



Evaluation of Physio-chemical and Antipyretic activity of Ananda Bhairava Rasa

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Abstract

Ayurveda is one of the systems of medicine in the world. Among so many systems of medicine it has its own identities. Ananda Bhairava Rasa is one such formulation explained in the Classical text Sidha Prayoga Latika found to be effective in Jwara. The aim of the present study was to develop Ananda Bhairava Rasa and evaluates its physicochemical and antipyretic activity. Raw materials were screened and collected. Preparation of Ananda Bhairava Rasa as per the Classical text Sidha Prayoga Latika was done. The physico-chemical properties namely Hardness, uniform weight, friability, loss on drying, water soluble extractive value, alcohol soluble extractive value, moisture content, pH, total ash, acid soluble ash and disintegration time of Ananda Bhairava Rasa were determined. The antipyretic activity of Ananda Bhairava Rasa vatis were evaluated against brewer's yeast induced pyrexia on Albino rats. The findings of physico-chemical properties of Ananda Bhairava Rasa were under normal value. The Ananda Bhairava Rasa vatis demonstrated significant antipyretic activity.

1 Introduction

Ayurveda defines swastha as one whose physical, spiritual, social and environmental aspects are in a good harmony. Medicinal plants and well processed minerals are assuming greater importance since age old. Indian medicinal plants and their derivatives have been an invaluable source of therapeutic agents to treat various disorders.

In Ayurvedic classics, it is said that Jwara is king of all the diseases. It appears as an independent disease and also as a lakshana of various diseases. In Jwara human beings not only suffer physically but also mentally causing stress, worry and anxiety. No other disease is so severe, so complicated and so difficult to treat as jwara. According to modern science, Pyrexia is defined as body temperature above the normal range due to an increase in the temperature regulatory set-point. Pyrexia is either a symptom of a disease or disease itself¹. It is essential to find an inexpensive, effective and safe remedy which is commonly available and easily administered. In modern science though many antipyretic drugs such as Paracetamol have been used to treat fever but various side effects like

dyspepsia, ulceration, haemorrhage in Gastro intestinal tract, nausea, rashes, heart burn, epigastric distress occurs.

Number of Antipyretic drugs have been mentioned in ayurvedic classical text. Anandabhairava rasa is one such formulation explained in the Classical text SidhaPrayogaLatika found to be effective in Jwara. Ananda Bhairava Rasa is a unique herbo-mineral formulation explained in JwaraChikitsaAdhyaya in various text books. It is a potent formulation having Hingula, Vatsanabha, Maricha and Tankana given bhavana with Nimbuswarasa .

Ananda Bhairavi rasa, which comes under Kharaleeyarasayana, which is the typical combination of drugs to combat the Jwara. Ananda Bhairavi rasa contains shodhitaHingula, shodhitavatsanabha, Tankana, Maricha. Kharaleeyarasayanas medicaments are having both herbal and mineral drugs. Because the herbal constituents will nullify the untoward effects of the minerals and increases the potency of the minerals forming the herbomineral complex^{2,3}.

In the view to contribute to a safe, effective and economical antipyretic formulation, present study is planned to evaluate pharmaceutico-analytical study of Anandabhairava rasa and experimental evaluation for its anti-pyretic activity.

2 Materials and Methods

2.1 Materials

Raw materials were screened and collected.

The formulation selected for the present study **Ananda Bhairava Rasa** was prepared in the P.G Laboratory of Rasa Shastra- Bhaishajya Kalpana, RamaKrishna Ayurvedic Medical College, Bengaluru.

Paracetamol (standard drug), Propylene glycol (control/vehicle), Wister Strain Albinorats, Baker's yeast (to induce pyrexia), Normal saline 0.9% (to prepare yeast solution)

2.2 Methods

2.2.1 Preparation of Ananda Bhairava Rasa

- The homogenous mixture of shodita Hingula, shodita Vatsanabha churna and Maricha churna and Tankana was prepared by mardana.
- Required quantity of Nimbu Swarasa is added and mardana was done.
- The vati of 62.5 mg or ½ Ratti was prepared by smearing hands with goghruata.
- 20 vatis were prepared and kept for drying in shade.

The compositions of Ananda Bhairava Rasa are illustrated in table 1.

Table 1: Ingredients of AnandaBhairava Rasa with their proportions and quantity

Ingredients	Quantity
Shodita Hingula	200 gms
Shodita Vatsanabha	200 gms
Shodita Tankana	200 gms
Maricha	200 gms
Nimbu Swarasa	200 ml

Observations

- The whole mixture turned to red colour.
- At one stage the mixture becomes a single bolus not sticking to the surface of khalva yantra.
- At this stage small amount of mixture was taken in between hands smeared with ghruta and tried to roll in to vatis and this was achieved.

2.2.2 Physio-chemical evaluation

Hardness test

The hardness can be roughly determined by Monsanto Hardness test. It is a soft portable hardness tester. It consists of a spring, which can be compressed by moving the screw knob forward. Reading was noted from the scale, which indicates the pressure required in Kg or in pounds to break the tablet. Hardness of 4 Kg considered suitable for handling of vati.

Uniform weight of tablets

The average weight was determined by weighing 20 tablets. The tablets are also weighed singly. The deviation from the average weight in each case is calculated and expressed as a percentage. Not more than two of the tablets deviate from the average weight by a greater percentage.

Friability

Friability test was performed by Friability test apparatus. A number of tablets are weighed and placed in the tumbling chamber, which is rotated for four minutes of 100 revolutions. During each revolution the tablets fall from a distance of 6 inches to undergo shock. After 100 revolutions the tablets are again weighed and loss in weight indicates the friability. The acceptable limit of weight loss should be not more than 0.8%.

Loss on drying

Pipette out of quantity of drug to yield about 1gm of dry matter mix with a few ml of water and transfer quantitatively to the dish containing prepared sand with aid of water. Mix the sample thoroughly with the sand.

Dry at a temperature not more than 110°C under pressure not more than 50 mm of Hg. Making trail washing at 2 hours interval. Towards end of drying period until successive weighing do not differ by more than 2 mg. Calculate the total solid from the loss of weight on drying.

Water and alcohol soluble extraction

10 grms of powder was weighed separately and added into a 100 ml conical flask. About 25ml of distilled water and alcohol added into it and kept on a rotator shaker (140 rpm) for 24 hrs. After 24hrs it was filtered and dried in hot air oven set at 80°C for 24hr and weighed again, the difference in the weight was determined and water soluble and alcohol soluble extractive were calculated^{4,5}.

Moisture content

1 gm of powder dried at 80°C for 24 hrs in air oven. After 24h, the powder was weighed again and the difference in the weight was determined. The percentage of moisture was calculated.

pH value

The 1% and 10% powder in water was kept on shaker for 5 h with 140 rpm and filtered. The filtrate was analyzed for the pH using pH meter⁶.

Total ash content

The clean and dry crucible (Silica) was weighed and 10 gm of powder was weighed in crucible and powder was turns into ash. The crucible was cooled and weighed again. The difference in the weighed was noted and percentage of total ash was calculated⁷.

Acid soluble Ash

1 gm of ash was added into 10ml of concentrated H₂SO₄. The mixture was kept on a shaker with 140 rpm for 8h and filtered through filter paper. The ash remained in the paper was kept in a crucible (silica) and burnt ash again in a muffle furnace for 3-4 h. The weight of ash obtained was noted and percentage of acid soluble ash was determined⁸.

Determination of disintegration time

Each individual tablet was dropped into a 10-mL glass test tube (1.5-cm diameter) containing 2 mL distilled water, and the time required for complete tablet disintegration was observed visually and recorded using a stopwatch. The visual inspection was enhanced by gently rotating the test tube at a 45- angle, without agitation, to distribute any tablet particles that might mask any remaining undisintegrated portion of the tablets^{9,10}.

2.2.3 Antipyretic activity

Preparation of 15% yeast solution: For 15gm of freeze dried baker's yeast. 100ml of 0.9% normal saline was added and triturated thoroughly to make Homogeneous solution. Every time fresh yeast solution was prepared and used.

Total 18 albino rats of either sex weighing between 150 -200 g was taken and divided into 3 groups, each containing 6 animals. All healthy albino rats which were selected for the experiment was kept under fasting for 18 hrs. Initial normal rectal temperature of all the animals was recorded by using a digital thermometer. Fever was induced by using 20% of Brewer's yeast solution which was injected subcutaneously in all albino rats in the dose of 2g/10ml body weight and was placed in the cages kept for them. Then the rectal temperature of each rat was noted 18th hr after the injection of Brewer's yeast. After 18th hour of injection of yeast, corresponding test drug was administered to respective group. Rats of group 1st which was administered with distilled water served as Control. Animals of group 2nd will be administered with Paracetamol suspension at the dose of 100 mg/kgwt of body weight by using the feeding syringe to serve as reference Standard. Similarly, Rats of group 3rd was administered with the Ananda Bhairava Rasa [Trial group] in a dose of 11.25mg/kg body wt. After administering

corresponding drug to each group hourly rectal temperature of each rat was noted for 4 hours and after 24 hours.

2.3 Statistical analysis

The results are expressed as mean \pm SEM of six independent experiments. Statistical significance between the groups was evaluated by one-way analysis of variance (ANOVA) followed by Dunet's test. A P < 0.05 value was considered as statistically significant.

3 Results and Discussions

When mass had stopped sticking to the walls of khalvyantra and part of it was taken in between fingers it got flattened, edges of it were not cracking and finger prints were appreciating on its surface, then it was allowed to drying. Colour change was observed during the process.

Before the preparation, the weight of ingredient was 600 gms. After the preparation they weighed 580 gms. It was found that there was a loss of 20 gms after the preparation. The loss may be due to procedures like mardana.

3.1 Physio-chemical evaluation

After using classical parameters for identification (grahya-agrahya lakshanas) raw drugs were selected. A fragrant odour was appreciated at the end in the prepared sample. This may have been due to trituration with Nimbu swarasa.

3.1.1 Hardness

The prepared vatis showed hardness of 3.5+0.5 kg (Table 2).

3.1.2 Uniformity of weight

For the present study vatis were prepared manually 20 vatis taken randomly and weighed. The average weight was calculated. The weight variation falls within normal limits (Table 2).

3.1.3 Friability

The loss in total weight of the vatis due to friability was 0.15% (Table 2) and the friability value is less than 1% which ensures that formulated vatis were mechanically stable.

3.1.4 Loss on drying

Ananda Bhairava Rasa shows that the end product contains 2.69% of moisture content. This test was to detect the moisture and volatile content in the sample (Table 2).

3.1.5 Moisture content

The percentage of moisture content of vatis was found to be 2.69% w/w (Table 2).

3.1.6 pH

Mean pH value of the vati is 6.45 which shows that the prepared medicine was minor acidic nature (Table 2).

3.1.7 Solubility test

It is sparingly soluble in H₂O and slightly soluble in alcohol solvent. By this it is understood that Ananda Bhairava rasa may be absorb slowly in GIT. The values of water and alcohol solubility were demonstrated in table 2.

3.1.8 Total ash

It is the criteria for identity or purity of drugs. Total ash is inclusive of physiological ash derived from plant tissue and non physiological ash consists of residue of the extraneous matter such as sand, soil etc. adhering to the herb itself.

The herbal drugs were converted in to ash form resulting in to weight loss. The total ash and acid insoluble ash of vatis were 12.28% and 11.88%, respectively (Table 2).

Table 2: Physio-chemical evaluation of Ananda Bhairava Rasa vatis

Parameters	Values
Hardness in Kg	3.5±0.5
Weight variation	4.28%
Friability	0.15%
Loss on drying	5.84%
Alcohol extractive	24.54% w/w
Water extractive	69.44% w/w
Moisture content	2.69% w/w
pH at 10%	6.45±0.41
pH at 1%	7.17±0.23
Total Ash	12.28%
Acid insoluble ash	11.88%
Disintegration time (min)	3.82±0.05

Values are mean ± SEM of triplicate determinations

3.1.9 Disintegration test

It was observed that the vatis disintegrated in 3.82 min. As bhavana was done with Nimbukaswarasa, which contain acidic properties, this may act as loose binding agent and disintegrate fast.

3.2 Antipyretic activity

The test item Ananda bhairava Rasa (Group III) showed antipyretic activity in yeast induced hyperthermia in rats. The test item III (trial group) showed a decrease in rectal temperature from an initial 40.13±0.11 °C to 3.18 ± 0.07 °C tested at 24 hours after treatment. Paracetamol, tested as positive control showed a decrease in rectal temperature from initial 39.96±0.10 to 37.13±0.10 °C tested at 24 hours after

treatment. The untreated control rats showed mean rectal temperature of 40.13±0.11 °C to 37.42±0.11 °C tested at 24 hours (Table 3).

The antipyretic activity in control group and standard group when compared showed more significance in standard group P<0.005. The antipyretic activity in control group and trial group when compared showed more significance in trial group P<0.05. The antipyretic activity comparison between standard group and trial group showed more significance in standard group. i.e, paracetamol had more significant anti pyretic activity than Drug Ananda bhairava rasa at the tested concentration of 11.25 mg/kg BW.

Fever is a surrogate marker for disease activity in many infectious and inflammatory disorders. According to the classical view, the genesis of fever is induced by inflammatory mediators (i.e., cytokines, namely interleukin-1, interleukin-6, tumor necrosis factor, and others) that are predominantly released by activated peripheral mononuclear phagocytes and other immune cells. Due to the fact that direct access of the large hydrophilic cytokine proteins to the temperature-controlling brain structures within the pre-optic/anterior hypothalamic areas is prevented by the blood–brain barrier, the mechanisms described below have been suggested for producing pyrexia.

Cytokines which are transported by the bloodstream could act at sites lacking a tight blood–brain barrier, the so-called circumventricular organs. Alternatively, circulating cytokines could interact with their specific receptors on brain endothelial cells or perivascular cells and thereby stimulate these cells to release pyrogenic mediators into the abluminal brain tissue. It has been proposed that fever-promoting cytokines are transported from the blood into the brain via specific carriers.

An assumed manifestation of a febrile response produced by these mechanisms is termed as the humoral hypothesis of fever induction. Within the brain, prostaglandin E₂ (PGE₂), produced by cyclooxygenase (COX)-2, is regarded as the principle downstream mediator of fever acting on thermosensitive or thermointegrative hypothalamic neurons.

Paracetamol is an analgesic but is also an effective febrifuge. It is a poor inhibitor of cyclooxygenase in the presence of peroxides that are found in inflammatory lesions. In contrast, its antipyretic effect may be explained by its ability to inhibit cyclooxygenase in the brain, where peroxide tone is low. Further, it does not inhibit neutrophil activation. In supra-pharmacologic doses it inhibits NF-kB stimulation of inducible nitric oxide synthase.

In the present study both the control group and trial group the rise in temperature was consistent and significant in comparison to the initial values.

Both the drug samples paracetamol and Ananda Bhairava rasa produced very good antipyretic effect in a dose-dependant

manner and the observed effect of Ananda Bhairava Rasa was almost similar to that in the paracetamol treated group.

Table 3: Antipyretic activity of Ananda Bhairava Rasa vatis

Groups	Rectal temperature at time (hrs) post 18 hrs of Yeast infusion							
	0	1	2	3	4	8	16	24
Group I (Control)	40.13±0.06	40.23±0.05	40.31±0.10	40.18±0.18	39.63±0.12	39.36±0.10	38.91±0.15	38.42±0.11
Group II (Standard)	39.96±0.10	38.85±0.45	38.11±0.31*	37.73±0.21*	37.40±0.14*	37.20±0.31	37.13±0.14	37.13±0.10
Group III (Trial)	40.13±0.11	39.78±0.10	38.54±0.07*	38.03±0.42*	37.73±0.16*	37.40±0.18	37.26±0.12	37.18±0.07

Values are expressed as mean ± SEM (Number of animals, n=6); significantly different at *P<0.05 when compared with control group

4 Conclusion

The formulation Ananda Bhairavi Rasa has multiple pharmacotherapeutic properties. Among them Jwaragna property was studied with respect to antipyretic activity. Jwara is one of the basic pathology in all most all diseases. Jwaragna property of drug has multi systemic actions. All the analytical values of Ananda Bhairavi rasa found almost in normal limits as per physico-chemical analysis reports. The antipyretic activity of Ananda Bhairavi Rasa was compared with standard drug Paracetamol. Hence forth Ananda Bhairavi Rasa can be used clinically for antipyretic activity which is comparatively safer compared to Paracetamol.

5 Conflict of interest

We declared that we have no conflict of interest.

6 Author contributions

VN and JKB have carried out the research work in the laboratory. VN compiled and analyzed the data of present work. Both authors approved the final manuscript.

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