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Research Article

IN-VIVO EVALUATION OF ANTI-INFLAMMATORY ACTIVITY OF *PANCHA SHIRISHANAMA AGAD*

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ABSTRACT

Keet Visha (insect bite venom) triggers an acute inflammatory response mediated by innate immune activation and the release of biochemical mediators. These mediators promote vasodilation, increased vascular permeability, and leukocyte recruitment, leading to the hallmark signs of inflammation i.e., redness, swelling, heat, pain, and loss of function. *Pancha Shirishanama Agad*, a classical Ayurvedic formulation, is traditionally indicated for managing symptoms associated with *Keet Visha*, although its anti-inflammatory potential remains experimentally unverified. **Objective:** To evaluate the anti-inflammatory efficacy of *Pancha Shirishanama Agad* in the carrageenan-induced paw edema model in Wistar albino rats. **Materials & Method:** Wistar strained albino rats were randomly parted into three groups: Group 1 served as control (distilled water), Group 2 served as test drug (*Pancha Shirishanama Agad* at 9.86mg/kg), and Group 3 served as standard drug (diclofenac sodium at 10mg/kg). Carrageenan (0.1ml, 1%) was injected into the left hind paw to induce edema on the 7th day, one hour after drug administration. Paw volume was recorded at 0th, 1st, 2nd, 3rd, 6th, and 9th hour post-injection with the help of a plethysmometer. The data were analysed using one-way ANOVA, followed by Dunnett's post hoc test for multiple comparisons. **Results:** *Pancha Shirishanama Agad* showed a time-dependent anti-inflammatory effect, with maximum inhibition of 33.68% at the 9th hour compared to 39.96% with diclofenac sodium. Statistically significant results were observed particularly during the late inflammatory phase ($P<0.05$). **Conclusion:** *Pancha Shirishanama Agad* demonstrates significant anti-inflammatory activity, especially in the late phase of inflammation, likely through COX inhibition and cytokine modulation. It offers a promising herbal alternative for inflammation management.

INTRODUCTION

Human beings are continually exposed to a wide range of threats that compromise health and survival, including both endogenous pathologies and external factors such as animal bites and stings. Among these, *Keet Visha* (insect bite) is a notable cause of localized or systemic envenomation and trauma, frequently resulting in acute or chronic inflammatory responses. *Vedana* (pain), *Shopha* (swelling), *Kandu* (itching), and *Jwara* (fever) are the main common symptoms of *Keet damsha*.[1] According to *Acharya Sushruta*, *Keet Visha* is characterized as mild and moderately hot in potency, predominantly involving *Vata* and *Kapha doshas*.[2] The clinical manifestation varies with *Doshic* predominance: *Vata*-dominant bites exhibit intense pricking pain; *Pitta*-dominant bites are marked by burning sensation, redness, and rapid spread resembling fruits like *Peelu* or *Kharjoora*; while *Kapha*-dominant bites result in mild pain and swelling like ripe fruit of *Udumbara*.[3]

The inflammatory response is mediated by the activation of tissue-resident immune cells, particularly macrophages and mast cells, which release a variety of pro-inflammatory mediators including histamine, serotonin, bradykinin, prostaglandins, leukotrienes, and cytokines such as TNF-α and interleukins (IL-1β,

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IL-6).[4-5] These mediators contribute to vasodilation, increased vascular permeability, and neutrophil recruitment, ultimately leading to the cardinal signs of inflammation i.e., redness (rubor), swelling (tumor), heat (calor), pain (dolor), and loss of function (functio laesa).[6]

Although conventional therapies for inflammation and pain, such as opioids and nonsteroidal anti-inflammatory drugs (NSAIDs), are widely used, their long-term use is associated with adverse effects and toxicity.[7] This has driven interest in identifying safer, plant-based alternatives. *Agad Tantra*, a specialized branch of Ayurveda, focuses on the treatment of poisoning and toxicological conditions, and includes various formulations for *Keet Visha* and its associated symptoms.

One such formulation is *Pancha Shirishanama Agad*, a classical Ayurvedic remedy described by *Acharya Sushruta*.[8] It is indicated in toxic conditions resulting from insect bites and the symptoms emerging due to their bite. The present study aims to evaluate the anti-inflammatory potential of *Pancha Shirishanama Agad* using the carrageenan-induced paw edema model in rats, a well-established model for assessing acute inflammation and screening anti-inflammatory agents.

MATERIAL AND METHOD

Test Formulation

Pancha Shirishanama Agad is prepared using a coarse powder of five parts of the *Shirish* plant- roots, flowers, stem bark, leaves, and seeds-combined with a fine powder of *Trikatu (Shunthi, Maricha,* and *Pippali*), five types of salts known as *Pancha Lavana* (*Saindhav, Sauvarchal, Samudra, Vid, and Audbhidha*), and honey (*Madhu).* Traditionally, it is administered as a decoction *(Kwatha)*. [8] All the contents are shown in Figures 1(a-d).

Collection and Authentication of Raw Drugs

Leaves, stem bark, root and flowers of *Shirish* were procured from the botanical garden of Rishikul campus, UAU, Haridwar. *Shirish* seeds, *Shunthi, maricha, Pippali, Pancha lavana* and *Madhu* were purchased from local market of Dehradun, Uttarakhand. All the raw materials of *Pancha Shirishanama Agad* were authenticated by the eminent experts of PG department of *Dravya Guna*, Rishikul campus, Uttarakhand Ayurved University, with a reference number (DG/RC/UAU-232).

Preparation of Test Formulation

After establishing proper identity of all the drugs, the five parts of *Shirish* were converted to coarse powder (*Yavakuta*) after drying in the shade. *Trikatu* and *Pancha lavana* were finely pulverized to obtain a homogeneous powder. Coarse powder of *Shirish* 1 *Pala* (48gm) was added with sixteen parts of water and was subjected to mild heat maintaining temperature between 95°C and 100°C. Continuous stirring was performed to prevent sedimentation and thermal degradation of the contents. When the volume was reduced to one-eighth (1/8th) around 96ml, the contents were filtered through double folded clean cotton cloth into a stainless-steel container to obtain decoction. *Prakshepak Dravya trikatu churna* (1 *Yama*, 5gm) and *Pancha lavana* (1 *Yama*, 5gm) were added to the decoction and after cooling it down, *Madhu* was also added and mixed nicely. Prepared formulation shown in Figure 2(a-c). According to *Sharangdhara Samhita*, dose of *Kwatha* is 2 *Pala* (96ml) per day, so, it can be given in 2 doses of around 1 *Pala* (48ml) twice in a day. [9]

Animals

Wistar albino rats weighing between 150-350 gm of either sexes were used for the evaluation of anti-inflammatory activity. The animals were acclimatized to the condition of laboratory for 1 week before starting the iv-vivo experiment. The temperature and humidity were kept at optimum and the animals were exposed to natural day-night cycles with free access to food and water, as per the standard procedure and kept in their separate polypropylene cages as shown in Figure 3. The experimental protocol was approved by the Institutional Animal Ethical Committee (BMRL/DIC/CCSEA/IAEC/2024/I/05) as per the guideline of the Committee for Control and Supervision of Experiments on Animals, India. The Wistar strained albino rats were then randomly parted into three groups of six each. Individuality of rats was identified using picric acid markings as- H (head), B (back), T (tail), HB (head and back), BT (back and tail), and HT (head and tail) as shown in Figure 4. Group I, designated as the control, was administered distilled water. Group 2 was administered the test formulation, *Pancha Shirishanama Agad*, at a dose of 9.86mg/kg. Group 3 was administered the standard drug, diclofenac sodium, at a dose of 10mg/kg. All treatments were administered orally twice daily for a duration of 7 days. Drug administration shown in Figure 5 (a-b).

Dose Fixation

The dose of the test formulation was determined by following the conversion guidelines provided by Paget and Barnes. [10] Based on the human therapeutic dose of *Pancha Shirishanama Agad Kwatha* 96ml/day orally, it was converted to 9.86mg/kg body weight of rat. Each animal from all the groups were administered the drug by oral route with the help of a gastric oral gavage catheter 16 G. The dosage regimen

for all experimental groups is presented in the accompanying table 1, 2 and 3.

Table 1: Dose Chart of Each Rat in Group 1

Group 1- Control (Distilled Water)		
Markings	**Weight (gm)**	**Dose of Distilled Water (ml)**
H	276	1.38
B	250	1.25
T	300	1.5
HB	340	1.7
BT	284	1.42
HT	210	1.05

H- head, B- back, T- tail, HB-head back, BT- back tail, HT- head tail

Table 2: Dose Chart of Each Rat in Group 2

Group 2- Test Drug (*Pancha Shirishanama Agad* at 9.86mg/kg Dose)			
Markings	**Weight (gm)**	**Dose of *Pancha Shirishanama Agad***	
		ml	**mg**
H	325	1.404	3.20
B	350	1.555	3.55
T	341	1.47	3.37
HB	329	1.42	3.25
BT	318	1.37	0.60
HT	340	1.46	3.34

H- head, B- back, T- tail, HB- head back, BT- back tail, HT- head tail

Table 3: Dose Chart f Each Rat in Group 3

Group 3- Standard Drug (Diclofenac Sodium AT 10 MG/KG Dose)			
Markings	**Weight (gm)**	**Dose of Diclofenac sodium**	
		ml	**mg**
H	344	3.4	0.34
B	199	1.9	0.19
T	300	3.0	0.30
HB	305	3.05	0.305
BT	264	2.64	0.264
HT	207	2.07	0.207

H- head, B- back, T- tail, HB- head back, BT- back tail, HT- head tail

Anti-Inflammatory Activity

Carrageenan-induced paw edema in rats

In this model, carrageenan was employed as a phlogistic agent to induce acute paw edema in rats. As the study focused on acute inflammation, the experimental period was set to 7 days. On the 7th day, acute inflammation was induced by sub-plantar injection of 0.1ml freshly prepared 1% carrageenan (in sterile saline) into the left hind paw, one hour after drug administration. A plethysmometer was employed to record the baseline volume of the left hind paw up to the tibia-tarsal joint. The paw was submerged in a distilled water chamber, causing a change in pressure, which was digitally displayed on an electronic monitor. Paw volume was recorded at 0th, 1st, 2nd, 3rd, 6th and 9th hour after carrageenan injection and the results were expressed as an increase in paw volume relative to baseline measurements and compared with the control group. Figure 6 demonstrate paw before inducing edema and figure 7, 8 demonstrates paw edema in initial and late phase.

Statistical Analysis

The data were analysed using one-way ANOVA, followed by Dunnett's post hoc test for multiple comparisons. A p-value less than 0.05 was considered indicative of statistical significance

OBSERVATION

Tables 4, 5, and 6 present the paw volume measurements of individual rats in each group at various time intervals. Table 7 and 8 shows the effect of *Pancha Shirishanama Agad* on change in paw volume at 0th, 1st, 2nd, 3rd, 6th and 9th hour expressed as mean ± standard error of the mean (SEM). The mean percentage reduction in paw volume over time, relative to the control, is summarized in Table 9. It is calculated using the standard formula:

Percent Inhibition= (Vc−VtVc)×100

Where, *Vc* is a Mean paw volume in Control Group and *Vt* is a Mean paw volume in Test drug Group II

Table 4: Paw Volume of Each Rat in Group 1 at Different Time Interval

Group 1 – Control Group						
Marking	**0 hr**	**1st hr**	**2nd hr**	**3rd hr**	**6th hr**	**9th hr**
H	1.07	1.55	1.86	1.26	1.42	1.32
B	1.25	1.62	1.2	1.58	1.51	1.44
T	0.91	1.33	1.21	1.53	1.21	1.59
HB	1.24	2.36	1.78	1.71	1.61	1.89
BT	0.86	1.84	1.87	1.74	1.6	1.41
HT	0.96	1.85	1.59	1.54	1.39	1.55

H- head, B- back, T- tail, HB- head back, BT- back tail, HT- head tail

Table 5: Paw Volume of Each Rat in Group 2 at Different Time Interval

Group 2- Test Drug						
Marking	**0 hr**	**1st hr**	**2nd hr**	**3rd hr**	**6th hr**	**9th hr**
H	1.04	1.37	1.66	1.34	1.16	1.06
B	1.16	1.78	1.56	1.37	1.62	0.9
T	1.18	1.41	1.27	1.12	0.95	0.94
HB	1.21	1.78	1.59	1.74	1.42	0.97
BT	1.07	1.18	1.38	1.21	0.93	1.21
HT	1.06	1.02	1.15	1.09	1.28	1.02

H-head, B-back, T-tail, HB-head back, BT- back tail, HT- head tail

Table 6: Paw Volume of Each Rat in Group 3 at Different Time Interval

Group 3 – Standard Drug						
Marking	**0 hr**	**1st hr**	**2nd hr**	**3rd hr**	**6th hr**	**9th hr**
H	1.46	1.43	1.11	1.02	0.95	0.8
B	1.25	2.22	0.96	1.15	0.92	0.9
T	1.11	1.38	1.08	1.26	1.18	0.86
HB	1.22	0.92	1.1	1.01	1.02	1.06
BT	1.18	1.24	1.13	1.05	1.05	0.99
HT	1.12	0.16	0.97	0.97	0.84	0.94

H-head, B-back, T-tail, HB-head back, BT- back tail, HT- head tail

Table 7: Change in Paw Volume at 0th, 1st, 2nd, 3rd, 6th and 9th hour Expressed as Mean ± Standard Error of the Mean (SEM)

Observation Time	Group 1 Mean±SEM	Group 2 Mean±SEM	Group 3 Mean±SEM
0 hr	1.048±0.068	1.120±0.029	1.223±0.052
1st hr	1.758±0.144	1.423±0.126	1.225±0.276
2nd hr	1.585±0.127	1.435±0.082	1.058±0.030
3rd hr	1.560±0.070	1.312±0.097	1.077±0.044
6th hr	1.457±0.062	1.227±0.110	0.993±0.048
9th hr	1.533±0.082	1.017±0.045	0.925±0.038

Mean ± SEM *P<0.05

Table 8: Two-Way Anova Followed by Dunnett's Multiple Comparisons Test

Dunnett's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant	Summary	Adjusted P Value
0 hr					
Group 1 vs. Group 2	-0.07200	-0.3980 to 0.2540	No	ns	0.8374
Group 1 vs. Group 3	-0.1750	-0.5010 to 0.1510	No	ns	0.3789
1st hr					
Group 1 vs. Group 2	0.3350	0.008979 to 0.6610	Yes	*	0.0431
Group 1 vs. Group 3	0.5330	0.2070 to 0.8590	Yes	***	0.0008
2nd hr					
Group 1 vs. Group 2	0.1500	-0.1760 to 0.4760	No	ns	0.4828
Group 1 vs. Group 3	0.5270	0.2010 to 0.8530	Yes	***	0.0009
3rd hr					
Group 1 vs. Group 2	0.2480	-0.07802 to 0.5740	No	ns	0.1597
Group 1 vs. Group 3	0.4830	0.1570 to 0.8090	Yes	**	0.0025
6th hr					
Group 1 vs. Group 2	0.2300	-0.09602 to 0.5560	No	ns	0.2018
Group 1 vs. Group 3	0.4640	0.1380 to 0.7900	Yes	**	0.0037
9th hr					
Group 1 vs. Group 2	0.5160	0.1900 to 0.8420	Yes	**	0.0012
Group 1 vs. Group 3	0.6080	0.2820 to 0.9340	Yes	***	0.0001

ns – not significant, * - mild significant, **- moderate significant, *** - Highly significant

Table 9: Paw Edema Inhibition Percentage of all the Groups

Observation Time	Group 1- Control (Distilled water)	Group 2- Test Drug (*Pancha Shirishanama Agad*)	Group 3- Standard Drug (Diclofenac Sodium)
0 hr	-	6.87% ↓	16.68% ↓
1st hr	-	19.06% ↓	30.29% ↓
2nd hr	-	9.46% ↓	33.27% ↓
3rd hr	-	15.90% ↓	30.96% ↓
6th hr	-	15.77% ↓	31.83% ↓
9th hr	-	33.68% ↓	39.96% ↓

(a)

(b)

(c)

(d)

Figure 1: Showing contents of *Pancha Shirishanama Agad*
(a) *Shirish* root, flower, leaves, stembark and seed
(b) *Pancha Lavana (Saindhav, Sauvarchal, Samudra, Vid* and *Audbhidha*)
(c) *Trikatu* (*Shunthi* rhizome, *Maricha* fruit, *Pippali* fruit)
(d) *Madhu* (honey)

(a) (b) (c)

Figure 2
(a) *Pancha Shirishanama Agad* in raw course powder form
(b) Making of *Pancha Shirishanama Agad kwatha* (decoction)
(c) Prepared *Pancha Shirishanama Agad Kwatha* (decocotion)

Figure 3: Housing of Albino wistar rats in separate Polypropylene cages (n=6, each group)

Figure 4: Marking of rat with Picric acid as Tail (T) for identification

(a) (b)

Figure 5

(a) Administration of Standard drug Diclofenac sodium with oral Gavage catheter 16 G

(b) Administration of Test drug *Pancha Shirishanama Agad* with oral Gavage catheter 16 G

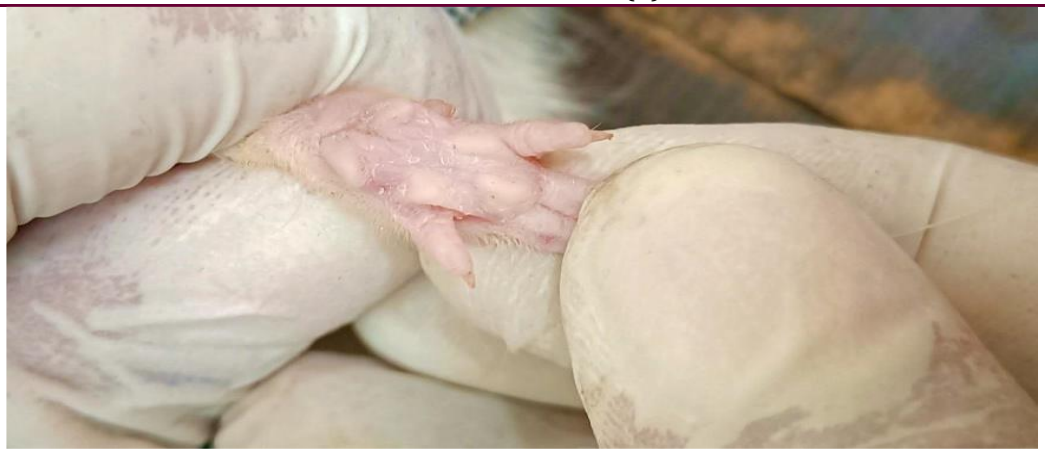

Figure 6: Left Hind paw of rat before inducing paw edema with Carragenan solution of 1%

Figure 7: Left Hind paw of rat after inducing paw edema with Carragenan solution of 1% (Initial Phase)

Figure 8: Left Hind paw of rat after inducing paw edema with Carragenan solution of 1% (Late Phase)

RESULT

The anti-inflammatory effect of *Pancha Shirishanama Agad* was evaluated using the carrageenan-induced paw edema model in Wistar albino rats. Measurements of paw volume were taken at defined intervals (0, 1, 2, 3, 6, and 9 hours) for the test formulation group (Group 2) and the standard drug group (Group 3), and results were compared with those of the control group (Group 1).

Effect on Early Phase of Inflammation (1st–2nd Hour)

The control group exhibited a significant rise in paw volume during the first hour due to the vasodilatory effects of histamine and serotonin. In the test group, a 19.06% inhibition of edema was observed at the 1st hour, indicating that *Pancha Shirishanama Agad* may possess mast cell stabilizing or antihistaminic activity,[11-12] possibly due to its phytoconstituents derived from the five parts of *Shirish* (Albizia lebbeck) and the *Trikatu*. However, the inhibition decreased to 9.46% at the 2nd hour, suggesting a relatively modest effect on bradykinin-mediated responses during the transition to prostaglandin involvement.

Effect on Late Phase of Inflammation (3rd–6th Hour)

With the progression of inflammation, the production of prostaglandins emerged as the main factor contributing to edema formation. The test formulation showed improved inhibition of 15.90% at the 3rd hour and 15.77% at the 6th hour, indicating that *Pancha Shirishanama Agad* starts to exert a delayed but gradually increasing anti-inflammatory effect, likely through COX modulation, antioxidant activity, or inhibition of pro-inflammatory cytokines.[13] The ingredients such as *Shunthi* [14] and *Pippali*[15] are known to possess such properties.

Effect on Resolution Phase (9th Hour)

At the 9th hour, the test group showed maximum edema inhibition of 33.68%, approaching the effect of Diclofenac sodium (39.96%). This substantial reduction in paw volume suggests that the test drug has a sustained and cumulative anti-inflammatory action, possibly supporting natural resolution mechanisms such as enhancing IL-10 activity, inhibiting reactive oxygen species (ROS), or modulating inflammatory gene expression. The contribution of *Pancha Lavana* (five salts)[16] and *Madhu* (honey) as bioavailability enhancers[17] of drug absorption and immune modulatory function[18] may help explain the delayed therapeutic effect.

DISCUSSION

Inflammation is a multifaceted biological process triggered by injury or infection, characterized by a sequence of molecular signalling events. The carrageenan-induced paw edema model employed in this research simulates acute inflammation by progressing through two distinct phases. The early phase (0–2 hours) is dominated by the release of histamine, serotonin, and bradykinin, while the late phase (3–6 hours and beyond) is mediated primarily by prostaglandins, leukotrienes, and pro-inflammatory cytokines such as TNF-α and IL-1β. In this context, the test group (*Pancha Shirishanama Agad)* showed a maximum inhibition of 33.68% at the 9th hour and the standard group (diclofenac sodium) demonstrated a greater inhibition of 39.96% at the same time point.

The traditional polyherbal formulation *Pancha Shirishanama Agad* exhibits notable anti-inflammatory activity when evaluated using the carrageenan-induced paw edema model. This study represents both early (histamine, serotonin, bradykinin-mediated) and late (prostaglandin, cytokine-mediated) phases of inflammation. The observed inhibition of paw edema at different time points supports the formulation's efficacy across this inflammatory spectrum. The early-phase inhibition (19.06% at 1st hour) suggests the presence of antihistaminic and mast cell-stabilizing constituents, while a maximum inhibition of 33.68% at the 9th hour indicates a prolonged pharmacodynamic response, possibly mediated through COX inhibition, anti-inflammatory cytokine regulation, and antioxidant action.

From an Ayurvedic standpoint, the formulation works through balancing *Vata* and *Kapha doshas*, reducing *Shotha* (inflammation), and eliminating *Ama* (toxins). The five parts of *Shirish* (*Albizia lebbeck*), having *Kashaya* and *Tikta rasa*, *Sheeta virya*, and *Katu vipaka*, act as *Vishaghna* and *Shothahara*, targeting early-phase mediators like histamine and serotonin. *Trikatu,* a combination of *Shunthi, Maricha,* and *Pippali,* is characterized by *Ushna Virya* and *Katu Rasa*, which enhance digestive fire (*Agni Deepana*), thereby improving drug absorption and bioavailability, along with exhibiting COX-2 inhibitory, antioxidant, and immunomodulatory properties. *Pancha Lavana*, with *Lavana rasa* and *Ushna virya*, improves electrolyte balance, detoxification, and tissue fluid redistribution. *Madhu* (honey), with its *Madhura* and *Kashaya rasa*, *Sheeta virya*, and *Ropana* properties, supports wound healing, prostaglandin inhibition, and free radical scavenging.

The formulation's progressive efficacy aligns with its initial suppression of histamine, followed by inhibition of prostaglandins and cytokines, and finally aiding tissue recovery and resolution. Thus, *Pancha Shirishanama Agad*, through its Ayurvedic mechanism and multi-target modern pharmacology, validates its traditional claim as a safe and effective anti-inflammatory remedy for inflammation in practice, though slightly less potent than the standard non-steroidal anti-inflammatory drug, diclofenac sodium.

CONCLUSION

The present experimental study demonstrated that *Pancha Shirishanama Agad*, a classical Ayurvedic polyherbal formulation, exhibits significant anti-inflammatory activity in the carrageenan-induced paw edema model in Wistar albino rats. While its initial effect on acute inflammatory mediators was modest, the formulation showed progressively increasing efficacy, achieving a maximum edema inhibition of 33.68% at the 9th hour, closely approaching the activity observed with the reference drug Diclofenac sodium was 39.96%.

These findings suggest that *Pancha Shirishanama Agad* exerts its anti-inflammatory effect primarily during the later stages of inflammation, potentially through a combination of anti-histaminic, COX-inhibitory, antioxidant, and immunomodulatory mechanisms. The delayed but sustained action may be attributed to the synergistic effects of its ingredients such as *Shirish*, *Trikatu*, *Pancha Lavana*, and *Madhu*.

In conclusion, *Pancha Shirishanama Agad* offers a promising herbal alternative for managing

inflammation, especially in conditions requiring prolonged therapeutic effect with minimal adverse effects. Further studies involving chronic inflammation models, detailed phytochemical profiling, and clinical trials can be planned to explore its full therapeutic potential and mechanism of action.

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