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Antipyretic and analgesic activity of *Amalakyadigana* - An experimental evaluation

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ABSTRACT

During the current times endurance to bear the symptoms of fever is less with a lowered pain threshold and the most of the individuals demand for instantaneous relief from pain and fever. Antipyretic and analgesic drugs that are being prescribed in modern medicine give instantaneous relief but public with some amount of awareness about the side effects wish to opt for safe and effective herbal remedies. This attitude necessitates the research for the evaluation of fast acting analgesics and antipyretics. Keeping this in view, a study has been planned to evaluate the antipyretic and analgesic properties of *Amalakyadigana* (total *Gana* - *Amalaki, Haritaki, Pippali, Chitraka*) in experimental animals.

Key words: *Fever, Analgesic, Research, Amalaki.*

INTRODUCTION

In Ayurveda there are numbers of formulations both herbal and herbo-minerals mentioned for treating *Jwara* viz., *Tribhuvankirtirasa*, *Mrityunjayarasa, Sudarshan Churna, Jwaraghnadashemani* (*Charaka*) etc. In *Charaka Samhita, Acharya* has described antipyretic drugs under the name of "*Jwaraharadashemani*" and analgesic drugs in "*Shoolaprashamana*", "*Vedanasthapana*" and "*Angamardaprasamana*"[1] groups, *Sushruta* suggests the *Ganas* namely *Guduchyadi, Amalakyadi, Dashamoola, Aragvadhadi, Patoladi, Sarivadi* and *Triphala* under *Jwarahara* action. And *Varunadi Gana, Virtarvadi Gana, Pippalyadi Gana*[2] and *Bruhatyadi Gana* are suggested for the management of *Shoola.*

Recent researches indicate herbs namely *Guduchi, Manjishtha, Parooshak, Nagavalli, Sariva, Vasa, Trivruta, Bharngi, Saptaparni, Katphala, Usheera, Dhanyaka, Rajapatha, Parpataka, Methika*[3] and formulations namely *Tribhuvankirtirasa, Agnisthayi Hingula, Santapanashak, Mritasanjivanirasa, Panchatiktavati, Jayavati, Samshamanivati, Patoladikashaya-Ghanvati- Arishta, Madhukadi Kashaya* are having antipyretic activity in animal experiments. In clinical studies herbs namely *Bhumyamalaki, Kiratatikta, Guduchi, Musta, Parpataka, Karanja, Tulasi, Maricha, Kantakikaranja, Nimba, Parijata, Sharpunkha, Dronapushpi, Pushkarmoola, Rasona, Tila, Bhramarchullika, Clerodendrum inermis, Leucas cephalotes*, and *Saptaparna* were studied in the treatment of *Jwara* while formulations namely *Tribhuvankirtirasa, Jwarakesarirasa, Hinguleshvara Rasa, Sudarshan Churna, Laxminarayanarasa, Tiktashatpala Ghruta, Ratnagiri Rasa, Panchatitka Ghanvati, Achintyashakti Rasa, Dashmoola, Chandanabalalakshaditaila, Bharangyadikwatha, Pippalyadighruta, Sutshekhar Rasa, Kiratadi Kwatha, Kshudradi Kwatha, Guduchisaptaka Yoga, Kalingakadi Yoga, Prataplankeshwar Rasa, Mustadikwatha, Vi-nine Yoga, Shatyadi Yoga,* and *Hartalbhasma* were studied in the

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treatment of *Jwara*. Certain herbs were screened for their analgesic activity namely *Parooshaka*, *Manjishtha*, *Saptaparni* and *Erandamoola*; some formulations were also studied namely *Santapanashaka*, *Mrutasanjivanirasa* and *Pindataila.*

PHARMACOGNOSTICAL STUDY

1. **Organoleptic study:** Dried powder of fruits *Emblica officinalis* Gaertn., *Terminalia chebula* Retz., *Piper longum* Linn. and root of *Plumbago zeylanica* Linn. Were evaluated by organoleptic characters like taste, odour, colour and touch.
2. **Macroscopic study:** The macroscopic characters of fruits *Emblica officinalis* Gaertn. *Terminalia chebula* Retz., *Piper longum* Linn.; and root of *Plumbago zeylanica* Linn. were studied systematically as per the standard textbook of Botany, as well as with the help of floras.
3. **Microscopic study:**

(a) Root and fruit:

Transverse section of root and fruit had been taken and photography had been done after proper mounting and staining with different staining reagents.

(b) Powder microscopy:

Powders of the samples of 4 drugs were studied microscopically for their characters.

4. Histochemical tests

Some Histochemical tests were performed to detect crystals, lignified elements and starch grains.

(a) Test for crystals of calcium oxalate (stain-1)

They are insoluble in glycerine and phenolic reagents but soluble in hydrochloric acid. On adding a drop of hydrochloric acid to the section/powder; calcium oxalate crystals were dissolved and effervescence was seen.

(b) Test for fibers (Lignified elements) (stain-2)

Section/powder was treated with phloroglucinol and hydrochloric acid. Sclerenchymatous fibres turned pink in colour.

(c) Test for starch grains (stain-3)

Sections and powder were treated with a drop of iodine. Starch grains turned blue when examined microscopically.

Fig. 1: *Amalaki* Fruit

Analytical Study

Physico-chemical analysis provides the objective parameters to fix up the standards for quality of raw drugs as well as finished products. With the help of analytical studies, it is possible to standardize the plant and drug, differentiate the adulterants, which is the need of present era. Analytical study is the application of a process or a series of processes in order to identify and authenticate or quantify a substance, the

ISSN: 2456-3110 ORIGINAL ARTICLE April 2023

components of a solution or mixture, or the determination of the structures of chemical compounds. Phytochemical investigations are important part, before pharmacological evaluation since it provides fundamental idea regarding its probable pharmacological active constituents. Physicochemical parameters provide important information regarding nature of drug either simple or compound formulation, its probable shelf-life, how to preserve the sample etc., which are very useful for converting a single raw drug into a dosage form. In the present study, powder of *Amalakyadigana* is analyzed through physico-chemical parameters and HPTLC studies.

Materials and Methods

Physico chemical analysis

Coarse powder of *Amalakyadigana* was used to carry out following parameters as mentioned in Ayurvedic Pharmacopoeia of India.[4]

- Loss on drying at 105°C.
- Ash value.
- Water soluble extractive value
- 4.Alcohol soluble extractive value.
- pH value

1. Determination of loss on drying

The loss on drying was determined by taking 2g accurately weighed sample, in a petri dish (tarred evaporating dish) and dried in an oven at 110°C till constant weight. The weight after drying was noted and loss on drying was calculated. The percentage was calculated on the basis of air-dried sample.

2. Determination of Ash value

1g accurately weighed sample was taken in a pre-weighed dried crucible. It was incinerated in a muffle furnace up to 450ºC. The crucible was taken out, self-cooled and weighed immediately. From the weight of the ash, the ash value was derived with reference to the air-dried drug. It was calculated and expressed as % w/w.

3. Determination of water soluble extractive

About 5g accurately weighed, sample was macerated with 100 ml of distilled water in a closed flask for 24 hours, shaking frequently during 6 hours and allowed to stand for 18 hours. Filtered rapidly, taking precaution against loss of solvent and 20 ml of the filtrate was evaporated to dryness in a tarred flat bottom shallow dish. First dried over water bath and then at 110°C in hot air oven, to constant weight and weight was noted down. From the weight of the residue the percentage of water soluble extractive was calculated with reference to air-dried sample.

4. Determination of alcohol soluble extractive

About 5g accurately weighed, sample was macerated with 100 ml of methanol of the specified strength (95%) in a closed flask for 24 hours; shaking frequently during 6 hours and allowed to stand for 18 hours. Taking precaution against loss of solvent, it was filtered and 20 ml of the filtrate was evaporated to dryness in a tarred flat bottom shallow dish and dried at 110°C to constant weight, and weight was noted down. From the weight of the residue the percentage of alcohol soluble Extractive was calculated with reference to air dried sample.

5. Determination of pH

A 5% w/v aqueous solution of the samples was prepared, it was filtered and pH of the filtrate was estimated by filter paper.

HPTLC:

Steps involved in HPTLC

- Selection of chromatographic layer.
- Sample and standard preparation.
- Layer pre-washing, Layer pre-conditioning.
- Application of sample and standard.
- Chromatographic development.
- Detection of spots.
- Scanning.
- Documentation of chromatic plate.

ISSN: 2456-3110 ORIGINAL ARTICLE April 2023

HPTLC of sample were carried out by employing different conditions as mentioned.

Chromatographic Conditions

Track-1: Methanolic extract of *Amalakyadigana*

Stationary phase: Precoated Silica gel GF 254 Plates (E-merck)

Application mode: CAMAG Linomat V Hamilton Syringe

Mobile phase: Toluene: Ethyl acetate: acetic acid (7.5: 2: 0.5)

Development chamber: CAMAG Twin trough chamber (20 x 10 cm-)

Chamber saturation: 30 min

Scanner: CAMAG Scanner III

Scanning mode: Linear at 254 nm and 366 nm

Spray Reagent: Vanillin : Sulphuric acid.

Detection: 1) Deutarium lamp, Mercury lamp

2) In day light after Spraying with Vanillin : Sulphuric acid

Data system: WINCATS software (Ver. 3.17)

Drying device: Oven

U.V. Spectrum: 200 nm to 700 nm

Densitometric evaluation of HPTLC plate

Densitometric scanning was performed with a Camag T.L.C. scanner III in reflectance absorbance mode at 254nm and 366nm under control of CATS software (V3.15 Camag). The slit dimension was 6 mm x 0.45 mm and the scanning speed was 10mm.

Table 1: Results of physico-chemical parameters of *Amalakyadigana.*

SN	Parameters	Values
1.	Loss of drying	7.146 % w/w
2.	Ash value	5.294 % w/w
3.	Water soluble extractive	42 % w/w
4.	Alcohol soluble extractive	34 % w/w
5.	pH	3.5 % w/w

Table 2: Results of HPTLC of *Amalakyadigana*

Sample	Solvent System	Detection under long UV Radiation [254]		Detection under long UV Radiation [366]	
		No. of Gf Spots	Rf Value	No. of Gf Spots	Rf Value
Amalkayadi Gana	Toluene: Ethyl acetate: acetic acid (7.5: 2: 0.5)	4	0.3, 0.18, 0.680.71	4	0.3, 0.18, 0.680.71

Experimental Models

Antipyretic activity

Yeast induced pyrexia in Wistar albino rats.

Analgesic activity

a) Formaldehyde induced paw licking in Wistar albino rats.

b) Radiant heat induced pain in in Wistar albino rats.

Test drug

a) Decoction of *Amalakyadigana*: Decoction was prepared by standard reference given in *Sharangadhar Samhita*, 50g of test drug taken and 400ml of water added in it boiled up to 4th part (100 ml). This decoction was administered orally at dose of 0.9 ml/200 g body weight.

b) Alcoholic extract of *Amalakyadigana*: Alcoholic extract was prepared by infusion (400 ml methanol and 40 g powder of sample) with occasional shaking, and filtered and evaporated the liquid portion. Yield was 18.69% w/w and the extract was diluted in distilled water.

Dose calculation: The dose for experimental study was calculated by extrapolating the human dose to animal dose based on the body surface area ratio using the

ISSN: 2456-3110 ORIGINAL ARTICLE April 2023

table of Paget and Barnes4 (1964) as follows, = Therapeutic human dose × Body surface area ratio (convertibility factor) for rat

Dose for Rats = Human dose × 0.018 as convertible factor for rat weighing 200gm.

Statistical analysis

The obtained data has been presented as Mean ± SEM, difference between the groups, statistically determined by student's 't' test for paired and unpaired data to assess the statistical significance between the groups. The value $P < 0.05$ is considered as statistically significant.

1) Brewer's yeast induced pyrexia method

This method is explained by Gujral*et al*. 1995 and also by Poonam*et al.* 1989. Brewer's yeast is used as a pyrogen& is adopted in experimental animals, albino rats for inducing pyrexia which is last up to 8-12 hours. Animals of either sex weighing between 200±20 g were divided in to three groups containing six in each group for this experiment as follow-

Group I - Normal Control

Group II - *Amalakyadigana* decoction (4.5ml/kg) (AGD)

Group III - Alcoholic extract of *Amalakyadigana* (50mg/kg) (AGAE)

Animals were kept on fasting overnight, but were provided with drinking water. Next morning, the initial rectal temperatures of all rats were recorded. Then fever was induced by injecting suspension of 12.5% dried Brewer's yeast in normal saline subcutaneously in a dose of 1ml/100g body weight. After one hour of induction of fever, the respective test dosage forms of drug were administered and distilled water to control group. The rectal temperature was recorded after 3hrs., 6hrs. and 9hrs. of drug administration. The difference between actual rectal temperature and initial rectal temperature were registered for each time interval. The maximum reduction in rectal temperature in comparison control group was recorded.

2) Analgesic activity

The analgesic activity of test drugs were evaluated against radiant heat induced pain in rats and formaldehyde induced paw licking in rats.

a) Tail flick method[5]

The tail flick response was measured with the help of an Analgesio meter (INSIF Ambala). Animals of either sex weighing between 200±20 g were divided in to four groups containing six in each group for this experiment are as follow:

Group I - Normal Control

Group II - Standard reference, Pentazocine sodium (20mg/kg,ip)

Group III - *Amalakyadigana* decoction (4.5ml/kg) (AGD)

Group IV - Alcoholic extract of *Amalakyadigana* (50mg/kg) (AGAE)

Tail flick response was evoked by placing rat tail over a nichrome wire heated electrically. The intensity of heat produced by nichrome wire was adjusted so that the base line tail flick latency averaged 3-4 s in all the animals. Cut off period of 15 s was observed to prevent the damage to the tail. Tail flick response was measured 3 times in each animal initially to obtain basal value. After initial reading, the test drugs and standard drug were administered to respective groups. Tail flick response was again recorded after 30, 60, 120, 180, 240 min. of drug administration.

The difference between actual values and initial values were registered for each time interval. The changes in tail flick response were calculated and results were compared with control group.

b) Formaldehyde induced pawlicking[6]

The effect of *Amalakyadigana* on formaldehyde-induced paw licking response was evaluated by adopting the procedure explained by Hunskar S *et al.* Formaldehyde injection produces inflammation in the rat paw, which produces pain as well as edema. The first half an hour after the injection of formaldehyde is important for observing analgesic activity as it was marked by pain. Wistar strain albino rats of either sex weighing between 200 ± 20g were used. The rats were divided into four groups of six each as follow:

Group I - Normal Control

Group II - Standard reference, Diclofenac sodium (5 mg/kg, ip)

ISSN: 2456-3110 ORIGINAL ARTICLE April 2023

Group III - *Amalakyadigana* decoction (4.5ml/kg) (AGD)

Group IV - Alcoholic extract of *Amalakyadigana* (50mg/kg) (AGAE)

Rats were provided with food and drinking water up to the start of the experiment. One hour after administration of drug, pain was induced by injecting 0.1ml of 1% formalin in distilled water in sub plantar aponeurosis region of left hind paw s/c. The duration of paw licking as an index of nociception was counted in periods of 0-10min (early phase), 11-20 min and 21-30 min (Late phase). The time taken for the onset of the response and frequency of paw licking during different time intervals for 30 min. were observed. The delay in onset and decrease in the frequency of paw licking after formaldehyde injection in test drug treated rats is considered to indicate analgesic effect of the drug

DISCUSSION

Analytical study shows some of the physicochemical parameters like loss on drying, ash value etc. which can give an idea about their values which can be used as standard for further study regarding the purity and strength of *Amalakyadigana* powder. Since no Pharmacological study has been carried out to test the efficacy of *Amalakyadi Gana* formulation with their antipyretic and analgesic activities. The present study was planned for experimental evaluation of antipyretic and analgesic activity of decoction and alcoholic extract *Amalakyadigana.*

Fever is a complex physiologic reaction to disease involving a cytokine-mediated rise in body temperature, generation of acute-phase reactants, and activation of numerous physiologic, endocrinologic, and immunologic systems. The temperature of the body is dependent on maintaining a balance between the production and dissipation of heat. Under normal circumstances, heat is generated internally during metabolic processes or when external environmental temperatures exceed those of the body. It is now clear that most antipyretics work by inhibiting the enzyme cyclooxygenase and reducing the levels of PGE2 within the hypothalamus. Recently, other mechanisms of action for antipyretic drugs have been suggested, including their ability to reduce pro inflammatory mediators, enhance anti-inflammatory signals at sites of injury, or boost antipyretic messages within the brain. Although the complex biologic actions of antipyretic agents are better understood, the indications for their clinical use are less clear. Brewer's yeast is a fungi containing lipo-polysaccharide, which is a cell wall component of gram negative bacteria. It binds with macrophages, releasing cytokines, interleukin – 1 etc. into the blood circulation, leading to antigen-antibody reaction. It reduces blood brain barrier and releases arachidonic acid mediated by the enzymes phospholipase, prostaglandin E2 synthase, and cyclo-oxygenase. Finally, synthesis and release of PGE2 into anterior hypothalamus resulting in pyrexia. AGD produced non-significant marked decrease in rectal temperature after 3 hours, 6 hours and 9 hours. Alcoholic extract of drugs produced non-significant and marked decreases in rectal temperature after 3 hours and 9 hours and there was statistically significant decrease in rectal temperature after 6 hours compared to control group was noted which is likely due to inhibition of the synthesis and/or release of local PGE2 into the preoptic area of anterior hypothalamus.

Analgesic activity

Considering the relationship between anti-inflammatory and analgesic effect, another objective of the present work was to study the anti-nociceptive activity of test drugs. The models investigating anti-nociception were selected based on their capacity to investigate both centrally and peripherally mediated effects. The tail flick method investigates the central activity, while formalin based model investigates both. In analgesic activity study, the analgesic testing protocol was selected such that both centrally and peripherally mediated effects could be ascertained by adopting formalin induced pain and tail flick response methods. Formalin injection to plantar aponeurosis of rats shows pain response in two phase's viz., initial and late phase. The initial phase lasts for 0-10 min. of formaldehyde injection; it is supposed to be mediated through modulation of neuropeptides17. The second phase, which is observed 20-30 minutes of

ISSN: 2456-3110 ORIGINAL ARTICLE April 2023

formaldehyde injection, is supposed to be mediated through release of inflammatory mediators like prostaglandin etc. Both the forms of test drug show statistically significant increase in latency onset of paw licking after formaline injection given in rats. Both forms of test drugs failed to inhibit first phase (0-10min) of formalin induced pain response while apparently inhibited the second phase of pain response (11-20min), but values were statistically non-significant. Again, test drugs failed to inhibit late phase (21- 30min) of formalin induced pain response compared to control group. Tail flick model, which is thermal induced nociception, indicates narcotic involvement, which is sensitive to opioid μ receptors (Abbott and Young, 1988). Both forms of test drug treated group did not show any significant increase in tail flick response compared to initial reading as well as control group up to 2 hours. However, both forms of test drug treated group shows non-significant increase in tail flick response after 180 and 240 mins compared to initial reading as well as control group.

Conclusion

The result of the present study suggests that decoction of *Amalakyadigana* produced marked decrease in rectal temperature compared to control group. Alcoholic extract of drugs produced pronounced non-significant and marked decrease in rectal temperature after 3 hours and 9 hours and there was statistically significant decrease in rectal temperature after 6 hours compared to control group. It may be due to inhibition of the synthesis and/or release of local PGE2 into the preoptic area of anterior hypothalamus. Both forms of test drug formulations produced non-significant increase in analgesic activity against radiant heat induced pain in rats which shows the ability of the drugs to prolong the reaction latency to thermally induced pain in albino rats as central analgesic activity. Further both forms of drug have no effect on first phase and have mild effect on second phase of pain response in formalin induced paw licking in rats.

References

1. Agnivesha, Charaka, Dridhabala, *Charaka Samhita*, Edited by Yadavjitrikamji Acharya, Reprint, Chaukhambha Surabharati Prakashana, Varanasi, 2011.
2. Vridhha Sushruta, Acharya Sushruta, Nagarjuna, Chandrat, *Sushruta Samhita*, With commentary *Nibandhsangraha* of Dalhanacharya, Edited by Acharyayadavaji trikamji, Reprint, Chaukhambha Surabharati Prakashan, Varanasi, 2008.
3. Vagabhata, *Astanga Samgraha,* Edited by Kaviraj Atridev Gupta, Chaukhambha Krishnadas Academy, Banaras,2005.
4. Ayurved Pharmacopeia of India part 2. Volume 2, appendix 5, Ministry of Health and Family Welfare, 2008.
5. O'Dell, T; Wilson, L; Napoli, M; White, H; Mirsky, J. (1960). "Pharmacology of a Series of New 2-Substituted Pyridine Derivatives with Emphasis on their Analgesic and Interneuronal Blocking Properties". *Journal of Pharmacology and Experimental Therapeutics*. 128: 65–74. PMID 14428053.
6. Sarika, A.; *et al*. Anti-inflammatory activity of Lactobacillus on carrageenan-induced paw edema in male wistar rats. *International Journal of Inflammation*. 2012, (2012-01-10), 2012(1), 752015.

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