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Standardization of an Ayurvedic Bhasma preparation and its evaluation as a potential haematinic agent

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ABSTRACT

Background: Ayurveda arrangement of solution was very valued and worked on amid the brilliant time of Indian history. Three unique measurements of lauhabhasma were assessed for the haematinic movement and contrasted and that of Ferrous sulfate. The outcomes recommend that lauhabhasmas indicates noteworthy haematinic movement. **Aim:** The present study was designed as standardization of an Ayurvedic Bhasma preparation and its evaluation as a potential Haematinic Agent. **Materials and Methods:** It was evaluated by the terms of physiochemical parameters including colour, odour, taste, pH, total ash, acid insoluble ash, loss on drying, particle size determination, determination of floating property and determination of fineness and metallic luster in direct sunlight. For the pharmacological evaluations, rats were divided into 6 groups and administered as follows. Group 1: 1% Acacia; Group 2: 1% Acacia glue containing 1ml of supreme liquor for each 50 ml; Group 3: 1 ml of oral single dosage of 20mg/kg body weight/day ferrous sulfate; Group 4: I ml of oral single dosage of 10 mg/kg body weight/day of lauhabhasma; Group 5: 1 ml of oral single dosage of 15 mg/kg body weight/day of lauhabhasma and Group 6: 1 ml of oral single dosage of 20 mg/kg body weight/day of lauhabhasma. It was examined by different parameters such as RBCs, WBCs (Monocyte, Basophil, Eosinophil, lymphocytes) count, PCV, MCV, hemoglobin value, Mean Cell Hemoglobin Concentration (MCHbC) and Mean cell hemoglobin (MCHb).**Results:** In all the parameters, Ayurvedic preparation exhibited a potential hematinic effect**. Conclusion:**In conclusion, this examination may infer that the lauhabhasma might be exceptionally compelling ayurvedichaematinic operator and can be helpful in treatment of iron deficiency.**Future aspect:** In future, it might be a well-researched and promising source to subside anemia and various blood disorders.

Keywords: Packed cell volume (PCV), Hemoglobin (Hb), Red blood cells (RBCs) and White blood cells (WBCs).

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INTRODUCTION

Ayurveda arrangement of solution was very valued and worked on amid the brilliant time of Indian history. This arrangement of meds perceives the significance of metallic smaller scale supplements in our body. Lack or lopsidedness of these metallic small scale supplements in body brings about the indication of ailments. This idea has incredible significance in show day circumstance as our reality faces major natural unsettling influences and therefore more up to date infections. Ayurveda lays more accentuation on the counteractive action and treatment of illnesses by keeping a harmony amongst eating regimen and way of life of an individual relying on the earth he or she lives in. The need of metallic smaller scale supplements for idealize wellbeing is a subject for escalated contemplate [1,2]. Customary pharmaceuticals are utilized as a part of the treatment of different interminable issue and for the change of prosperity of people. In Ayurveda, metals, for example, Iron, Copper, Zinc, and Lead, and so on., are utilized as a part of numerous arrangements, in the wake of changing the metals into nonmetallic structures. The solutions so arranged are ordered under a gathering called Rasauṣadhis. Rasauṣadhis have been endorsed by Ayurvedic doctors since long with uncommon notices of danger. Bhasmas are one of a kind arrangements including metallic/mineral arrangements calcined utilizing warmth to change metals into non lethal organometallic shapes. In arrangements of these Bhasmas, the starter procedure which causes detoxification without hurting its restorative properties (guṇas) is called as 'Śodhana'. The present investigation was intended to set up the lethality profile of Bhasma [3]. Weakness is a condition portrayed by diminish in the aggregate sum of red platelets (RBCs) or hemoglobin in the blood underneath the typical range for the given age and sex of the patient. This by and large outcomes in brought capacity of the blood down to convey oxygen. The ordinary scope of hemoglobin in females is 14±2.5 g/dl, in guys is 15.5±2.5g/dl and in newborn children is 16.5±3 g/dl. It can be caused by any condition bringing about a huge reduction in the aggregate body

erythrocyte mass. Ayurvedic treatment of diabetes incorporates a few home grown medications and furthermore a couple of mineral arrangements including bhasmas. LauhaBhasma is the most as often as possible utilized Ayurvedic arrangement of metallic iron. It is one of the old arrangements for press suppliments and can be set up by established and additionally mechanical strategies [4]. Bhasma are remarkable ayurvedic metallic/minerals readiness, treated with home grown juice or decoction and uncovered for Ayurveda, which are known in Indian subcontinent since 7thcentury A.D. what's more, generally suggested for treatment of an assortment of unending diseases. Creature's subsidiary, for example, horns, shells, quills, metallic, nonmetallic and herbals are ordinarily managed as Bhasma [5]. Ayurveda is one of the most seasoned frameworks of prescription, honed in Indian sub-landmass. Ayurveda is a settled investigation of antiquated Indian legacy. The word Ayurveda infers the signifying "investigation of life". This science is trusted and built up through a great many years. At the season of Charaka and Sushuruta restorative plants were fundamentally utilized for the planning of therapeutic operators[6].

MATARIALS AND METHODS

1. Physical standardization

Determination of aggregate fiery debris, Determination of misfortune on drying and Determination of pH.

2. Pharmacological Studies

Here the examples are tried for particular pharmacological action utilizing creature models.

i) Animals

Swiss mice of either sex weighing around 18g (youthful ones, matured two months) and 25g (more established ones, matured 28 weeks) will be utilized as a part of the present investigation. The creature will be acquired from infection free little creature house. They will be acclimatized to the research center conditions for 5 days before social investigations. The creatures had free access to sustenance and water and will be kept up under 12:12 hours light and dull cycles. Every one of the readings will be taken amid a similar time i.e. between 6-8 PM. Institutional Animals Ethics Committee(IAEC) had endorsed the exploratory convention and care of creatures was taken according to the CPCSEA rules, Animal welfare division, Ministry of Environment and timberland, Govt. of India. The creatures will be kept in polypropylene confines under standard research facility condition. The creature house will be kept up at 27°c ±2°c temperature and 50 to 60% mugginess. The creatures will be gotten from CPCSEA endorsed creature place of establishment.

ii) Acute oral toxicity study

Intense danger contemplates were led to decide the protected dosage according to OECD-423 rules. Medications were regulated orally to medium-term fasted creatures. After medication organization the creatures were watched constantly for 60 minutes, oftentimes for the following four hours, and afterward following 24 hours. After organization, Irwin's test was directed, where the creatures were watched for net social changes. The dangerous dosage was dictated by watching the death rate in the medication treated gatherings. From this the helpful measurements was chosen for the further investigation.

iii) Planning of measurements

Promoted arrangements of Lauhabhasma were acquired from neighborhood ayurvedic medicinal market. Phenyl hydrazine and ferrous sulfate were acquired from SD Fine Chemicals, Mumbai, India. Every other substance were utilized of AR review and utilized as got.

The dosage for exploratory investigation of the medications was figured by extrapolating the human measurements to creature dosage in view of the body surface territory proportion. Medication suspension was set up in 1% gum acacia arrangement in water and arrangement of phenyl hydrazine was set up by dissolving it in total liquor (50mg of every 1ml) and including acacia suspension [4,7]. Every single other concoction utilized for think about were obtained from rumored Indian providers and will be of AR review.

3. Experimental Design

Group 1: Normal Control Group (1% acacia glue containing 1ml of total liquor for every 50 ml).

Group 2: Anemic Control Group (1% acacia glue containing 1ml of supreme liquor for each 50 ml).

Group 3: Standard Group (1ml of 20mg/kg body weight/day ferrous sulfate).

Group 4: Test Group 1 (1ml of the 10 mg/kg body weight/day of lauhabhasma).

Group 5: Test Group 2 (1ml of the 15 mg/kg body weight/day of lauhabhasma).

Group 6: Test Group 3 (1ml of 20 mg/kg body weight of lauhabhasm).

4. Inspecting Schedule

The examination was done for 21 days. The blood (1-2ml) was pulled back in EDTA from the retro-orbital plexus, under slight chloroform anesthesia, on day 0 preceding phenyl hydrazine organization, and on days 1, 7, 14 and 21, days after phenyl hydrazine organization i.e. acceptance of paleness.

5. Assessment of hematological parameters

The hematinic movement was assessed by surveying different blood parameters like red platelet (RBC) tally, hemoglobin (HB) fixation and stuffed cell volume (PCV). The mean cell volumes (MCV), mean cell hemoglobin (MCH), mean cell hemoglobin focus (MCHC) and white platelets (WBC) tally. The parameters were dictated by utilizing Auto cell counter 920E (Swelab Ltd) (8,9).

RESULT

A. Physical standardization

Determination of Total, Acid soluble and water soluble ash value

Table 1 depicts the determination of total ash, acid soluble and water soluble ash values found in the ayurvedic preparation as potent hematinic agent.

Table 1: Determination of Total, Acid soluble and water soluble ash value

Substance	Total ash (%)	Acid soluble (%)	Water soluble (%)
Ayurvedic formulation	11.24	7.29	3.95

Determination of Loss on Drying (LOD)

Table 2 depicts the loss on drying of the substance.

Table 2: Determination of LOD

Substance	Weight of formulation (g)	Loss on drying (g)
Ayurvedic formulation	3.5	0.8

B. Pharmacological evaluation

Acute oral toxicity test

Table 3: Acute oral toxicity test

S.N.	Treatment	Mortality Observation		
		0 Days	7 Days	14 Days
1.	Control group (Distilled water)	No toxic, behavioural& mortality changes observed	No toxic, behavioural& mortality changes observed	No toxic, behavioural& mortality changes observed
2.	Test group (herbal formulation 5000mg/kg)	No toxic, behavioural& mortality changes observed	No toxic, behavioural& mortality changes observed	No toxic, behavioural& mortality changes observed

Determination of RBC Count

The following table depicts the determination of RBC Count-

Table 4: RBC Count

S.N.	Treatment (Group)	RBC Count[Million (cells/mcL)±SEM] 1st day	7thday	14th day	21th day
1.	Normal Control	6.3±0.2	6.3±0.4	6.6±0.1	6.8±0.7
2.	Anemic Control	6.2±0.1	6.2±0.3	5.8±0.7	5.2±0.5
3.	Standard (20mg/kg)	6.4±0.4	6.8±0.4	7.6±0.1	7.9±0.7
4.	Test 1 (10mg/kg)	6.2±0.6	6.3±0.5	6.7±0.7	6.8±0.4
5.	Test 2 (15mg/kg)	6.3±0.1	6.3±0.7	6.8±0.4	7.1±0.6
6.	Test 3 (20mg/kg)	6.4±0.5	6.6±0.8	6.9±0.1	7.6±0.8

Estimation of hemoglobin count

The hemoglobin concentration was observed by Sahli's method and depicted in the table mentioned below-

Table 5: Estimation of hemoglobin level

S.N.	Treatment (Group)	Hemoglobin level (g/100ml±SEM) 1st day	7thday	14th day	21th day
1.	Normal Control	13.2±0.2	13.3±0.5	13.8±0.6	14.1±0.2
2.	Anemic Control	12.8±0.6	12.1±0.7	11.8±0.8	11.5±0.4
3.	Standard (20mg/kg)	12.4±0.5	13.6±0.8	13.9±0.3	14.8±0.1
4.	Test 1 (10mg/kg)	12.5±0.2	12.6±0.4	12.8±0.7	13.6±0.1
5.	Test 2 (15mg/kg)	12.4±0.8	12.8±0.4	13.2±0.6	13.7±0.7
6.	Test 3 (20mg/kg)	12.3±0.1	13.1±0.2	13.6±0.7	14.2±0.3

Determination of Packed Cell Volume (PCV)

Table 6: Determination of PCV

S.N.	Treatment (Group)	PCV (Percentage± SEM) 1st day	7thday	14th day	21th day
1.	Normal Control	42.2±0.2	42.8±0.6	42.6±0.8	44.9±0.1
2.	Anemic Control	41.2±0.3	41.4±0.6	38.2±0.7	41.8±0.9
3.	Standard (20mg/kg)	44.6±0.1	46.9±0.2	47.6±0.4	49.6±0.7
4.	Test 1 (10mg/kg)	41.5±0.5	42.2±0.2	45.6±0.7	46.7±0.1
5.	Test 2 (15mg/kg)	42.2±0.2	42.2±0.7	43.5±0.9	44.1±0.3
6.	Test 3 (20mg/kg)	44.3±0.1	44.9±0.3	47.2±0.5	48.1±0.9

Determination of Mean Cell Volume (MCV)

Table 7: Determination of MCV

S.N.	Treatment (Group)	MCV (Pg± SEM) 1st day	7thday	14th day	21th day
1.	Normal Control	20.2±0.2	20.5±0.3	20.9±0.5	21.2±0.4
2.	Anemic Control	19.8±0.4	20.3±0.8	19.4±0.6	18.6±0.4
3.	Standard (20mg/kg)	20.6±0.4	22.9±0.2	23.3±0.1	23.8±0.3
4.	Test 1 (10mg/kg)	19.5±0.7	19.9±0.5	20.8±0.7	21.7±0.1
5.	Test 2 (15mg/kg)	20.2±0.6	21.2±0.6	22.5±0.3	22.1±0.1
6.	Test 3 (20mg/kg)	20.3±0.4	21.3±0.7	22.2±0.4	22.5±0.8

Determination of Mean Cell Hemoglobin (MCHb)

Table 8: Determination of MCHb

S.N.	Treatment (Group)	MCHb (Pg± SEM) 1st day	7thday	14th day	21th day
1.	Normal Control	20.2±0.1	20.5±0.7	20.9±0.8	21.2±0.2
2.	Anemic Control	19.8±0.7	20.3±0.4	19.4±0.7	18.6±0.1
3.	Standard (20mg/kg)	20.6±0.5	21.2±0.2	22.3±0.7	22.8±0.8
4.	Test 1 (10mg/kg)	19.5±0.4	19.9±0.8	20.8±0.4	21.7±0.1
5.	Test 2 (15mg/kg)	20.2±0.1	21.2±0.7	22.5±0.3	22.1±0.4
6.	Test 3 (20mg/kg)	20.3±0.8	21.5±0.4	20.3±0.1	21.5±0.7

Determination of Mean Cell Hemoglobin Concentration (MCHC)

Table 9: Determination of MCHC

S.N.	Treatment (Group)	MCHC (g/dl± SEM) 1st day	7thday	14th day	21th day
1.	Normal Control	26.2±0.2	26.5±0.1	26.9±0.4	27.2±0.7
2.	Anemic Control	25.8±0.7	25.3±0.5	25.1±0.6	24.6±0.1
3.	Standard (20mg/kg)	26.6±0.1	26.5±0.2	27.3±0.1	28.4±0.5
4.	Test 1 (10mg/kg)	25.5±0.6	25.9±0.3	25.8±0.5	26.7±0.5
5.	Test 2 (15mg/kg)	25.2±0.7	25.9±0.1	26.3±0.5	27.5±0.9
6.	Test 3 (20mg/kg)	26.3±0.2	26.5±0.1	27.3±0.1	27.8±0.7

Determination of White Blood Cells Count (WBC)

Table 10: Determination of WBC Count- Neutrophils

S.N.	Treatment (Group)	Neutrophils (10^3/mm^3± SEM) 1st day	7thday	14th day	21th day
1.	Normal Control	22.2±0.2	22.5±0.1	22.9±0.4	23.4±0.6
2.	Anemic Control	22.8±0.1	22.3±0.3	22.6±0.6	22.3±0.5
3.	Standard (20mg/kg)	22.6±0.1	23.5±0.2	24.3±0.3	25.9±0.6
4.	Test 1 (10mg/kg)	22.5±0.1	22.9±0.2	23.3±0.8	23.7±0.4
5.	Test 2 (15mg/kg)	22.2±0.7	22.5±0.8	22.5±0.7	22.7±0.4
6.	Test 3 (20mg/kg)	22.3±0.4	23.5±0.1	23.3±0.2	24.8±0.3

Determination of WBC Count- Basophils

Table 11: Determination of basophils count

S.N.	Treatment (Group)	Basophils (10^3/mm^3± SEM) 1st day	7thday	14th day	21th day
1.	Normal Control	1.0±0.1	1.0±0.3	1.0±0.2	1.0±0.1
2.	Anemic Control	1.0±0.3	1.0±0.4	1.0±0.6	1.0±0.4
3.	Standard (20mg/kg)	1.0±0.7	1.0±0.6	1.0±0.6	1.0±0.7
4.	Test 1 (10mg/kg)	1.0±0.7	1.0±0.2	1.0±0.4	1.0±0.9
5.	Test 2 (15mg/kg)	1.0±0.2	1.0±0.1	1.0±0.7	1.0±0.6
6.	Test 3 (20mg/kg)	1.0±0.1	1.0±0.6	1.0±0.4	1.0±0.2

Determination of WBC Count- Monocytes

Table 12: Determination of Monocytes

S.N.	Treatment (Group)	Monocytes (10^3/mm^3± SEM) 1st day	7thday	14th day	21th day
1.	Normal Control	3.0±0.2	3.1±0.1	3.4±0.6	3.6±0.4
2.	Anemic Control	3.0±0.4	3.1±0.3	2.8±0.5	2.7±0.4
3.	Standard (20mg/kg)	3.1±0.1	3.3±0.1	4.1±0.3	4.7±0.7
4.	Test 1 (10mg/kg)	3.0±0.3	3.0±0.5	3.4±0.3	3.7±0.2
5.	Test 2 (15mg/kg)	3.0±0.1	3.3±0.3	3.6±0.1	3.8±0.6
6.	Test 3 (20mg/kg)	3.1±0.1	3.4±0.4	3.8±0.8	4.2±0.4

Determination of WBC Count- Lymphocytes

Table 13: Determination of Lymphocytes

S.N.	Treatment (Group)	Lymphocytes (10^3/mm^3± SEM) 1st day	7thday	14th day	21th day
1.	Normal Control	61.0±0.1	61.4±0.4	62.0±0.7	62.5±0.4
2.	Anemic Control	62.0±0.5	62.4±0.4	61.0±0.6	60.8±0.5
3.	Standard (20mg/kg)	62.0±0.4	63.4±0.7	64.0±0.6	65.2±0.4
4.	Test 1 (10mg/kg)	62.2±0.5	62.8±0.1	62.8±0.8	62.5±0.7
5.	Test 2 (15mg/kg)	62.4±0.8	62.8±0.4	62.4±0.7	63.7±0.4
6.	Test 3 (20mg/kg)	62.0±0.5	63.1±0.4	63.9±0.8	64.7±0.1

Determination of Eosinophil

Table 14: Determination of Eosinophil

S.N.	Treatment (Group)	Lymphocytes (10^3/mm^3± SEM) 1st day	7thday	14th day	21th day
1.	Normal Control	2.8±0.1	3.4±0.8	3.6±0.5	3.9±0.4
2.	Anemic Control	3.2±0.2	3.1±0.6	2.8±0.4	2.8±0.8
3.	Standard (20mg/kg)	3.1±0.8	3.4±0.9	3.8±0.4	4.2±0.7
4.	Test 1 (10mg/kg)	2.8±0.1	3.1±0.4	3.1±0.7	3.5±0.9
5.	Test 2 (15mg/kg)	2.8±0.6	3.1±0.8	3.4±0.7	3.5±0.1
6.	Test 3 (20mg/kg)	2.8±0.4	3.2±0.5	3.7±0.4	3.9±0.4

DISCUSSION

The physiochemical parameter viz ash content and loss on drying were studied to quantify the medicinal importance of drugs ; Acid soluble ash value was compared with water soluble ash value and found more. It shows its lipophilic nature that influences its absorption. loss on drying shows about the water content and volatile substances present in the formulation. After incubation the total weight was found 2.7g that proved that it contains only 0.8g of volatile and water contents. In Acute oral toxicity test no significant differences in body weight gain were observed No toxic, behavioral & mortality changes observed. No altered growth or abnormal changes were detected during the macroscopic analysis of the rat organs. It demonstrated a safe and convenient drug for further use. It assures the safety data along with the efficacy. The hematinic movement was assessed by surveying different blood parameters like red platelet (RBC) tally, hemoglobin (HB) fixation and stuffed cell volume (PCV). The mean cell volumes (MCV), mean cell hemoglobin (MCH), mean cell hemoglobin focus (MCHC) and white platelets (WBC) tally. The parameters were dictated by utilizing Auto cell counter 920E (Swelab Ltd). Total 06 groups were identified and evaluated including Normal control, anemic control, standard, test 1, test 2 and test 3. They were evaluated for its hematinic potential after 1, 7, 14 and 21 days. Effect was calculated and compared with the standard and control group. They demonstrated a significant

level of effect when compared to standard. It proved that the marketed ayurvedic formulation possess a significant hematinic potential. It showed a dose dependent response because maximum response was noted at dose 20mg/kg. It also proves about its good preparation and standardization of marketed ayurvedic preparation. The standardization and pharmacological potential of the product is so significant and safe. It assures that this ayurvedic formulation can be further used in the treatment of mild to severe anemia.

CONCLUSION

In-vivo experimentation revealed safety and good efficacy of traditional ayurveda-based therapy for the treatment of anaemia. The study needs to be extended to explore other traditional preparations and medicinal plants. This examination may infer that the lauhabhasma might be exceptionally compelling ayurvedic haematinic operator and can be helpful in treatment of iron deficiency.

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