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Pharmacological Study

Experimental evaluation of antipyretic and analgesic activities of *Amalakyadi Gana*: An Ayurvedic formulation

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Abstract

Introduction: In Ayurvedic classics, the symptom fever is considered as a separate disease called *Jwara*. Acharya Sushruta has mentioned *Amalakyadi Gana* for treatment of all types of *Jwara*, which contains four drugs namely *Amalaki* (*Emblica officinalis* Gaertn.), *Haritaki* (*Terminalia chebula* Retz.), *Pippali* (*Piper longum* L.), and *Chitraka* (*Plumbago zeylenica* L.). **Aims:** To evaluate the antipyretic and analgesic activity of *Amalakyadi Gana* in experimental animals. **Materials and Methods:** Decoction and alcohol soluble extract of *Amalakyadi Gana* were used in the present study. Antipyretic activity of dosage forms were carried out against yeast-induced pyrexia in Wistar albino rats. Analgesic activity was evaluated using radiant heat model and formalin induced paw licking in Wistar albino rats. **Results:** In yeast-induced pyrexia model, both dosage forms of test drug produced marked decrease in rectal temperature after 3 h, 6 h, and 9 h among which extract produced statistically significant decrease after 6 h compared to control group. In the tail flick method, both forms of test drug showed insignificant increase in tail flick response after 180 and 240 min compared to control group and in formalin induced paw liking model decoction form of test drug significantly increased the latency of onset of paw licking and decreased the paw licking in early phase while alcoholic extract produced insignificant effect compared to control group. **Conclusion:** Decoction and alcoholic extract of *Amalakyadi Gana* has moderate antipyretic activity in rats, which may be due to inhibition of the synthesis and/or release of local PGE_2. Further, *Amalakyadi Gana* has mild analgesic effect through central and peripheral mechanism. The result of the present study provide further scope for development of new palatable dosage form and tested clinically for better efficacy.

Key words: *Amalaki, Amalakyadi Gana,* analgesic, antipyretic, *Chitraka,* decoction, *Haritaki, Pippali*

Introduction

Amalakyadi Gana is an Ayurvedic formulation described by *Sushruta* in *Sushruta Samhita*.[1] It contains four herbs namely *Amalaki* (*Emblica officinalis* Gaertn.), *Haritaki* (*Terminalia chebula* Retz.), *Pippali* (*Piper longum* Linn.) and *Chitraka* (*Plumbago zeylenica* Linn.). This formulation is indicated in all types of *Jwara* (fever). Ingredients of this *Gana* are also individually used in treatment of *Jwara*. Many Ayurvedic compendia such as *Bhaishajyaratnavali*, *Yogarathnakara*, *Gadanigraha*, *Chakradatta* and *Chikitsakalika* have quoted the same *Gana* for the management of *Jwara*,[2-5] which reflects the importance of this group of drugs in clinical practice. From the ingredients of *Amalakyadi Gana*, *Amalaki* is proved to be anti-inflammatory and antipyretic by experimental studies[6] while *Chitraka* and *Haritaki* are proved to be analgesics in experimental studies.[7,8]

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Jwarahara drugs are considered as antipyretics in modern medical science. All the antipyretic drugs, which reduce the elevated body temperature by inhibition of prostaglandin synthesis, are also reducing the pain sensation by same mode of action. Non-steroidal anti-inflammatory drugs (NSAIDs) are most frequently used antipyretic and analgesic agent in current times but associated with many side effects.[9] Indigenous drugs possessing fewer side effects should be looked for as a better alternative for the treatment of pain, inflammation, and pyrexia.[10] Thus in the light of above and considering wide usage of *Amalakyadi Gana*, it was thought useful to undertake antipyretic and analgesic activity of two dosage forms *Amalakyadi Gana* in rats to substantiate its ancient claim.

Materials and Methods

Drug and chemicals

Fruits of *Amalaki* and *Haritaki* were collected in the month of January (2012) and *Chitraka* was collected in the month of March (2012) from Sasoi Botanical Garden, Gujarat Ayurved University, Jamnagar. *Pippali* was procured from local market of Jamnagar in March (2012). The drug materials were authenticated and voucher specimens of each drug (Phm 6067/2011, Phm 6033/2011, Phm 6036/2011 and Phm 6034/2010 for *Amalaki*, *Haritaki*, *Chitraka* and *Pippali* respectively) submitted to Pharmacognosy Laboratory, IPGT and RA, Gujarat Ayurved University, Jamnagar, India. Ingredients were cleaned properly to remove any type of contamination. Washed ingredients were dried and coarse powder was prepared and stored in airtight container until further use. Pentazocine Lactate IP (Fortwin brand, Ranbaxy Laboratories Limited, India) and Diclophenac sodium (Voveran D brand, Novartis India Limited, India) were used as standard drugs.

Preparation of test drugs

Decoction of *Amalakyadi Gana* was prepared by standard reference given in *Sharangadhara Samhita*.[11] Coarse powder (50 g) of mixture and 400 ml water was added; boiled on low to medium heat until the liquid portion was reduced to 1/8th part of the original volume (50 ml) and filtered. Alcoholic extract of *Amalakyadi Gana* was prepared by infusion method.[12] A volume of 400 ml methanol was added to 40 gm coarse powder of sample with occasional shaking, filtered, and liquid portion was evaporated. Yield was 18.69% w/w.

Animals

Wistar albino rats (200 ± 20 g) of either sex were used for the study. The rats were acclimatized to laboratory condition for 7 days before commencement of the experiment. The animals were maintained under standard laboratory conditions in terms of 12 h light and dark cycle, temperature (23 ± 2°C) and relative humidity (50–60%) with access to Amrut brand rat pellet feed and drinking water *ad libitum*. Food was withdrawn overnight before and during the experimental hours. The experimental protocol was approved by the Institutional Animal Ethical Committee (IAEC/12/2013/07) as per guideline of Committee for the Purpose of Control and Supervision on Experiments on Animals, India.

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 table of Paget and Barnes.[13] Accordingly human therapeutic dose of decoction (50 ml/day) converted to rat dose as 4.5 ml/kg and alcoholic extract (500 mg/day) converted to rat dose as 45 mg/kg, orally. Rat dose of test drug was fixed on the basis of human therapeutic dose mentioned in the literature.[11]

Antipyretic activity

Rats were randomized in to three groups, each consisting of six animals. Group I control group, received distilled water in dose of 5.0 ml/kg. Group II and III were test drug treated groups which received *Amlakyadi Gana* decoction (AGD) (4.5 ml/kg) and alcoholic extract of *Amlakyadi Gana* (AGAE) (45 mg/kg). 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 1 ml/100 g body weight of rats. After 1 h of induction of fever, the respective test drugs were administered and distilled water was given to control group. The rectal temperature was recorded by digital telethermometer (EIE Instruments, Ahmedabad) after 3 h, 6 h and 9 h of drug administration. The difference between final and initial rectal temperature were registered for each time interval. The maximum reduction in rectal temperature in comparison to control group was recorded.[14]

Analgesic activity by radiant heat model

Rats were randomized into four groups, each consisting of six animals. Group I control group, received distilled water in dose of 5.0 ml/kg. Group II and III were test drug treated groups which received AGD (4.5 ml/kg) and AGAE (45 mg/kg). Group IV kept as standard group treated with Pentazocine sodium (20 mg/kg, ip). Tail flick response was evoked by placing rat tail over a nichrome wire heated electrically in tail flick analgesiometer (INSIF, India). 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 drugs were administered to treated groups and distilled water to control group. Tail flick response was again recorded after 30, 60, 120, 180 and 240 min of drug administration.[15]

Table 1: Effects of test drugs on Brewer's yeast-induced pyrexia in rats

Groups	Increase in pyrexia (°C)					
	After 3 h	Percentage change	After 6 h	Percentage change	After 9 h	Percentage change
Control	0.98±0.38	-	1.43±0.37	-	1.52±0.45	-
AGD	0.33±0.22	66.33↓	1.20±0.27	16.08↓	1.05±0.27	30.92↓
AGAE	0.45±0.29	54.08↓	0.48±0.21*	66.43↓	0.97±0.19	36.18↓

Data: Mean±SEM. *$P<0.05$ when compared with control group. ↓: Decrease, AGD: *Amlakyadi Gana* decoction, AGAE: Alcoholic extract of *Amlakyadi Gana*

Analgesic activity against formaldehyde induced paw liking

Rats were randomized into four groups, each consisting of six animals. Group I control group, received distilled water in dose of 5.0 ml/kg. Group II and III were test drug treated groups which received AGD (4.5 ml/kg) and AGAE (45 mg/kg). Group IV kept as standard group treated with Diclophenac sodium (5 mg/kg, ip). One hour after administration of test drug and distilled water to control group, pain was induced by injecting 0.1 ml of 1% v/v formalin in distilled water in sub plantar aponeurosis region of left hind paw subcutaneous. The duration of paw licking as an index of nociception was counted in periods of 0–10 min (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 drug.[16]

Statistical analysis

The obtained data have been presented as mean ± standard error of mean. Difference between the groups statistically determined using 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.

Results

Table 1 represents the data related to effect of AGD and AGAE on rectal temperature after Brewer's yeast induced pyrexia in rats. AGD produced non-significant decrease in rectal temperature after 3 h (66.33%), 6 h (16.08%) and 9 h (30.92%). AGAE produced non-significant decreases in rectal temperature after 3 h (54.08%) and 9 h (36.18%) and statistically significant decrease in rectal temperature after 6 h (66.43%) compared to control group.

Table 2 summarizes the data related to effect of test drugs on tail flick response at different intervals in rats. The standard drug shows significant increase of the tail flick response after 30 min and 60 min compared to initial reading as well as control group. AGD and alcoholic extract of *Amlakyadi Gana* treated groups did not show any significant increase in tail flick response up to 2 h compared to initial reading as well as control group, however, showed nonsignificant increase in tail flick response after 180 and 240 min compared to initial reading as well as control group.

The data related to effect of test drug on formalin-induced paw liking response is given in Table 3. All the treated groups increased latency of onset in paw liking, among them AGD treated group shows statistically significant increase in latency of onset of paw liking response. Standard drug, (Diclofenac sodium) showed statistically significant decrease in paw liking response at 0–10 min (42.15%) and 21–30 min (64.53%) interval while insignificant decrease at 11–20 min (73.60%) interval. AGD showed statistically significant decrease in paw liking response at 11–20 min interval and insignificant decrease in paw liking response at 0–10 min (7.23%) and 21–30 min (20.0%) interval. Alcoholic extract of *Amlakyadi*

Table 2: Effects of test drugs against radiant heat-induced pain model in rats

Groups	Duration of latency of tail flick response (s)									
	Initial	30 min	Percentage change	60 min	Percentage change	120 min	Percentage change	180 min	Percentage change	240 min	Percentage change
Control	2.11±0.07	2.50±0.22	-	2.33±0.21	-	2.50±0.34	-	2.17±0.17	-	2.33±0.21	-
Pentazocine	2.11±0.21	8.50±2.09*	240.0↑	3.83±0.79	64.38↑	2.83±0.31	13.20↑	2.67±0.33	23.04↑	2.33±0.33	-
AGD	2.87±0.78	2.23±0.32	10.8↓	2.88±0.41	23.60↑	2.41±0.50	2.36↓	3.30±0.59	52.07↑	3.12±0.59	33.90↑
AGAE	2.90±0.37	2.17±0.27	15.20↓	2.81±0.65	20.60↑	2.51±0.58	-	3.05±0.53	40.55↑	3.04±0.41	30.47↑

Data: Mean±SEM; *$P<0.05$ when compared with control group. ↓: Decrease, ↑: Increase, AGD: *Amlakyadi Gana* decoction, AGAE: Alcoholic extract of *Amlakyadi Gana*

Table 3: Results of formalin-induced paw liking

Groups	Latency of onset (s)	Percentage change	Number of paw licking at time intervals					
			0-10 min	Percentage change	11-20 min	Percentage change	21-30 min	Percentage change
Control	22.16±4.24	-	13.83±1.08	-	2.50±0.99	-	7.50±1.33	-
Diclofenac sodium	41.66±3.47*	87.99↑	08.00±1.84*	42.15↓	0.66±0.33	73.60↓	2.66±0.80*	64.53↓
AGD	46.67±12.80*↑	110.6↑	12.83±1.05	7.23↓	0.00±0.00*	100.00↓	6.00±1.43	20.00↓
AGAE	26.16±5.74↑	18.05↑	13.83±1.67	0.00	1.50±0.85	40.00↓	10.17±3.16	35.60↑

Data: Mean±SEM. *P<0.05 when compared with control group. ↑: Increase, ↓: Decrease, SEM: Standard error of mean, AGD: *Amlakyadi Gana* decoction, AGAE: Alcoholic extract of *Amlakyadi Gana*

Gana showed insignificant decrease in paw liking response at 11–20 min interval (40.0%) while non-significant increase at 21–30 min interval (35.60%) while no effect was found at 0–10 min interval when compared with control group.

Discussion

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.[17] 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.[18] 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.

Decoction produced non-significant decrease in rectal temperature after 3 h, 6 h and 9 h. AGAE drugs produced non-significant and marked decrease in rectal temperature after 3 h and 9 h and there was statistically significant decrease in rectal temperature after 6 h which is likely due to inhibition of the synthesis and/or release of local PGE2 into the preoptic area of anterior hypothalamus.[19,20]

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.

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 neuropeptides.[21] The second phase, which is observed 20–30 min of formaldehyde injection, is supposed to be mediated through release of inflammatory mediators like prostaglandin. Both the forms of test drug show statistically significant increase in latency of onset of paw licking after formalin injection in rats. Both forms of test drugs failed to inhibit first phase (0–10 min) of formalin induced pain response while apparently inhibited the second phase of pain response (11–20 min) and again, failed to inhibit late phase (21–30 min) of formalin induced pain response in rats.

Tail flick model, which is thermal induced nociception, indicates narcotic involvement, which is sensitive to opioid micro receptors.[22] Both forms of test drug treated group did not show any significant increase in tail flick response in early phase of 2 h. However, drug shows non-significant increase in tail flick response after 180 and 240 min compared to initial reading as well as control group which suggest mild analgesic effect through central mechanism.

Conclusion

The result of the present study suggests that decoction and alcoholic extract of *Amalakyadi Gana* has moderate antipyretic activity in rats may be due to inhibition of the synthesis and/or release of local PGE2 into the preoptic area of anterior hypothalamus. Further, both dosage forms of *Amalakyadi Gana* have mild central analgesic effect in rats and have no effect on first phase of formalin induced pain while mild effect on second phase of pain response in rats which suggest the mild analgesic effect through central and peripheral mechanism. The result of the present study corroborate with the ancient claims made on *Amalakyadi Gana* for the management of *Jwara* which open further scope for development of new palatable dosage form and can be tested clinically for better efficacy.

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Conflicts of interest
There are no conflicts of interest.

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हिन्दी सारांश

आमलक्यादि गण के ज्वरहर और शूलहर प्रभाव का परीक्षण

मनोज जे. तिम्बडिया, के. निष्ठेश्वर, रबिनारायण आचार्य, मुकेश बी. नारिया

ज्वर और शूल ऐसी अवस्थायें है जो सामान्य जनसमुदाय की जीवनशैली और स्वास्थ्य को बहुत ही प्रभावित करती है। आयुर्वेद में बुखार लक्षण को ज्वरनामक अलग रोग से वर्णित किया गया है। सुश्रुताचार्य ने द्रव्यगुणसंग्रहणीय अध्याय में आमलक्यादिगण सर्वज्वर की चिकित्सा के लिये दिया है। आमलक्यादिगण ४ औषधियों से बना है, आमलकी, हरीतकी, पिप्पली और चित्रक। इस अध्ययन में आमलक्यादि गण के ज्वरहर तथा शूलहर प्रभाव का चूहों पर परीक्षण किया गया। ज्वरहर प्रभाव का अध्ययन यीष्ट इन्ड्युस्ड पाईरेक्सिया मोडेल पर किया गया और शूलहर प्रभाव का अध्ययन रेडीयन्ट हीट मोडेल पर तथा फ़ोर्मेलीन द्रावितसत्व के रुप में उपयोग किया गया। इस परीक्षण से आमलक्यादिगण ज्वरहर और शूलहर साबित हुआ।