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Research Article

"PREPARATION AND PHYSICO-CHEMICAL ANALYSIS OF PRATAPMARTAND RASA AND ITS ASSESSMENT OF ANTIPYRETIC ACTIVITY IN ALBINO RATS."

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ABSTRACT

Pratapmartand Rasa is one of the herbomineral compound explained in *Rasendrasarsangraha* and indicated in *Jwara* disease. To establish standards for quality control and provide scientific data to establish antipyretic activity by experimental study an attempt was made.

The whole study was taken up with a view of validation and setting up SOP (Standard Operative Procedure) in the preparation of *Pratapmartand Rasa*. The main underlying aim for the same being setting of standards - based on various parameters, mainly analytical in nature. The major problem in standardization of any Ayurvedic drug is unavailability of any set standards with which to compare a drug. This variation in processes, process being the same, a variation in the media used which are invariably herbs, and many other dependent, derived modifications created by, the workers, has lead to the conception of 'process validation'. The Experimental study was carried out in manner to do a comparative analysis of antipyretic activity between standard drug (Paracetomal) and trail drug (i.e.*Pratapmartand Rasa).*

The work was conducted in two steps. First consist standardization of raw material, process and final product. Second step consist evaluation of anti pyretic activity of *Pratapmartand Rasa* in albino rats. In step 1 *Shodhana*of *Tankana, Hnigula, vatsanabha* and *Jaypal* was done according to classic. Preparation of *pratapmartanda Rasa* was done with reference of *Rasendrasarasangraha* Jwara Adhyay 2. In analytical study the ingredients and *Pratapmartanda Rasa* examined for Ayurvedic and modern parameter like AAS etc. After that its antipyretic activity study was examined on Wister Strain albino rats taking as test group, control group, standard drug group each consisting 6 albino rats.

Keywods: *Pratapmartand Rasa, Jwara, AAS*

INTRODUCTION

Rasaushadhis are appreciated for their smaller dosages, quicker effectiveness, long durability, palatability and high efficacy etc. Thus the *Rasaushadhi* preparation's plays an important & major role in curing the ailing human beings. On internal administration of metals and minerals i.e. *Rasausadhis*, in unprocessed or misprocessed form, they are very toxic but when scientifically *Shodhana* and *Marana* of these substances are done with some special processes, they became non-toxic or least toxic with low untoward effects and can be used therapeutically with high gratitude of efficacy, for this standardization of *Rasausadhis* is very necessary. So in the present study humble attempt was made in such point of view.

Jwara is a prime disease mentioned in *Ayurveda* as well as contemporary science. *Jwara* being a commonest symptom which accompanies almost all the Constitutional diseases have been termed as *Rogapati* by *Aacharyas.* The management of *Jwara* is still a complexity. The antipyretic efficient *ayurvedic* formulations are available in

herbal as well as *Rasaushdhis* form, but potent antipyretic drug with respect to antipyretic activity still a need of *ayurvedic* Pharmacopeia.

The animal study is a confirmed & suggestive measure with respect to drug doses, toxicity, adverse effect etc with least bias compare to clinical one. A number of *Jwarahara* drugs have been mentioned in *Ayurvedic* Classics. *Pratapmartand Rasa* explained in *Rasendrasarasangraha* has been indicated in all types of *Jwara*. Therefore *Pratapmartand Rasa,* which is formulated with *Shudha Hingula, Shudha Vatsanabha, Shudha Tankana, Shudha Jayapala* together help to reduce *Jwara*. Hence this study will be under taken for scientific validation of *Pratapmartand Rasa* on its antipyretic activity.

AIMS AND OBJECTIVES

Aim: -

1. To establish the physico-chemical standards for *Pratapmartand Rasa.*
2. To assess the antipyretic activity of *Pratapmartand Rasa* in albino rats.

Objectives:-

1. To prepare *Pratapmartand Rasa* as described in *Rasendrasarasangraha.*
2. To establish the Physico-chemical standards of *Pratapmartand Rasa.*
3. To assess the anti-pyretic activity of *Pratapmartand Rasa* in albino rats.

MATERIAL AND METHOD

Present study was conducted by following steps

Step 1 -

A. Raw material standardization.
B. Process standardization.
C. Final product standardization.

Step 2 -

Evaluation of antipyretic activity of *Pratapmartand Rasa* in albino rats.

A) Raw Material Standardization-

1. Authentication of raw *Hingula,Tankana,Vatsanabha,Jayapala&Aadraka.*

B) Process Standardization-

a) *Shodhana* of *Hingula* in *Aadraka swarasa.*
b) *Shodhana* of *Vatsanabha in Gomutra.*
c) *Shodhana* of *Vatsanabha* in *Godugdha* by *Dola Yantra* -method.
d) *Shodhana* of *Jayapala* in *Godugdha* by *Dola Yantra* method.

Preparation of *Pratapmartand Rasa* as described in *Rasendrasarasangraha.*

C) Final Product Standardization-

Standardizationof *Pratapmartand Rasa.*

- **Method of Preparation of *Pratapmartand Rasa:***

Table Showing Material Taken for Preparation of *Pratapmartand Rasa*

SR. NO	Material	Quantity
1.	*Shodhit Vatsanabha*	50 gm
2.	*Shodhit Hingula*	100 gm
3.	*Shodhit Jayapala*	150 gm
4.	*Shodhit Tankana*	200 gm

Equipements :

*Khalva yantra 2.*Weighing machine 3.*Vastra 4.*Spoon.

- **Method :**
  1. Above mentioned ingredients were taken in dry and clean *khalva yantra.*
  2. Mixed well and triturated up to vary fine powdered.
  3. Pills of *Pratapmartand Rasa* prepared.
  4. The pills were dried in shade and after complete drying they were collected and preserved in air tight container.

EXPERIMENTAL STUDY :

- **Aims and Objectives of Study**
  - To assess the anti-pyretic activity of *Pratapmartand Rasa* in albino rats.
  - To provide a potent, safe and economic antipyretic medicine.
- **Materials :**

1) Pratapmartand Rasa (Test Drug) 2) Paracetamol (Standard Drug)
3) Carboxy methyl cellulose (Control vehicle) 4) Distilled water
5) Albino rats (Wister Strain) 6) Baker's yeast (to induce pyrexia)
7) Normal saline 0.9% (to prepare yeast solution)

Equipment's and Glass Wares :

1. Digital tele thermometer (to record the rectal temperature of albino rats), Glass beakers, 18 No. Needle, 26 No. Needles, Disposable syringes, Stop watch, Hand gloves, Glass rod.

Method :

1. Healthy adult male albino rats (Wister Strain) of 90-120 days old, weighing from 150-200 gms were taken for experimental study.
2. The animals were maintained under strict laboratory condition with controlled environment of temperature, humidity, light and dark cycles.
3. Animals were fed with balanced pellet diet as prescribed by CFTRI, Bengalor (Central Food Technological Research Institute) and water ad libitum.
4. Maximum number i.e. 03 animals per cage were maintained. Animals under different groups of experiments were caged separately.The animals were selected from central animal house of Shri B.M. Kankanwadi Ayurved Mahavidyalaya Shahapur-Belgaum, considering inclusive and exclusive criteria.

EXPERIMENTAL PROTOCOL:

Sample Size :18 albino rats were taken for the experimental study, distributed 6 in each group. Three groups were for antipyretic study.

INCLUSIVE AND EXCLUSIVE CRITERIA:

- **Inclusive Criteria:**
  1. Adult healthy male albino rats.
  2. Albino rats weighing 150-200 gms.
  3. Albino rats between 90-120 days.

- **Exclusive Criteria:**
  1. Unhealthy Albino rats.
  2. Weight range below 150 gms and above 200 gms.
  3. Female albino rats.
  4. Albino rats of age below 90 days and above 120 days.

❖ **Dosage and Mode of Drug Administration:**

Animal dose = Human dose x 0.018.

❖ **Drug Schedule for Experimental Study:**

Antipyretic activity study:

Study	**Group 1**	**Group 2**	**Group 3**
Drug	Carboxy methyl cellulose	Paracetamol	*Pratapmartand Rasa*
Dose	1 ml	9mg/200gm body weight of rat	2.25mg/200gm body weight of rat
Dosage form	Suspension	Suspension	Suspension
Route	Oral	Oral	Oral

Anti-Pyretic study (On Yeast Induced Pyrexia in Albino Rats)

Study group :

Test Group - 2.25 mg Pratapmartand Rasa.
Standard group - 9 mg Paracetamol.
Control Group - 1 ml CMC.

❖ **Method:**

1. The animals were starved for 24 hrs and water ad libitum.
2. The digital tele thermameter cord was lubricated with borax glycerin and initial temperature of the chosen animals was recorded.
3. Preparation of 15% yeast solution For 15 gm of freeze dried baker's yeast (Prestige yeast manufactured by SAF yeast Co. Ltd., Mumbai). 100 ml of 0.9% normal saline was added and triturated though roughly make homogenous solution. Every time fresh yeast solution was prepared and used.
4. Induction of pyrexia was induced by the parenteral administration of 1 ml of yeast solution at the nape region of the rat.
5. The medicines (test drug, standard drug and control) were administered, after 18 hrs of administration of Baker's yeast.

Table Showing the Drug Schedule :*Antipyretic activity study:*

Sr. No	Group	No. of Rats	Drug	Dose
1	Test drug	6	*Pratapmartand Rasa*	2.25mg/200gms of rat
2	Control drug	6	Carboxy methyl cellulose	1 ml
3	Standard drug	6	Paracetamol	9mg/200gms of rat

6. The rectal temperature were noted usingtele thermameter at 30 minsintervals up to60 mins and then at 1 hr interval up to 5 hrs and then every 3rd hr. up to 9th hr.

OBSERVATION AND RESULT:

Shows Organoleptic Characters of *Pratapmartand Rasa :*

Sr. No.	Organoleptic character	Description.
1	Colour	Slightly light scarlet in colour
2	Odour	Faint
3	Taste	Pungent
4	Touch	Amorphous
5	Appearance	Powder

Weight of *Pratapmartand Rasa* = 500 gm

Shows Physico-chemical standards of *Pratapmartand Rasa:*

Sl. No.	Physical constants	Results
1.	Description	Slightly light scarlet in color
2.	Odor	Faint
3	Moisture content	5.37%
4	Ash content	3.88%
5	Acid insoluble Ash	0.41%
6	Water insoluble Ash	98.92%
7.	Bulk Density	2.68 gm/ml
8.	pH Value	7.02

Shows Pharmaceutical Standardization of *Pratapmartand Rasa* pills:

Pharmaceutical Standards	Results
Uniformity Of Weight	123.5 ± 1.4 mg.
Diameter test	5.5 ± 0.30 mm.
Hardness	3.50 kg
Friability	0.440%.
Disintegration Of *Pratapmartand Rasa* pills in acidic media of pH 3.2	26 min.
Disintegration Of *Pratapmartand Rasa* pills in Distilled Water of pH 7.1	28 min.
Disintegration Of *Pratapmartand Rasa* pills in alkaline media Water of pH 9.2	28.30 min.
Dissolution of *Pratapmartand Rasa* pills in acidic media of pH 3.2	1 hour 10 min.
Dissolution of *Pratapmartand Rasa* pills in Distilled Water of pH 7.1	1 hour 15 min.

Dissolution of *Pratapmartand Rasa* pills in Alkaline media of pH 9.2	1 hour 16 min.

- **In Atomic Absorption-Spectrophotometer:**

Sr. No	Content	Results
1.	Mercury	14.61
2.	Sulfur	8.38
3.	Sodium Content	0.31 PPM
4.	Calcium Content	0.28 PPM

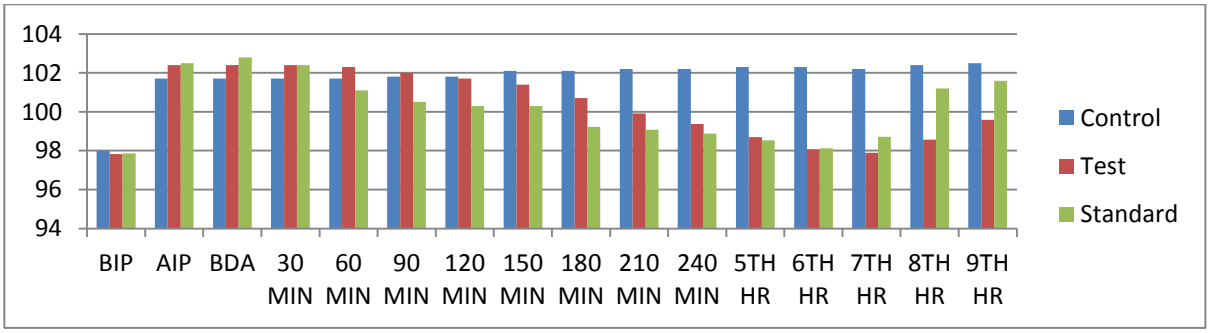
EXPERIMENTAL STUDY :

OBSERVATIONS:

After the administration of baker's yeast, all the albino rats were closely observed for their behavior and symptoms, and the observations noted in all the rats.

1. In all rats increase in temperature was noted after 18 hours of baker's yeast administration.
2. Rats were less active.
3. All the rats were facing downwards after 12 hours
4. Shivering was observed in most of the arts after 4 hours
5. Fur erected was noted.

Graph Showing the comparison between Group 1 (Control Group), Group 2 (Test Group) and Group 3 Standard Group.



CONCLUSION

1. *Pratapmartand Rasa is slightly light scarlet in colour, faint odour, smooth surface, pungent in taste and sparingly soluble in Distilled Water.*
2. *Pratapmartand Rasa (Test Drug) has shown significant antipyretic activity from 1st hour to 8th hour of drug administration.*
3. *Paracetamol (Standard Drug) has shown significant antipyretic activity from 1st hour to 6th hour of drug administration.*
4. *In Control Group antipyretic activity was not observed throughout the study.*
5. *Pratapmartand Rasa shown anti-pyretic activity up to 8th hour, where as standard drug paracetamol shown anti-pyretic only up to 6th hour, from this observation it clearly indicates Pratapmartand Rasa has demonstrated more significant anti-pyretic activity and was sustained up to 8th hour in comparison with paracetomal up to 6th hour.*

6. *Rats were healthy and active in test group due to presence of elements and phytochemical constituents in PratapmartandRasa than control and standard.*
7. *Pratapmartand Rasa contains elements Hg, S, Na and Ca which might have contributed for reducing the elevated body temperature.*
8. *Organoleptic characters, physical constants, elemental analysis, AAS reports and antipyretic activity results may contribute to establish standards for Pratapmartand Rasa.*

This study has given scope for further experimental and clinical study too.

DISCUSSION

Pratapmartand Rasa is a *khalviya Rasayana* prepared by process of trituration. Trituration was carried out in *Pashana khalva* as it is neutral towards ingredients of *Pratapmartand Rasa* .The mixing of contents was done in descending manner for homogenisation. In Trituration shear forces occur which reduces the segregation by thinning of dissimilar layers of solid material.

In this study, all are taken for analysis in which carried out Atomic absorption-Spectrophotometer (AAS), pharmaceutical, Organoleptic, Physico-Chemical characteristics.

The appearance of Raw *Hingula* before *shodhana* was shining bright scarlet in color and after *Shodhana* in *Ardraka Swarasa* it became Brick red in Color. The % of Mercury was 86.12 and % of sulfur 13.09 in Raw *Hingula* and After *shodhana* % of Mercury in *Shodhit Hingula* was 87.29% and sulfur 13.48%

Physico-chemical analysis of *Pratapmartand Rasa* was;

Organoleptic characteristics of *Pratapmartand Rasa* shows Slightly light Scarlet in colour, due to colour of *Hingula* in the ingredients. Faint in odour, pungent taste, amorphous touch and in the powdered appearance. *Pratapmartand Rasa* shows, Moisture Content were 5.37%, Bulk density 2.68 gm/ml and pH value of was 7.02. Total Ash value of *Pratapmartand Rasa* was 3.88% and acid insoluble Ash was 0.41, Water insoluble Ash 98.92%

Pratapmartand Rasa was prepared in pill form and was subjected for pharmaceutical standardization. Average weight was 123.5 mg ± 1.4 mg. Average diameters was 5.5 mm ± 0.30 mm. Hardness of pills was 3.50 kg, the friability of pills was 0.440%. Disintegration time of pills in distilled water, acidic media and alkaline media was 28 minutes, 26 minutes and 28.30 minutes respectively.

Dissolution Time of *Pratapmartand Rasa* in distilled water, acidic media and alkaline media was 1 hour 15 minutes, 1 hour 10 minutes and 1 hour 16 minutes respectively. It is noted that *Pratapmartand Rasa* disintegrated and dissolves earlier in acidic media as compared to other Medias.

- In Atomic Absorption-Spectrophotometer:

Content	**Raw *Hingula***	***Shodhit Hingula***
% Of Mercury	86.12%	87.29%
% of Sulfur	13.09%	13.48%

Content	***Raw Tankana***	***Shodhit Tankana***
% Assay of Borax	93.65%	95.38%
% of Sodium	15.41%	18.28%

Pratapmartand Rasa was subjected for elemental analysis by using Atomic absorption-Spectrophotometer. Analysis was carried out at Late Prin. B.V.Bhide Foundation (For Education & Research in Chemistry, Ayurved & Allied Sciences) Pune.

Elementals detected by elemental analysis were Mercury 14.61%, Sulfur 8.38%, Sodium content 0.31 PPM and Calcium Content 0.28 PPM were noted in *Pratapmartand Rasa,* might have contributed for the antipyretic activity.

Results of organoleptic characters, Physical constants, pharmaceutical constituents, elemental analysis and AAS may contribute to establish standards for standardization and quality control.

Experimental Study (Antipyretic Study):

Pratapmartand Rasa is a herbomineral drug used in *Jwara* chikitsa and prepared according to *Rasendrasarsangraha.* Experimental study on albino rats was carried out, to assess the antipyretic activity. The statistical analysis was carried out by 'ANOVAs' Test followed by Bonferronis Multiple comparison Test.

The antipyretic study was carried out by using yeast induced hyperpyrexia method. 18 male albino rats were selected and distributed 6 in each group and maintained at constant temperature of 24-25^0 C for 24 hours before the experimental study. Initial rectal temperature was recorded individually before inducing pyrexia. Pyrexia was induced by subcutaneous injection of 2 ml of 15% Baker's yeast solution in 0.9% normal saline.

After 18 hours of yeast solution administration rise in temperature was recorded in control group, test group, and standard group and from 97.986 ± 0.451 to 101.663 ± 0.694, 97.816 ± 0.553 to 102.391 ± 0.717, 97.986 ± 0.451 to 101.663 ± 0.694 respectively. Then drugs were administered orally to each group in the form of suspension prepared with compound powder of Carboxy methyl cellulose. Then rectal temperature was recorded every half hour for four hours and hourly for next five hours.

From study it was noted that antipyretic activity of *Pratapmartand Rasa* was found up to 8th hour where as in standard group it was up to 6th hour. It was found to be highly significant compared to that of control group, both group have shown sustained antipyretic activity. It was also noted that standard drug shows rapidly antipyretic activity but it remains up to 6 hrs and Test drug shows delay action compare to standard but it was up to 8th hrs. This action may be due to disintegration time of *PratapmartandRasa* is more than paracetamol.

It was also noted from the study that onset of antipyretic activity of paracetamol started, at one hour of drug administration till 6th hour. Whereas the antipyretic activity of *Pratapmartand Rasa* was also observed at one hour after drug administration and continued till 8th hour. This may be due to longer duration of action.

Antipyretic activity of *Pratapmartand Rasa* may be mainly due to *Agneedeepaka, Amapachak, Swedajanaka* and *Jawaraghna* properties in the ingredients of *Pratapmartand Rasa*.

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