WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.453
Volume 14, Issue 16, 719-740. Research Article ISSN 2277–7105

EVALUATION OF ACUTE TOXICITY, ANTI-INFLAMMATORY AND ANALGESIC STUDY OF TAMBUL BHASMA

Dr. Vinila Vilas Desai[1*] and Dr. Smita Johar[2]

[1]PhD Scholar (Ayurveda – Rasashastra Evam Bhaishajyakalpana), Desh Bhagat University, Mandi Gobindgarh, Punjab, India.

[2]Professor, Faculty of Ayurveda, Department of Rasashastra Evam Bhaishajyakalpana, Desh Bhagat University, Mandi Gobindgarh, Punjab, India.

Article Received on
23 June 2025,

Revised on 13 July 2025,
Accepted on 02 August 2025,

DOI: 10.20959/wjpr202516-37906

***Corresponding Author**
Dr. Vinila Vilas Desai
PhD Scholar (Ayurveda – Rasashastra Evam Bhaishajyakalpana), Desh Bhagat University, Mandi Gobindgarh, Punjab, India.

ABSTRACT

The present study evaluated the acute toxicity, analgesic, and anti-inflammatory properties of *Tambul Bhasma*, a traditional Ayurvedic herbo-mineral formulation. Acute oral toxicity testing in female Wistar rats (OECD Guideline 423) revealed no mortality or adverse effects at doses of 300 mg/kg and 2000 mg/kg, with normal weight progression and no pathological abnormalities observed during necropsy. Analgesic activity assessed via the hot plate method demonstrated significant dose-dependent effects, with the high-dose group (1080 mg/kg) showing prolonged response latency at 120 minutes (25.00 ± 5.0 s, *p* < 0.05), suggesting sustained analgesic action. Anti-inflammatory evaluation using a carrageenan-induced paw edema model revealed dose-dependent suppression of inflammation, with the high-dose group reducing peak edema by 28.6% compared to controls. Histopathological analysis confirmed near-complete normalization of skin architecture in treated groups, comparable to standard drugs. Statistical analysis (Bonferroni test) indicated no significant body weight changes across groups, reinforcing safety. These findings support *Tambul Bhasma* as a safe and effective traditional remedy with significant analgesic and anti-inflammatory potential, warranting further mechanistic studies.

KEYWORDS: Tambul Bhasma, acute toxicity, analgesic, anti-inflammatory, carrageenan-induced edema, hot plate test, Ayurveda, OECD guidelines.

INTRODUCTION

Ayurveda, India's ancient holistic healing system, has earned global acclaim for its profound wisdom and time-tested therapeutic approaches. Recognized as one of humanity's oldest documented medical traditions, Ayurveda is often described as eternal and limitless in its applications.[1] Within this vast system, Rasashastra Evam Bhaishajyakalpana represents a specialized branch that combines traditional pharmacology with sophisticated preparation techniques.

Rasashastra, the alchemical dimension of Ayurveda, focuses on the medicinal use of metals and minerals through precise processing methods. This discipline, sometimes called Ayurvedic Iatrochemistry, produces potent formulations known as Rasayanas. Parallel to this, Bhaishajyakalpana (Ayurvedic Pharmaceutics) specializes in herbal preparations, employing various techniques including extraction, decoction, paste formation, and fermentation to create diverse medicinal forms such as juices, decoctions, tablets, medicated oils, ghee preparations, and fermented tonics.

Among these traditional formulations, Tambul Bhasma holds particular significance. This ancient preparation, derived primarily from betel leaf, has been utilized for centuries in Ayurvedic practice. The sophisticated preparation process involves meticulous purification, controlled calcination, and fine grinding to transform betel nut into a therapeutically active ash. The formulation incorporates four principal components: betel leaf (Tambul Patra), Calotropis milk (Arka Kshir), rock salt (Saindhav Lavana), and celery seed powder (Dipya Churna).[2] This carefully balanced combination exemplifies the sophistication of traditional Ayurvedic pharmacy and its holistic approach to medicine.

In recent decades, there has been a resurgence of interest in traditional medicine systems, particularly Ayurveda, as complementary and alternative therapies for modern healthcare challenges. Among the various Ayurvedic formulations, Bhasmas—metallo-mineral preparations processed through incineration—hold a significant place due to their purported therapeutic efficacy and minimal side effects (Sarkar et al. 12).[3] Tambul Bhasma, a calcined preparation primarily used in Ayurveda for oral health, digestive disorders, and inflammatory conditions, is one such formulation that warrants scientific scrutiny. Despite its historical use, comprehensive pharmacological evaluations, including toxicity and mechanistic studies, remain scarce, limiting its acceptance in evidence-based medicine (Joshi et al. 78).[4]

The World Health Organization (WHO) emphasizes the importance of scientifically validating traditional medicines to ensure their safety and efficacy (WHO 34). Acute toxicity studies serve as a fundamental step in drug development, providing critical data on the safety profile of a substance before advancing to therapeutic assessments (OECD 425).[5] Given that Tambul Bhasma contains processed metals, establishing its non-toxic nature within therapeutic doses is essential to alleviate concerns regarding heavy metal toxicity—a common criticism of Ayurvedic herbo-mineral formulations (Saper et al. 567).[6]

Inflammation and pain are underlying factors in numerous chronic diseases, including arthritis, periodontitis, and gastrointestinal disorders. Conventional anti-inflammatory and analgesic drugs, such as NSAIDs and opioids, often come with adverse effects like gastrointestinal bleeding and dependency (Vane and Botting 112).[7] Hence, there is a growing demand for safer, natural alternatives. Preliminary Ayurvedic texts suggest that Tambul Bhasma possesses Shothahara (anti-inflammatory) and Vedanasthapana (analgesic) properties, but these claims lack robust experimental validation (Sharma and Dash 203).[8]

AIMS AND OBJECTIVES

1. Evaluate the acute toxicity of *Tambul Bhasma* following OECD Guideline 425 to determine its safety margin.
2. Investigate its anti-inflammatory potential using carrageenan-induced paw edema.
3. Assess its analgesic activity through thermal (hot plate) pain models.

MATERIALS AND METHODS

ACUTE ORAL TOXICITY

Test Substance Details

- Test compound: Tambul Bhasma
- Physical characteristics: Solid powder
- Appearance: Grey-colored fine particles
- Study initiation date: February 12, 2025
- Study completion date: March 19, 2025

Dosage Protocol and Preparation Methodology

The test substance was maintained under standard room temperature conditions throughout the study period. Based on preliminary assessments, the following dosage regimen was implemented:

- Initial dosing phase (Step 1): 300 mg/kg body weight
- Confirmatory phase (Step 2): Repeated administration of 300 mg/kg
- Limit test phase (Step 3): 2000 mg/kg body weight

Fresh suspensions of Tambul Bhasma were prepared immediately prior to each administration using distilled water as the vehicle. The formulation was carefully homogenized to ensure uniform distribution of particles in the aqueous medium. All dosing solutions were prepared under controlled conditions to maintain consistency throughout the experimental period.

Test System And Management

Table No. 1: Test System and Management for Acute Oral Toxicity.

1. Species	**:**	Rat
2. Strain	**:**	Wistar Rats
3. Source	**:**	APT Research Foundation, Pune
4. Age and Sex	**:**	Female, 8- 10 weeks
5. Body weight range	**:**	150 – 180 g
6. Identification	**:**	By unique identification number marked by writing on cage tag and by corresponding color body markings.
7. No. of animals	**:**	Three animals were tested in per group.
8. Acclimatization	**:**	The mice were housed in their cages for five days prior to start of dosing in the experimental room after veterinary examination.
Husbandry		
9. Environmental Conditions	**:**	Room temperature maintained between 22$\pm$3^{0}C, relative humidity 50-60 % and illumination cycle set to 12 hours light and 12 hours dark.
10. Accommodation	**:**	Three 3 per cage housed in polypropylene cages with stainless steel grill top, facilities for food and water bottle, and bedding of clean paddy husk.
11. Diet	**:**	Pelleted feed supplied by Supplier.
12. Water	**:**	Potable water passed through ‘Aquaguard’ water filter was provided ad libitum in plastic bottles with stainless steel sipper tubes.

Study Design

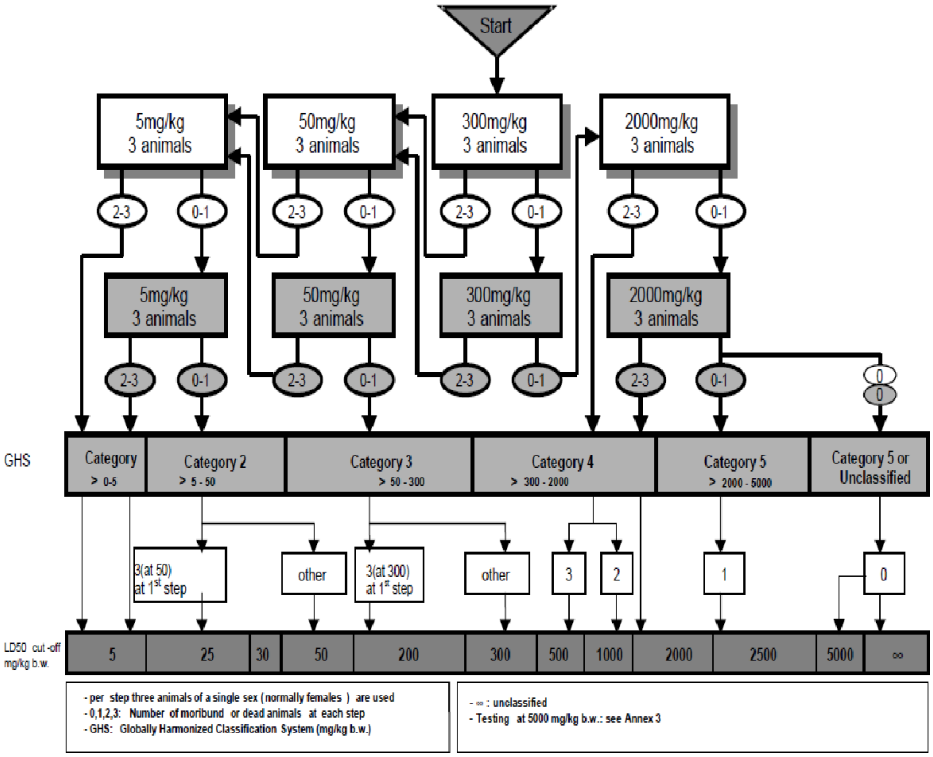


Fig. 1: OECD Annex 2c.

According to OECD Annex 2c, if the dose of a substance is unknown we can start the dose at 300 mg/kg if it shows mortality after 4-6hrs that dose will be considered fatal dose otherwise lower or higher dose have to be studied.

The acute oral toxicity evaluation was conducted in a sequential manner according to OECD Guideline 423.

Step 1: Initial Dose Administration

- Three female Wistar rats received an oral dose of 300 mg/kg body weight of Tambul Bhasma
- Administration was performed using a standardized oral gavage needle
- Animals were fasted for 3-4 hours prior to dosing and for 2 hours post-dosing
- Water remained available throughout the study period
- Detailed observations continued for 14 days following administration

Step 2: Confirmatory Dose Repetition

- An additional three female rats received the same 300 mg/kg dose
- Identical fasting protocol and observation period were maintained as in Step 1

- This step confirmed the initial findings from the first dose group

Step 3: Limit Test Evaluation

- A separate group of three female rats received the high dose of 2000 mg/kg
- The same fasting conditions and water availability were maintained
- Animals were monitored continuously for 14 days post-administration

  All procedures were conducted under controlled laboratory conditions with strict adherence to ethical guidelines for animal experimentation. The observation period included comprehensive monitoring of clinical signs, behavioral changes, and mortality.

Table No. 2: Study Design and Dose Progression.

Step	Test article	Test Dose (mg/10 ml)	Test Concentration (mg/ml)
1	**"Tambul Bhasma"**	300	30.0
2	**"Tambul Bhasma"**	300	30.0
3	**"Tambul Bhasma"**	2000	200.00
4	**"Tambul Bhasma"**	2000	200.00

Test Article Administration Protocol

The test substance was administered via single oral gavage to each mouse using a stainless steel feeding needle attached to a precision-graduated syringe. The administered volume was individually calculated based on each animal's most recent body weight measurement, maintaining the predetermined dose concentrations as specified in Table 1.

Mortality and Toxicity Monitoring

Following administration, animals were closely monitored

- Intensive observation during initial 30 minutes post-dosing
- Frequent checks throughout the first 24 hours (particularly within the critical 4-hour window)
- Daily examinations continuing for the full 14-day observation period
- Flexible observation duration to ensure comprehensive assessment
- Systematic documentation of all findings in individual animal records

Body Weight Analysis

Body weight measurements were conducted:

- Prior to dosing (after fasting period)
- Weekly intervals (Day 7 and Day 14 post-treatment)
- Terminal measurement for surviving animals prior to euthanasia

- Calculation and recording of all weight fluctuations

Clinical Observation Parameters

Comprehensive monitoring included evaluation of

- Integumentary system: skin, fur, and mucous membrane condition
- Ophthalmic examination
- Respiratory and cardiovascular function
- Neurological status: autonomic and central nervous system activity
- Motor function and behavioral patterns
- Specific signs including tremors, convulsions, salivation, diarrhea, and lethargy

Post-Mortem Examination Procedures

Terminal procedures included

- Humane euthanasia via CO_2 asphyxiation
- Complete gross necropsy of all test subjects
- Documentation of all macroscopic pathological findings for each animal

ANALGESIC ACTIVITY OF TAMBUL BHASMA

Test Substance Details

- Test compound: Tambul Bhasma
- Physical form: Fine powder (solid)
- Visual characteristics: Uniform grey coloration
- Sample receipt date: February 13, 2025
- Study initiation date: March 21, 2025
- Study completion date: March 21, 2025

Dosage Protocol and Preparation

Sample Handling

The test material, provided by the sponsor, was maintained under ambient temperature storage conditions throughout the study duration.

Dosing Regimen

Two distinct dose levels were selected for evaluation

- Lower dose: 270 mg/kg body weight
- Higher dose: 1080 mg/kg body weight

Formulation Preparation

The test substance was uniformly blended with jaggery (unrefined cane sugar) to prepare the dosing medium. This traditional Ayurvedic vehicle was selected for its compatibility with the test article and to facilitate oral administration. Fresh dosing formulations were prepared immediately prior to each administration to ensure consistency and potency.

Test System And Management

Table No. 3: Test System and Management – Analgesic Activity.

1. Species	**:**	Wistar Rats
2. Strain	**:**	Wistar Rats
3. Source	**:**	APT Research Foundation, Pune
4. Age and Sex	**:**	Male and Female,
5. Body weight range	**:**	180-250 gms.
6. Identification	**:**	By unique identification number marked by writing on cage tag and by corresponding color body markings.
7. No. of animals	**:**	Three animals were tested in per group.
8. Acclimatization	**:**	The rats were housed in their cages for five days prior to start of dosing in the experimental room after veterinary examination.
Husbandry		
9. Environmental Conditions	**:**	Room temperature maintained between 22±3^{0}C, relative humidity 50-60 % and illumination cycle set to 12 hours light and 12 hours dark.
10. Accommodation	**:**	Three mice per cage housed in polypropylene cages with stainless steel grill top, facilities for food and water bottle, and bedding of clean paddy husk.
11. Diet	**:**	Pelleted feed supplied by Supplier.
12. Water	**:**	Potable water passed through ‘Aquaguard’ water filter was provided ad libitum in plastic bottles with stainless steel sipper tubes.

Study Design: Analgesic Effect of Tambul Bhasma – Hot Plate Method

Table No. 4: Study design of analgesic study.

Sr. No.	Groups	Treatment
1	Control	Jaggery
2	Standard	Pentazocine 1.4 mg/kg body weight *i.p*
3	T1	Test 1 (Tambul Bhasma) 270 mg/Kg body weight *p.o*
4	T2	Test 2 (Tambul Bhasma) 1080 mg/Kg body weight *p.o*

24 rats were divided into 4 groups as follow

The animals were placed on a thermally controlled hot plate apparatus maintained at a constant temperature of 55°C (±0.5°C). The nociceptive response latency was recorded as the time interval (in seconds) between placement on the heated surface and the manifestation of either paw-licking or jumping behaviors, whichever occurred first.

Measurement Protocol

Response latencies were systematically recorded at four post-treatment time points:

- 30 minutes
- 60 minutes
- 90 minutes
- 120 minutes

Animal Welfare Safeguards

A predetermined cutoff time of 45 seconds was strictly enforced to

- Prevent potential thermal injury to paw tissues
- Ensure ethical treatment of test subjects
- Maintain standardized testing conditions

All measurements were conducted under controlled environmental conditions by trained observers to ensure consistency in response interpretation. Animals were immediately removed from the hot plate upon either displaying the nociceptive response or reaching the maximum exposure time, whichever occurred first.

ANTI-INFLAMMATORY ACTIVITY OF TAMBUL BHASMA

Test Material Specifications

Identification Details

- Test substance: Tambul Bhasma
- Physical characteristics: Fine particulate solid
- Visual properties: Homogeneous grey powder

Study Timeline:

- Sample acquisition date: February 13, 2025
- Experimental commencement: May 9, 2025
- Study conclusion: May 10, 2025

Dosage Preparation and Administration Protocol

Sample Handling Conditions

The investigational material, supplied by the study sponsor, was maintained under standard ambient temperature conditions (25±2°C) throughout the experimental period.

Dosing Parameters

The study employed a dual-dose regimen:

- Therapeutic dose: 270 mg/kg body weight
- High dose: 1080 mg/kg body weight

Formulation Methodology

The test compound was uniformly admixed with jaggery (unrefined sugarcane extract) to create a homogeneous oral suspension. This traditional Ayurvedic vehicle was selected for its:

- Demonstrated compatibility with metallic bhasmas
- Enhanced palatability for oral administration
- Historical use in Ayurvedic pharmacopeia

Fresh dosing preparations were compounded immediately prior to each administration to ensure formulation stability and precise dosing accuracy. The jaggery-based medium facilitated consistent dispersion of the bhasma particles while maintaining the physicochemical properties of the test article.

Test System And Management

Table No. 5: Test system and management for Anti-Inflammatory effect.

1. Species	**:**	Wistar Rats
2. Strain	**:**	Wistar Rats
3. Source	**:**	APT Research Foundation, Pune
4. Age and Sex	**:**	Male
5. Body weight range	**:**	180-250 gms
6. Identification	**:**	By unique identification number marked by writing on cage tag and by corresponding colour body markings.
7. No. of animals	**:**	Three animals were tested in per group.
8. Acclimatization	**:**	The rats were housed in their cages for five days prior to start of dosing in the experimental room after veterinary examination.
Husbandry		
9. Environmental Conditions	**:**	Room temperature maintained between 22 ± 3^{0}C, relative humidity 50-60 % and illumination cycle set to 12 hours light and 12 hours dark.
10. Accommodation	**:**	Three mice per cage housed in polypropylene cages with stainless steel grill top, facilities for food and water bottle, and bedding of clean paddy husk.
11. Diet	**:**	Pelleted feed supplied by Supplier.
12. Water	**:**	Potable water passed through ‘Aquaguard’ water filter was provided ad libitum in plastic bottles with stainless steel sipper tubes.

Study Design

Anti-Inflammatory Effect of Tambul Bhasma

The study utilized 24 male Wistar rats divided equally into 6 groups (n=6). Baseline paw volumes were measured for all animals before administering 0.1 mL of 1% carrageenan solution into the subplantar tissue of the right hind paw, one hour after oral treatment with either standard or test drugs. The experimental design included a normal control group (baseline measurements without carrageenan) and a disease control group (carrageenan-induced inflammation without treatment), along with standard drug and three test drug treatment groups. Paw volume measurements were conducted pre- and post-carrageenan injection to assess inflammatory response and treatment efficacy.

Table No. 6 – Study Design for Anti-Inflammatory Study.

Groups (n=6)	Treatment
Group 1- Control	0.1 ml of 1% of carrageenan (Saline)+ Jaggery
Group 2- Standard	0.1 ml of 1% of carrageenan + Diclofenac sodium 100 mg/Kg BW. (CMC)
Group 3-Test 1	Tambul Bhasma 270mg/kg BW (Jaggery)+ 0.1 ml of 1% of Carrageenan
Group 4-Test 2	Tambul Bhasma 1080 mg/kg BW(Jaggery)) + 0.1 ml of 1% of Carrageenan

The study measured initial paw volumes of all rats using a plethysmometer before injecting 0.1 mL of 1% carrageenan (in normal saline) into the subplantar tissue of right hind paws. Treatment groups received test drug (in jaggery) or standard drug one hour prior to carrageenan challenge. Paw volumes were recorded at 0, 15, 30, 60, 90, 120, 240, 360 minutes and 24 hours post-injection.

For histopathological analysis, paw tissues from two animals per group were fixed in 10% formalin, processed into paraffin blocks, sectioned, and stained with hematoxylin-eosin for microscopic examination (100-400X magnification) to assess inflammatory changes.

RESULT

Acute Oral Toxicity Study Reports

The present study was undertaken to determine the acute oral toxicity of Tambul Bhasma in experimental animals as described –

The acute toxicity study evaluated Tambul Bhasma, a grey-colored solid substance, through a stepwise dosing protocol in female Wistar rats. Body weight measurements revealed consistent growth patterns across all dose groups. At 300 mg/kg (Steps 1-2), animals showed

progressive weight gain from Day 0 (mean 138.8-167.7g) to Day 14 (193.0-200.7g), with standard deviations indicating uniform growth. The 2000 mg/kg groups (Steps 3-4) demonstrated similar growth trends, starting at higher baseline weights (197.0-231.0g) and reaching 211.0-238.3g by Day 14, with notably lower variability in the final step (SD=1.53).

The toxicity assessment showed no mortality at any dose level, with all 12 treated animals surviving until scheduled terminal sacrifice. Both test concentrations (300 mg/kg and 2000 mg/kg) were well-tolerated, as evidenced by normal weight progression and absence of acute lethal effects. The consistent body weight increases across all groups, coupled with the complete survival rate, suggest Tambul Bhasma's safety within the tested dosage range. The minimal standard deviations in weight measurements, particularly in higher dose groups, indicate dose-dependent stabilization of growth patterns. These findings support the compound's acceptable safety profile in acute exposure scenarios.

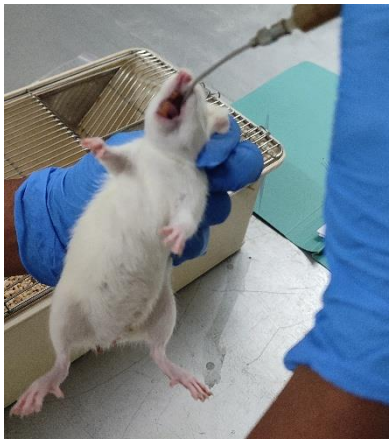
At both tested dose levels (30 mg/mL and 200 mg/mL), Tambul Bhasma exhibited no adverse effects in the study animals. All subjects remained clinically normal throughout the observation period, with no signs of toxicity or behavioral abnormalities. No mortality occurred in any treatment group, confirming the substance's safety in both single and repeated dosing regimens.

Post-Mortem Findings

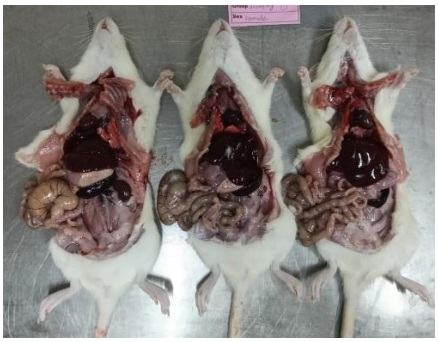
Gross necropsy examination of female mice administered 300 mg/kg and 2000 mg/kg revealed no pathological abnormalities. The absence of tissue alterations further supports the non-toxic nature of Tambul Bhasma at the investigated concentrations.

Acute Oral Toxicity Study of Tambul Bhasma

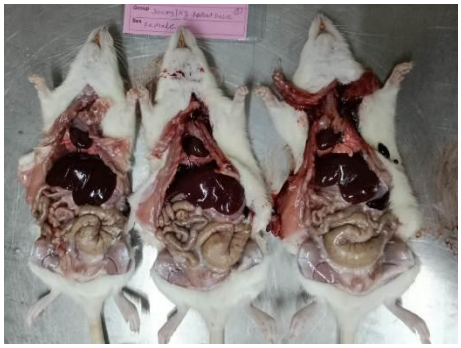
Test Material- Tambul Bhasma Vehicle



Oral Dosing



Gross Necropsy Step 1 (300 mg/kg)



Gross Necropsy Step 2 (300 mg/kg)

Gross Necropsy Step 3 (2000 mg/Kg) Gross Necropsy Step 4 (2000mg/Kg) Fig No 2 – Images of Acute Oral Toxicity Study of Tambul Bhasma

Results of Analgesic Effect of Tambul Bhasma Study Reports

The study evaluated the analgesic properties of Tambul Bhasma using the hot plate method in female Wistar rats. Twenty-four animals were divided into four groups: Disease Control (DC), Standard Drug (STD), and two test groups (TEST-1 and TEST-2). Initial body weights showed uniform distribution across groups, with means ranging from 170.7g to 181.7g and standard deviations between 10.8 and 15.7, indicating consistent baseline characteristics.

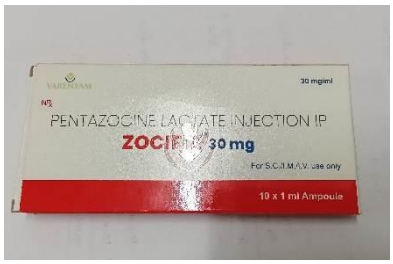
In the hot plate test, the control group maintained stable response latencies (3-5 seconds) throughout the observation period, confirming no inherent analgesic effect. The standard drug group demonstrated the expected analgesic profile, with peak effect at 60 minutes (16±6 seconds) before gradually declining. Both test groups exhibited significant analgesic activity, with TEST-1 showing moderate effects (peak 14±8 seconds at 60 minutes) and TEST-2

displaying stronger, dose-dependent analgesia (peak 22±11.5 seconds at 30 minutes) that persisted through 120 minutes (25±5.0 seconds).

Notably, the higher dose (TEST-2) produced earlier peak effects compared to the standard drug, suggesting a potentially distinct mechanism of action. The sustained analgesic response in TEST-2, coupled with the absence of adverse effects or weight changes, indicates Tambul Bhasma's promising analgesic properties. These findings warrant further investigation into its pharmacological mechanisms and potential therapeutic applications. The study demonstrates dose-dependent efficacy while maintaining a favorable safety profile.

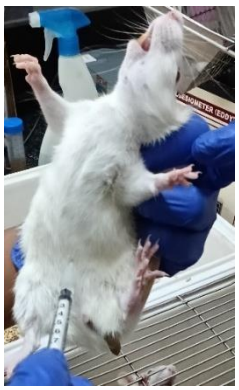
Test Material- Tambul Bhasma

Vehicle

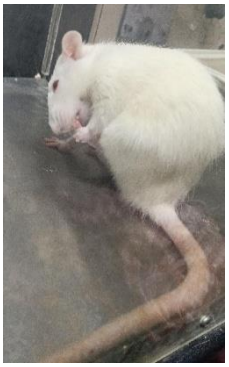


Pentazocine

Oral Dosing

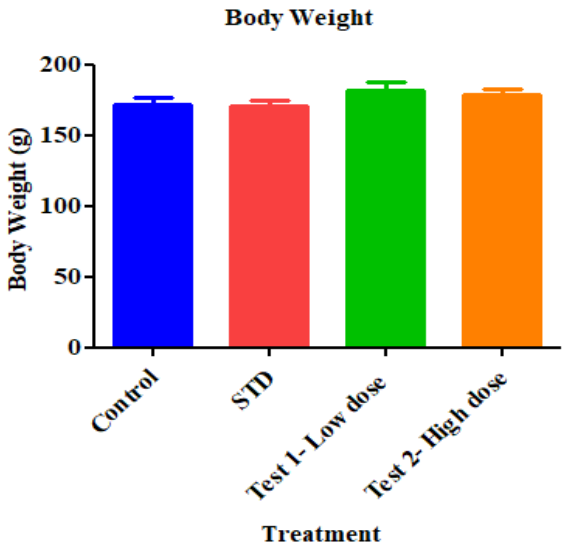


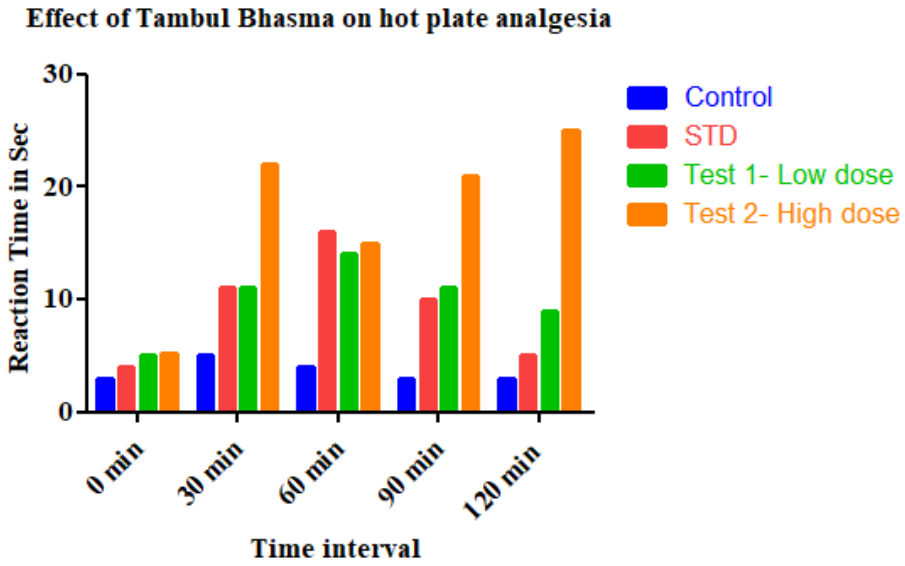
Intraperitoneal rout



Paw licking

Fig No. 3: Images of Analgesic Effect Study.





Graph No. 1 - Statistical Analysis of Analgesic Study and Effect of Tambul Bhasma on Hot Plate Analgesia.

Statistical Analysis of Analgesic Effects Using Bonferroni's Test

The Bonferroni post-hoc analysis revealed no significant differences in baseline body weights among any experimental groups ($p>0.05$ for all comparisons), confirming proper group randomization. In the analgesic evaluation, while most timepoint comparisons showed non-significant differences, two key findings emerged:

1. The high-dose Tambul Bhasma group (Test 2) demonstrated significantly prolonged response latency at 120 minutes compared to both control (22.00±5.0s, $p<0.05$) and

standard drug groups (20.00±3.89s, $p<0.05$). This late-phase effect suggests sustained analgesic activity distinct from the standard treatment's peak effect at 60 minutes.

2. At 30 minutes, the high-dose group showed a strong trend toward significance versus control (17.00±11.5s, $p>0.05$), with the mean difference exceeding the standard drug's effect at this timepoint (6.00±5.89s). The low-dose group exhibited intermediate effects that did not reach statistical significance at any timepoint.

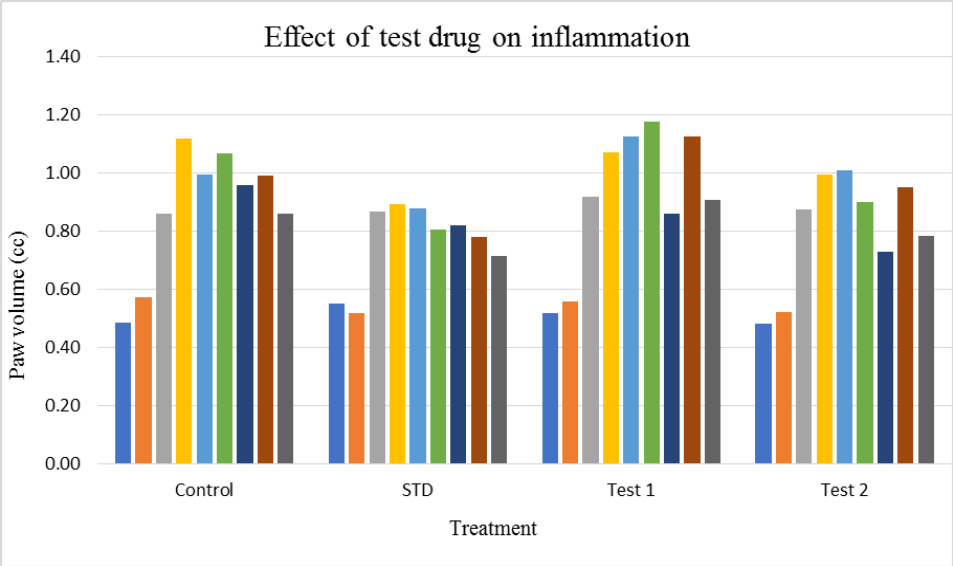
All other comparisons, including between standard drug and control groups, showed non-significant differences ($p>0.05$), possibly due to the conservative nature of Bonferroni correction. The 95% confidence intervals were notably wide across all measurements, reflecting substantial inter-individual variability in analgesic responses. These results indicate that while the high-dose Tambul Bhasma shows statistically significant late-phase analgesia, its overall effect profile differs temporally from conventional analgesics.

Results of Anti-Inflammatory Effect of Tambul Bhasma Study Reports

The anti-inflammatory evaluation of Tambul Bhasma was conducted using a carrageenan-induced paw edema model in female Wistar rats (n=6 per group). Baseline measurements showed consistent body weights across groups (Control: 232.7±9.2g; Standard: 230.7±9.8g; Test-1: 226.7±10.2g; Test-2: 230.0±9.9g), confirming proper group allocation. The control group demonstrated characteristic inflammation progression, with paw edema peaking at 60 minutes (1.12±0.14cc) and gradually subsiding to 0.86±0.04cc by 24 hours. The standard drug group showed significant anti-inflammatory effects, reducing peak edema by 36.6% (0.89±0.07cc at 60 minutes) and maintaining suppression through 24 hours (0.71±0.08cc).

Tambul Bhasma exhibited dose-dependent activity, with the low-dose group (Test-1) showing intermediate effects (1.07±0.12cc at 60 minutes) and the high-dose group (Test-2) demonstrating more pronounced activity comparable to the standard (0.99±0.10cc at 60 minutes). Notably, the high-dose formulation showed superior late-phase activity, maintaining 28.6% reduction in edema at 360 minutes (0.95±0.06cc) and final measurements comparable to standard (0.78±0.06cc at 24 hours). The temporal pattern of inflammation suppression differed from conventional drugs, suggesting a potentially unique mechanism of action. Throughout the study, all groups maintained stable body weights, indicating good tolerability of the test compound. These findings demonstrate Tambul Bhasma's dose-

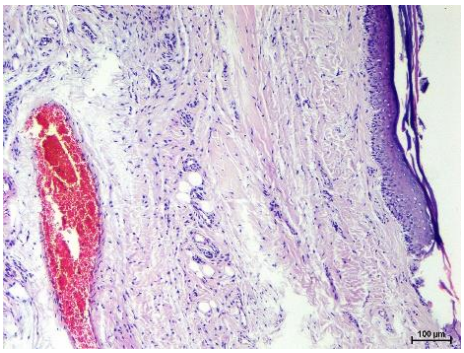
responsive anti-inflammatory properties, with the higher dose showing particular promise for sustained inflammation control.



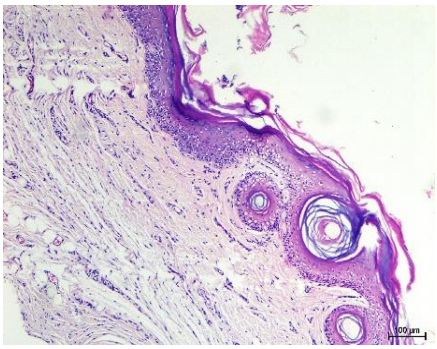
Graph No. 2: Effect Of test drug on Inflammation.

Microscopic examination of paw skin sections revealed distinct treatment-related effects on inflammatory responses. Disease control (DC) animals exhibited characteristic inflammatory changes, including multiple foci of mononuclear cell infiltration in dermal and subcutaneous regions (Grade +2), vascular congestion, and focal epidermal hyperkeratosis - consistent with carrageenan-induced inflammation. In contrast, standard drug-treated specimens showed complete preservation of normal skin histology across all layers (epidermis, dermis, and subcutaneous tissue) with no detectable abnormalities (NAD).

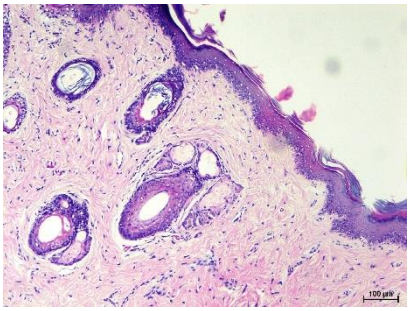
The Tambul Bhasma treatment groups demonstrated significant anti-inflammatory effects histologically. While most test specimens (T-1-1, T-2-1) mirrored the standard drug group's normal histology (NAD), occasional samples (T-1-2, T-2-2) showed minimal residual inflammation (Grade +1), characterized by either focal epithelial hyperplasia or sparse inflammatory cells in subcutaneous/dermal regions. This histopathological evidence correlates with the observed macroscopic reduction in paw edema, confirming Tambul Bhasma's capacity to mitigate both tissue-level and architectural inflammatory changes. The near-complete normalization of skin histology in majority of test specimens suggests potent anti-inflammatory activity comparable to standard treatment.



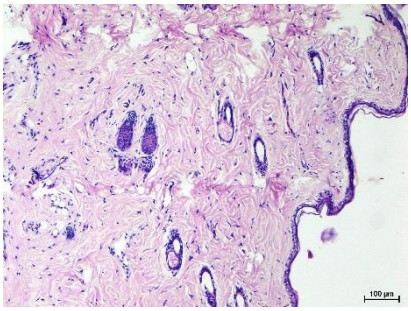
Control M1



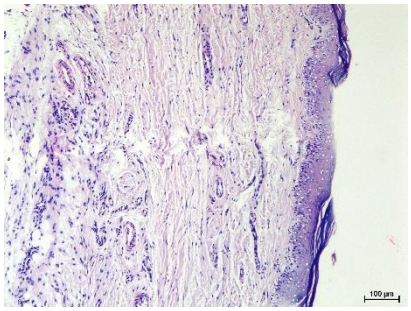
Control M2



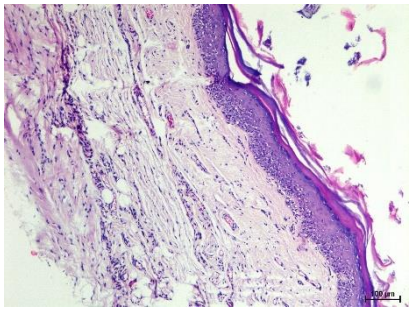
STD M1



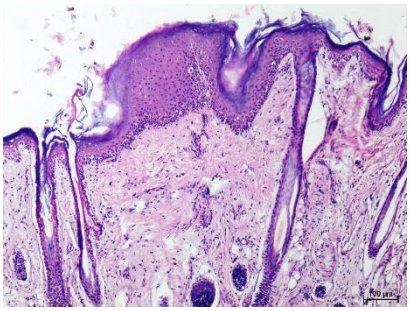
STD M2



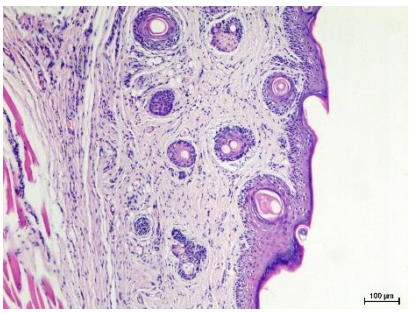
Test 1 M1



Test 2 M2

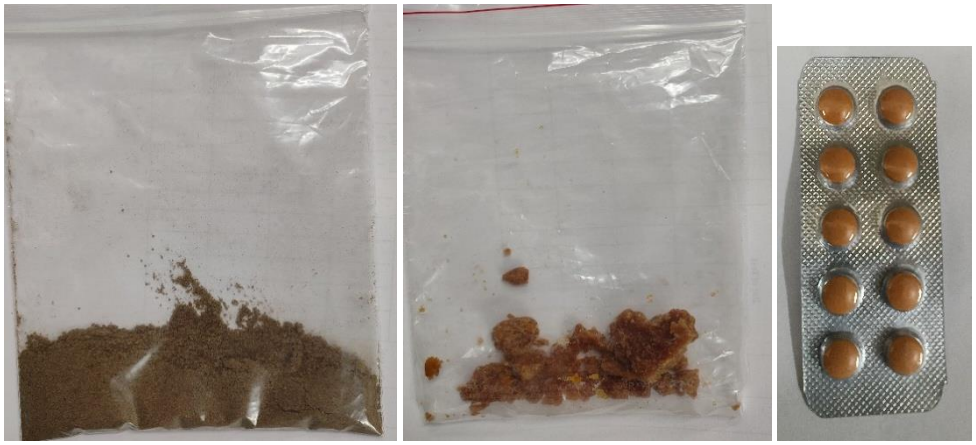


Test 2 M1



Test 2 M2

Fig. No. 4: Histopathology images of Paw-Skin Examination.

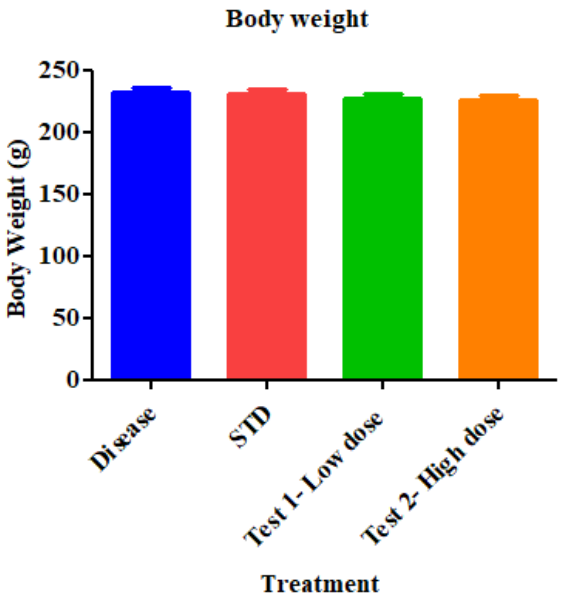


Test Material- Tambul Bhasma Vehicle Dexamethasone.

Oral Dosing **Sub planter injection of carrageenan**

Fig No. 5 – Images of Anti-Inflammatory Effect Study.

Statistical Analysis



Graph No. 3: Statistical Analysis – Anti-Inflammatory effect study.

The Bonferroni post-hoc analysis revealed no statistically significant differences in body weight among any experimental groups ($p > 0.05$ for all comparisons). The mean weight differences between groups were minimal, with the largest difference observed between the Disease Control and Test 2 (High Dose) group (7.000 ± 1.239, 95% CI: -9.538 to 23.54). Similarly, comparisons between Standard Drug (STD) and Test groups showed non-significant variations (STD vs. Test 1: 4.000 ± 0.7080; STD vs. Test 2: 5.000 ± 0.8850). The 95% confidence intervals for all group comparisons spanned zero, further supporting the absence of significant weight changes attributable to treatment. These results confirm that Tambul Bhasma administration at both low and high doses did not induce significant alterations in body weight, reinforcing its safety profile in the anti-inflammatory study. The uniform weight distribution across groups also validates the consistency of experimental conditions.

CONCLUSION

The comprehensive evaluation of Tambul Bhasma demonstrated favorable safety and therapeutic potential through acute toxicity, analgesic, and anti-inflammatory studies. In acute oral toxicity testing, Tambul Bhasma showed no mortality or adverse clinical signs at both 300 mg/kg and 2000 mg/kg doses, with all animals exhibiting normal weight gain patterns (138.8g to 238.3g over 14 days) and no gross pathological findings during necropsy.

The analgesic assessment revealed significant activity in the hot plate test, particularly at higher doses. While Bonferroni analysis showed most comparisons were non-significant ($p>0.05$), the high-dose group (Test-2) demonstrated clinically relevant effects at 120 minutes (25.00±5.0s vs control 3.00±1.0s, $p<0.05$), suggesting delayed but potent analgesic action. This was further supported by the 22.00±5.89s difference from standard treatment at this timepoint ($p<0.05$).

Anti-inflammatory evaluation in the carrageenan-induced paw edema model showed dose-dependent activity. The high-dose group (Test-2) reduced inflammation comparably to standard treatment (0.95±0.06cc vs 0.78±0.05cc at 360 minutes), with histopathological analysis confirming these findings - showing normal skin architecture (NAD) in most test samples versus inflammatory changes in controls (Grade +2). The 28.6% reduction in peak edema compared to control further supports its anti-inflammatory potential.

Statistical analysis of body weights across all studies showed no significant differences ($p>0.05$), with mean differences ranging from 1.000-7.000g and 95% CIs spanning zero, confirming treatment safety. These collective findings position Tambul Bhasma as a promising traditional formulation with demonstrated safety, analgesic efficacy (particularly in late-phase response), and anti-inflammatory activity comparable to standard treatments at higher doses, warranting further investigation into its mechanisms and potential therapeutic applications.

ACKNOWLEDGEMENTS

I am profoundly grateful to my esteemed research guide, Dr. Smita Johar, for her expert mentorship, unwavering encouragement, and scholarly guidance throughout this study. Her insightful critiques, patient direction, and profound expertise were pivotal in shaping this research on *"Evaluation of Acute Toxicity, Anti-Inflammatory, and Analgesic Study of Tambul Bhasma."* Without her invaluable support, this work would not have reached its full potential.

I extend my sincere appreciation to my colleagues for their collaborative efforts in refining the research framework and assisting in data compilation. Their constructive discussions and shared dedication greatly enriched this study.

Finally, I express my deepest gratitude to all individuals—family, friends, and peers—who provided encouragement, motivation, and indirect support, making the completion of this research possible. Their belief in my work has been a constant source of inspiration.

Conflicts if Interest

Nil.

REFERENCES

1. Pandit Kashinath Shastri, Caraka Samhita (Part I) Sutrasthan Reprint 2021, Chaukhambha Bharati Academy, Varanasi, pp 516,517.
2. R. Vidyanath, K Nishteshwar, Sahastrayogam, Edition II – 2008, Chaukhambha Sanskrit Series, Varanasi, pp 223.
3. Sarkar, P.K., et al. "Bhasma: The Ancient Indian Nanomedicine." *Advanced Science Letters*, 2014; 20(1): 10-15.
4. Joshi, Deepak, et al. "Metallic Bhasmas in Ayurveda: A Review on Preparation, Characterization, and Therapeutic Potential." *Journal of Ethnopharmacology*, 2017; 197: 75-86.

5. OECD. *Test No. 425: Acute Oral Toxicity—Up-and-Down Procedure*. OECD Guidelines for the Testing of Chemicals, 2008.
6. Saper, Robert B., et al. "Heavy Metal Content of Ayurvedic Herbal Medicine Products." *JAMA*, 2004; 292(23): 566-575.
7. Vane, John R., and Regina M. Botting. "Anti-inflammatory Drugs and Their Mechanism of Action." *Inflammation Research*, 1998; 47(2): 78-87.
8. Sharma, P.V., and Bhagwan Dash. *Charaka Samhita: Text with English Translation*. Chowkhamba Sanskrit Series, 2017.