity has not yet been evaluated scientifically. Being a simple herbal syrup preparation, which consists of Pomegranate juice and honey as main ingredients, help in improving the haemoglobin level in the blood [(8)]. The presence of ascorbic acid and the iron in the preparation which is having the haemocombatible p^H 7.2 ± 0.6 assist in enhancing the erythropoietic mechanism in our body [(9)]. The fruit pomegranate is rich in various nutrients, minerals and vitamins per 100 grams of edible portion including iron about 0.3mg [(10)]. With these inputs from various studies, the haematinic activity of the herbal syrup "Madhulai Manappagu"is carried out to establish the pharmacological significance of this Siddha preparation in treating Iron deficiency anaemia particularly in children and reproductive age group women.

II. Materials And Methods:

Preparation of Madhulai Manappagu:

The formulation Madhulai Manappagu was prepared in the Gunapadam Laboratory of National Institute of Siddha vide O/o PF.No.112/Admn. Dated

Ingredients:

1. Madhulam Pazham Saru (Pomegranate Juice) - 500 ml
2. Thaen (Honey) - 500 ml
3. Panneer (Rose Water) - 500 ml
4. Kalkandu (Sugar Candy) - 500 gms

Process:

Pomegranate fruit juice was taken and filtered. Equal quantities of rose water and sugar candy were added to the juice, mixed well and filtered. The filtrate was boiled and stirred frequently. When aroma aroused, fire was put down and was allowed to cool. After it had cooled, honey was added to the syrup and was collected and stored in a clean glass container [(11)]. The container was sterilised with boiling hot water and then dried up in hot air oven.

Shelf life :

6 Months

PHARMACOLOGICAL STUDY OF MADHULAI MANAPPAGU IN ANIMAL MODEL

The animal study protocol was presented and got approval from Institutional Animal Ethical Committee of National Institute of Siddha, Chennai (1248/Go/Re/S/2009/CPCSEA 21.05.2015) vide IAEC Protocol number NIS/IAEC-11/2016/04. The studies were conducted in the animal house of National Institute of Siddha, Chennai, Tamilnadu, India.

Experiment animal's husbandry

Female Albino Wistar rats (6 - 8weeks and wt: 140–160g) were purchased from the Laboratory Animal Medicine – Centre for Animal Health Studies, Tamilnadu Veterinary and Animal Sciences University, Chennai and maintained in the animal house of National Institute Siddha, Chennai. Animals were inhoused individually in polypropylene cages in a ventilated room (air cycles: 12-15/min; 70:30 exchange ratio) under an ambient room temperature of 22±3°C and 40–70% relative humidity, with a 12-h light/dark artificial photoperiod. The animals received RO water ad libitum and fed with Rodent pellet ad libitum[(12)].

Induction of Anaemia:

The rats were grouped into four groups with six female rats in a group. Group I was kept as control group and the remaining eighteen rats were given Phenyl hydrazine (PHZ) by daily oral administration at the dose of 10 mg/kg for 8 days. On 9th day, blood was withdrawn from retro orbital sinus and analysed for haemoglobin concentration. The other parameters like RBC, MCH, MCV, MCHC, PCV, and Serum Iron were also noted. Haemoglobin concentration lower than 12 gm/dl were recruited for the study. They were grouped as Group II, III and IV.

Experimental design:

The animals with anaemia were randomly divided into three groups along with Group I. Six animals were allotted to each group and were grouped as group I as control, group II as anaemia control, group III receiving standard haematinic syrup in the dose of 10mg/kg body weight and group IV as test group receiving the trial drug in the dose of 800mg/kg/day. All the dose of the trial drug and standard syrup were administered once daily via oral route through gastric gavage for 28 days to the respective group of animals.

Group		Treatment
I	Control	No Treatment
II	Disease Control	No Treatment
III	Test group	MM at 800 mg/kg b. wt
IV	Standard Iron syrup	10 mg/kg b.wt

Tab: Haematenic activity – animal groups.

At the end of 28 days, rats were over night fasted, blood samples were collected from the retro-orbital puncture under anaesthesia with and without anticoagulant and used for haematological and biochemical

parameters. Hematological parameters like Hb, RBC, MCH, MCV, MCHC, PCV, and Serum Iron were analyzed.

Results of HAEMATENIC ACTIVITY of MADHULAI MANAPPAGU:

The effect of Madhulai Manappagu in blood Hb levels

Animal Group	Before Mean ± Std (Min- Max)	After Mean ± Std (Min- Max)	P value
Control	13.8 ± 1.48 (12.5 - 15.7)	13.57 ± 1.40 (12.2 - 15.2)	-
Disease Control	9.25 ± 0 .76 (8.4 - 10.4)	8.63 ± 0.67 (7.6 - 9.6)	a1***p<0.001
Std treated	10.47 ± 0.85 (9.3 - 11.6)	11.78 ± 0 .68 (11 - 12.6)	a2*p<0.05 b1***p<0.001
MM treated	10.2 ± 0.70 (9.4 - 11)	11.88 ± 0.85 (10.8 - 13.3)	a3*p<0.05 b2***p<0.001

Data were expressed as mean ± SD (n = 6). P value was calculated using one way ANOVA followed by Tukey's multiple comparison tests.

a1***p<0.001 significantly different when compared with control group.

a2*p<0.05 significantly different when compared with control group.
a3*p<0.05 significantly different when compared with control group.
b1***p<0.001 significantly different when compared with disease control group.
b2***p<0.001 significantly different when com

The effect of Madhulai Manappagu in blood RBC levels

Animal Group	Before Mean ± Std (Min-Max)	After Mean ± Std (Min- Max)	P value
Control	7.12 ± 0.95 (5.7- 8.3)	8.05 ± 0.93 (7.2 - 9.5)	-
Disease Control	6.3 ± 0.59 (5.5 - 7.2)	5.68 ± 0.50 (5.2 - 6.4)	a1***p<0.001
Std treated	7.97 ± 0.36 (7.5 – 8.5)	8.1± 0.32 (7.6 - 8.5)	b1***p<0.001
MM treated	7.22± 0.57 (6.4 - 7.8)	7.88 ± 0.85 (6.9 – 9.4)	b2***p<0.001

Data were expressed as mean ± SD (n = 6). P value was calculated using one way ANOVA followed by Tukey's multiple comparison tests.
a1***p<0.001 significantly different when compared with control group.

b1***p<0.001 significantly different when compared with disease control group.
b2***p<0.001 significantly different when compared with disease control group.

The effect of Madhulai Manappagu in blood MCH levels

Animal Group	Before Mean ± Std (Min- Max)	After Mean ± Std (Min- Max)	P value
Control	20.83 ± 1.73 (18.4- 22.8)	22.95 ± 0.91 (21.5- 24.1)	-
Disease Control	16.65 ± 0.49 (16 – 17.4)	16.45 ± 0.54 (16 - 17.5)	a1*p<0.05
Std treated	19± 1.80 (16.4- 20.5)	18.92 ± 3.87 (13.7- 23.5)	b1***p<0.001
MM treated	17.5± 0.57 (16.5 - 18)	17.73 ± 2.34 (16.2– 22.4)	b2**p<0.01

Data were expressed as mean ± SD (n = 6). P value was calculated using one way ANOVA followed by Tukey's multiple comparison tests.

a1*p<0.05 significantly different when compared with control group.

b1***p<0.001 significantly different when compared with disease control group.
b2**p<0.01 significantly different when compared with disease control group.

The effect of Madhulai Manappagu in blood MCV levels

Animal Group	Before Mean ± Std (Min- Max)	After Mean ± Std (Min- Max)	P value
Control	58.72 ± 1.98 (56.5 - 61.5)	60.98 ± 1.09 (59.5 - 62.7)	-
Disease Control	56.95 ± 5.94 (50 - 65.1)	53.15 ± 3.77 (50 - 59.4)	a1**p<0.01
Std treated	54.45 ± 1.26 (53 – 56.4)	54.53 ± 0.59 (52.8 - 55.4)	a2**p<0.01
MM treated	57.25 ± 0.43 (56.8 - 58)	57.42 ± 5.32 (51.5 – 67.4)	

Data were expressed as mean ± SD (n = 6). P value was calculated using one way ANOVA followed by Tukey's multiple comparison tests.

a1**p<0.01 significantly different when compared with control group.

a2**p<0.01 significantly different when compared with control group.

Table.6.5.5. The effect of Madhulai Manappagu in blood MCHC levels

Animal Group	Before Mean ± Std (Min- Max)	After Mean ± Std (Min- Max)	P value
Control	31.57 ± 1.33 (29.8 - 33.6)	32.58 ± 0.74 (31.7- 33.6)	-
Disease Control	23.72 ± 1.36 (22.4 - 25.4)	23.58 ± 1.35 (22.2 - 25.1)	a1**p<0.01
Std treated	24.6 ± 0.57 (24.7 – 26.4)	25.15 ± 0.27 (24.9 - 25.6)	a2**p<0.01
MM treated	27.93 ± 0.60 (27.1 - 28.6)	28.65 ± 2.49 (26.6 – 33)	a3**p<0.01

Data were expressed as mean ± SD (n = 6). P value was calculated using one way ANOVA followed by Tukey's multiple comparison tests.

a1***p<0.001 significantly different when compared with control group.

a3***p<0.001 significantly different when compared with control group.
b1***p<0.001 significantly different when compared with disease control group.
b2***p<0.001 significantly different when compared with disease control group.
c1**p<0.01 significantly different when compared with standard drug treated group.

The effect of Madhulai Manappagu in blood PCV levels

Animal Group	Before Mean ± Std (Min- Max)	After Mean ± Std (Min- Max)	P value
Control	41.22 ± 1.40 (40 - 43)	41.15 ± 1.32 (39.9 - 43)	-
Disease Control	39.43 ± 2.20 (36.2 - 42.8)	39.35 ± 2.38 (36.2 - 42.4)	-
Std treated	39.15 ± 0.99 (37.7 – 40.4)	40.98 ± 1.30 (39 - 42.3)	-
MM treated	38.88 ± 0.92 (37.6 - 40.1)	42.27 ± 2.13 (39.8 – 45.2)	-

Data were expressed as mean ± SD (n = 6). P value was calculated using one way ANOVA followed by Tukey's multiple comparison tests.

The effect of Madhulai Manappagu in serum Iron levels

Animal Group	Before Mean ± Std (Min- Max)	After Mean ± Std (Min- Max)	P value
Control	16.57 ± 1.04 (15.7 - 18.4)	16.53 ± 1.12 (15.6 - 18.1)	-
Disease Control	12.93 ± 0.96 (11.8 - 14.5)	12.17 ± 0.54 (11.5 - 12.9)	a1***p<0.001
Std treated	15.52 ± 0.71 (14.3 - 16.3)	16.37 ± 0.85 (14.9 - 17.2)	b1***p<0.001
MM treated	12.9 ± 0.85 (11.9 - 14.2)	14.8 ± 1.03 (13.6 - 15.9)	a3*p<0.05 b2***p<0.001 c1*p<0.05

Data were expressed as mean ± SD (n = 6). P value was calculated using one way ANOVA followed by Tukey's multiple comparison tests.

a1***p<0.001 significantly different when compared with control group.

a3*p<0.05 significantly different when compared with control group.
b1***p<0.001 significantly different when compared with disease control group.
b2***p<0.001 significantly different when compared with disease control group.
c1*p<0.05 significantly different when compared with standard drug treated group.

III. Discussion:

Haematenic Action:

The pharmacological study of Haematenic activity of MM was carried out in the female Wistar rats by inducing anaemia to the animals. The animals were grouped in to 4 groups maintaining 1st group as control, 2nd group as anaemia control. The 3rd group was administered with a standard iron syrup available in the market and the 4th group was administered the trial drug. The blood parameters Hb, RBC, MCH, MCV, MCHC, PCV and Serum Iron level[13] were analysed for the efficacy of MM in treating anaemia in animals.

In analyzing the Hb levels amongst the different groups of animals, extremely significant reduced changes were recorded between control and the disease control group ($p<0.001$) revealing the evidence of inducing anaemia in Group II. Induction of anaemia in the rats was observed from this inference. But the p values between the control and the treatment groups were found to be $p<0.05$ as these groups received the treatment. This revealed that the induced anaemia was on the process of improvement. The significant changes between the disease control and the other two groups received any one of the treatment with trial drug or standard, was noticed. This provides the information of efficacy of either the drug or the standard Iron syrup. But there was no significant changes between the standard drug treated animal and the trial drug administered group. This result showed that the trial drug and the standard iron syrup were almost equally efficacious in improving the blood Hb level.

In the analysis of RBC, extremely significant change was noted between the control and disease control groups ($p<0.001$) (inference: anaemia induced) and extremely significant changes between the groups of disease control and the treatment groups ($p<0.001$). This gave the inference of increase in RBC levels in the treated groups. But no changes between control and treatment groups were recorded giving the inference of reversal of RBC levels in the treatment groups. This result showed the increase in RBC levels in the trial drug and standard drug treated animals.

Analysing the MCV values showed very significant changes between control and disease control.($p<0.01$); very significant changes between control and standard treatment group ($p<0.01$). From this, anaemia was present in disease control and the induced anaemia in the standard drug treated groups was getting reduced when compared with the disease control. But the trial drug treated group, the MCV value was almost normal as that of in control group ($p>0.05$). This revealed that the trial drug improved the MCV levels after treatment.

Regarding MCHC values, the extremely significant values were noted between control group and the other groups. ($p<0.01$). These results showed that MCHC were reduced when anaemia was induced and the treatment with standard and the test drugs improved the MCHC values.

In analyzing the PCV values statistically no significance was observed as the results of the after treatment values were analysed. But on observing the mean values of each group it is evident that there was improvement in each group of animals treated with standard and trila drugs.

In iron levels, there were significant changes between the control group and other groups while there were also significant changes observed between the disease control and the treated groups. The significance

between the standard and the trial drug was not of much importance when observing the before treatment values of both the groups.

IV. Summary

Summarizing all the observations it may be evident that the trial drug MM improves the values of Hb and Serum iron showing significant improvement in these parameters between control and disease control and between the treated groups and disease control groups.

In analyzing the percentage of improvement of HB between before and after treatment with the trial drug, it was found to be 16.41% . The percentage of improvement in standard iron syrup was about 12.52%.

V. Conclusion

The haematenic pharmacological activity study revealed the efficacy of the trial drug in experimental controlled animals in enhancing the haemopoiesis and thereby proved its efficacy in treating iron deficiency anaemia[14, 15, 16].

The haematenic activity of the drug showed 16.41% improvement of Hb levels in experimental animals.

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