World Journal of Advanced Research and Reviews
eISSN: 2581-9615 CODEN (USA): WJARAI
Cross Ref DOI: 10.30574/wjarr
Journal homepage: https://wjarr.com/

(RESEARCH ARTICLE)

Check for updates

Evaluation of anti- inflammatory and analgesicactivity of Bhasmas in experimental animal models

Sivaram K [1,*], Lakshmi Anusha V [2], Sunitha N [2] and Thangabalan B [3]

[1] *M Pharm student, SIMS College Of Pharmacy, Mangaldas Nagar, Guntur, India.*
[2] *Associate Professor, SIMS College Of Pharmacy, Mangaldas Nagar, Guntur, India.*
[3] *Professor, SIMS College Of Pharmacy, Mangaldas Nagar, Guntur, India.*

World Journal of Advanced Research and Reviews, 2024, 22(02), 832–847

Publication history: Received on 02 April 2024; revised on 11 May 2024; accepted on 13 May 2024

Article DOI: https://doi.org/10.30574/wjarr.2024.22.2.1438

Abstract

Acute toxicity studies of both the bhasmas showed no mortality at 2000 mg/kg.The Scanning electron microscopy of both Tamra and Lauha bhasma indicated the nanomaterial characteristics which justifies claim of Ayurvedic bhasmas as ancientnanomaterials. Tamra and Lauha bhasma were found to be effective as anti-inflammatory agent inacute models of inflammation. It inhibits all phases of inflammation induced by carrageenan. Tamra and Lauha bhasma were found to be effective as anti-inflammatory agent inchronic model of inflammation. It significantly inhibits wet as well as dry weightof cotton pellet granuloma at highest doses only. Analgesic activity of both Tamra and Lauha bhasma in hot plate and tail immersion models were found to be significant at 120-240 mins.

Analgesic activity of both Tamra and Lauha bhasma in acetic acid models were found to be non-significant in nature, while found to be significant at late phase in formalin induced model. Tamra and Lauha bhasma also were found to have significant anti-arthritic activityin animal study.The whole pharmacological investigations were indicated that activity was only significant at therapeutic and more than therapeutic dose but nonsignificant at doselower than therapeutic doses. The obtained results indicates the anti-inflammatory and analgesic potential of both these bhasmas probably by inhibiting inflammatory mediators.

Keywords: Tamra; Lauha bhasm; Carrageenan; Ayurvedic; Inflammation

1. Introduction

Bhasmas are proven their safety into animal studies which motivates the researchers towards their theraputic effectiveness into chronic ailments. Inflammation and inflammatory response:

Inflammation is one of the body defense mechanism to get rid of causative agent which causes cell injury and further cell necrosis or it is protective response by the host body to inflammatory stimulus (Florey 1970, Majno 1975). There are four important cardinal signs of inflammation as indicated in figure 5.

Basically, there are two types of inflammation which are described as follows: (Lawrence T, 2002)

* Corresponding author: Sivaram K

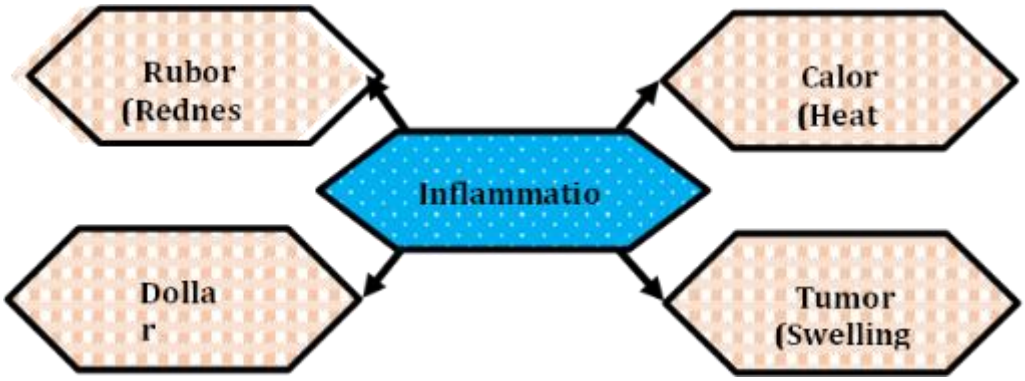


Figure 1 Cardinal signs of inflammation

2. Methodology

Toxicity and doses of Tamra bhasma (TB) and Lauha bhasma (LB):

2.1. Acute toxicity study

This test was performed accordingly with OECD guideline 425. Healthy, young, and non preganant female rats were selected for the study. Animals were administered with 2000 mg/kg dose of Tamra bhasma (TB) and Lauha bhasma (LB) and observed for any mortality for first 6 hrs continuously and intermittently thereafter. The results of this study indicated that both the bhasmas does not showed any mortality at 2000mg/kg body weightgiven orally in the rats, and hence the drugs were considered to be safe for further pharmacological screening.

Table 1 Dose selection for animal study

Name of drug	Selected doses
Tamra bhasma (TB)	5.5 mg / kg (Theraputic equivalent dose, TED)
	2.75 mg / kg (Theraputic equivalent dose, TED / 2)
	11 mg / kg (Theraputic equivalent dose, TED x 2)
Lauha bhasma (TB)	11 mg / kg (Theraputic equivalent dose, TED)
	5.5 mg / kg (Theraputic equivalent dose, TED / 2)
	22 mg / kg (Theraputic equivalent dose, TED x 2)

2.2. Acid insoluble ash

The ash of both bhasmas were taken into two different crucibles boiled for 5 minutes with 25 ml of dilute hydrochloric acid, the collected insoluble matter on ash less filter paper (whatman filter paper), washed with hot water and transferred to crucible and ignite to get constant weight. Allow the residue to cool for 30 mins and calculate percentage of acid insoluble ash as %w/w.

2.3. Loss on drying

This test was performed determine the moisture content in the sample. One gram of exactly weighed sample (Tamra bhasma (TB), Lauha bhasma (TB) was taken in a previously weighed petri dish and dried in an oven at 105°C for 5 hrs and weigh. Then the petridish was taken out, weighed after self-cooling and from the weight loss the percentage of loss on drying was calculated and expressed as %w/w.

2.4. Pharmacological study

After the verification of genuine quality of both bhasmas they were subjected to their folk claimed anti-inflammatory and analgesic activity.

Table 2 Animal grouping and dosing

Group	No of animals	Name of group	Treatment	Dose
I	6	Control	Gum acacia/water	2ml/kg
II	6	Standard	Diclofenac	40 mg/kg
III	6	TB treated group	Tamra bhasma (TB)	2.75 mg/kg
IV	6	TB treated group	Tamra bhasma (TB)	5.5 mg/kg
V	6	TB treated group	Tamra bhasma (TB)	11 mg/kg
VI	6	LB treated group	Lauha bhasma (LB)	5.5 mg/kg
VII	6	LB treated group	Lauha bhasma (LB)	11 mg/kg
VIII	6	LB treated group	Lauha bhasma (LB)	22 mg/kg

2.5. Anti-inflammatory activity

2.5.1. Carrageenan induced paw edema method

The test was carried out in accordance with the previously described method. Animals were grouped and dosed as mentioned in table 10. Thirty minutes after thedrug/vehicle treatments, λ-carrageenan (Phlogistic agent) was injected subplantarly (0.1ml of 1 %) constituted in 0.9 % sodium chloride into the right hind paws of the experimental rats. The inflammation induced by the carrageenan was quantified by measuring the paw volume using a UGO Basile plethysmometer and calculated % inhibition of paw edema.

2.5.2. Cotton pellet-induced granuloma

This method was utilized to study the effect of Tamra Bhasma on the subacute stage of inflammation as per method of Hejjaj et al. with slight modifications. Thirty minutes after the administration of the drugs/vehicle, 10mg±1 mg sterilized autoclaved cotton wasaseptically implanted into the axilla and groin regions of the rats by making small cuts using blunt forceps after inducing an anesthetized state using anesthetic ether. All the drug/vehicle treatments were administered once a day for the next 7 days. On the eighth day, all the rats were anesthetized again with anesthetic ether. The implanted surgical cotton pellets were removed surgically, cleaned of extraneous matter and dried in a hot airoven at 60°C. The dried pellets were weighed and the percentage inhibition of dry weight granuloma tissue calculated.

CFA-induced arthritis: Arthritis was induced by injecting CFA (0.1 ml suspension of heat-killed Mycobacterium tuberculosis) into the sub-plantar tissue of the right hind paw of each rat after it wasanesthetized with ketamine (120 mg/kg) and anesthetic ether. Animals were grouped anddosed as mentioned in table 10 except utilized dose of diclofenac (10mg/kg) in presentmodel. Thirty minutes after the drug/vehicle treatments, CFA was injected into the righthind paw (primary lesion). The day of this treatment was designated day 0. All thetreatments (drug/vehicle) were continued over the next 27 days. On the 28th day, the ratswere subjected to an array of evaluations related to their blood and organs.

2.5.3. Paw volume measurement

On 7th, 14th, 21th, 28th day paw volume was measured with help of UGO basile plethysmometer.

2.5.4. Body weight measurement

On 7th, 14th, 21th, 28th day body weight was measured with help of normal animal weighing balance.

2.5.5. Blood estimations

On 28th day all rats were subjected to light ether anesthesia and blood was collected through retro-orbital plexus. Collected blood was further centrifuged to isolate serum at 3000 rpm for 15 mins. On blood and serum several estimations were performed listed as follows:

Hematological estimations: RBC, WBC, ESR and HB count

Biochemical estimations: Alanine transaminase (ALT), Aspartate transaminase (AST), C- reactive protein (CRP), Rheumatoid factor (RF), creatinine and urea estimations were performed by commercial kit methods and as per manufacturer instructions.

Cytokine estimations: TNF-alpha and IL-1beta estimations were performed as perthe manufacturer instructions.

Paw scores: Arthritic score and mobility score were assigned to injected paws of the rats by visual observation method. Scores were assigned to all the paws of rats and individual scores were given to each paw.

Radiographic examination: It was performed same as per method described by Wejikoon HS with slight modifications.

Radiology was performed on 28th day on all animals on x-ray machine (Fuji)

Organ weights: Thymus and spleen were isolated from rats and subjected to their weight measurement with normal weighing balance.

Histopathological examination: Towards the end, ankle joints of rats were removed and put into the liquid paraffin and subjected to its histopathology. Histology was performed at histopathological center in Aurangabad, Maharashtra.

2.5.6. Analgesic activity

Hot plate analgesic test method

Animal grouping and dosing of animals were done as described in Table. 10 except standard drug used. Indomethacin (10 mg/kg) used as reference standard. Thirty mins afterall drug/Vehicle treatments, each rat was placed individually on hot plate set at temperature 55^{0}c to assess animal's tolerance to thermal noxious stimulus. Time taken by

rat to jumped out, paw licking, lifting from hot plate were considered as analgesic behavior were recorded as reaction time.

Tail immersion analgesic test method

Animal grouping and dosing of animals were done as described in Table. 10 except standard drug used. Indomethacin (10 mg/kg) used as reference standard. Each rat had their tail immersed in water bath thermostatically set at temperature 55°c. The reaction time (in seconds) for individual rat to withdraw its tail indicates its pain tolerance to thermal stimulus. The time was measured by stopwatch started at onset of immersing tail (1-2- cm) of each rat. The measurement were done at 30 min, 60 min, 120 min & 240 min post drug administration. The obtained results of drug treatment were compared control.To avoid tail injury, cut-off time for tail immersion is 180 sec.

Acetic acid induced writhing analgesic test method:

Animal grouping and dosing of animals were done as described in Table. 10 except standard drug used (Aspirin, 5mg/kg). After 30 mins post drug treatments, writhing was induced by acetic acid (0.1ml, 0.6%) injected intra-peritonially. Number of writhing's/animal was counted individually for next 20 mins. Percentage protection against writhing by drug was calculated by a formula:

% Protection: Wc –Wt / Wc X 100

Where, Wc = mean writhing's for control, Wt = mean writhing's for standard/test

Formalin induced pain analgesic test method: Animal grouping and dosing of animals were done as described in Table. 10 exceptstandard drug used (Indomethacin, 10mg/kg). After 30 mins, pain was instigated byinfusing 0.05 mL of 2.5% formalin in distilled water in the Subplantar of the correct rearpaw of rats. All the animals were put in cages and continuously observed for number ofpaw lickings on injected rear paw divided in two phases i.e. initial phase (0-15 mins), latephase (15-30 mins). Licking of paw as an indicator of nociception.

2.6. Statistical analysis

The data generated were expressed as the mean ± SEM. The control group and the drug- treated groups were compared. All statistical difference between two means was resolute by One-way ANOVA, followed by Dunnett's multiple

comparison test. The Kruskal- Wallis test followed by Dunn's multiple comparison test was used to score the data. Data were considered statistically significant if $P < 0.05$.

3. Results and discussion

In present investigation, Tamra bhasma (TB) and Lauha bhasma (LB) were obtained from the market. Hence, their quality check is utmost necessary to take it for further animal studies. So for said purpose, several techniques were adopted and their results are summarized as follows.

3.1. Physicochemical characterization

Evaluation of Tamra bhasma (TB) by traditional parameters:

Rekhapurnatvam: When a pinch of Tamra bhasma (TB) was held between the thumb and forefinger and rubbed, it was observed to enter in lines of fingers and wasn't washed off easily with water

Varitaratavam:A little quantity of Tamra bhasma (TB) was sprinkled over cold water and it was observedthat bhasma particles were floated on surface of cold water in beaker (Figure 14A).

Nisvadutvam: A small quantity of Tamra bhasma (TB) was placed on the tongue and it was found to betasteless.

3.2. Acid insoluble ash

This test was performed to assess acid insoluble inorganic matter in sample. It was foundto be 7.4% w/w for Tamra bhasma (TB).

3.3. Loss on drying

This test was performed to assess moisture content in sample. It was found to be 0.36 %w/w for Tamra bhasma (TB).

Table 3 Characterization of Tamra bhasma (TB) and Lauha bhasma (LB) bytraditional parameters

Rekhapurnatvam	TB & LB entered in lines of finger & wasn't washed off easily.
Varitaratvam	TB & LB particles were floated on surface of cold water
Nisvadutvam	TB & LB was found to be tasteless when putted on tongue
Acid insoluable ash	Tamra Bhasma = 7.4 Lauha Bhasma = 10.5
Loss on drying	Tamra Bhasma = 0.36 Lauha Bhasma = 0.34

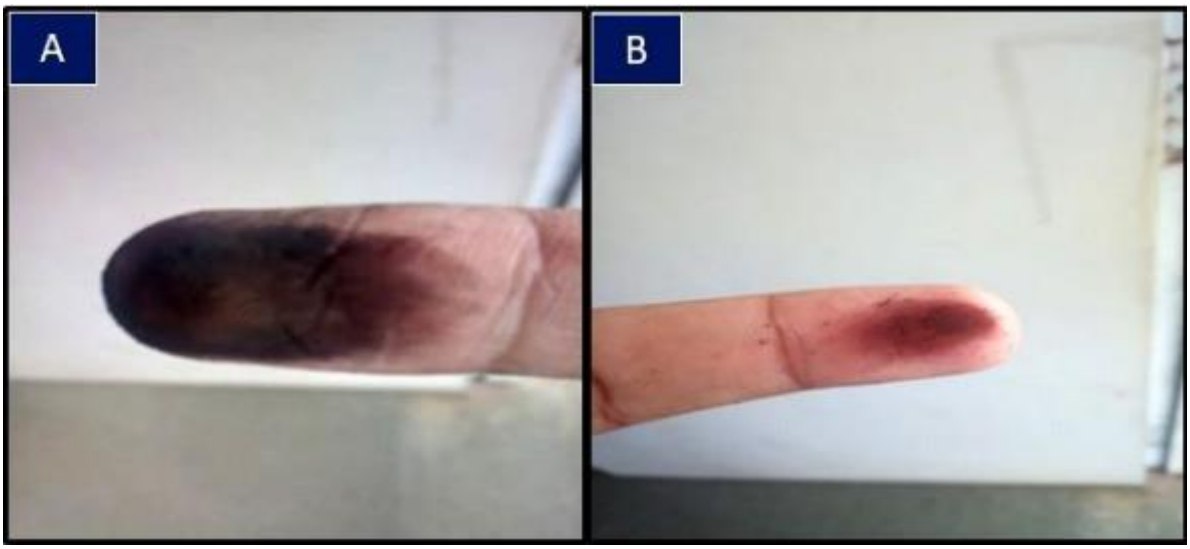


Figure 2 Rekhapurnatvam of studied bhasmas. Figure 2A Tamra bhasma (TB), Figure 2B = Lauha bhasma (LB)

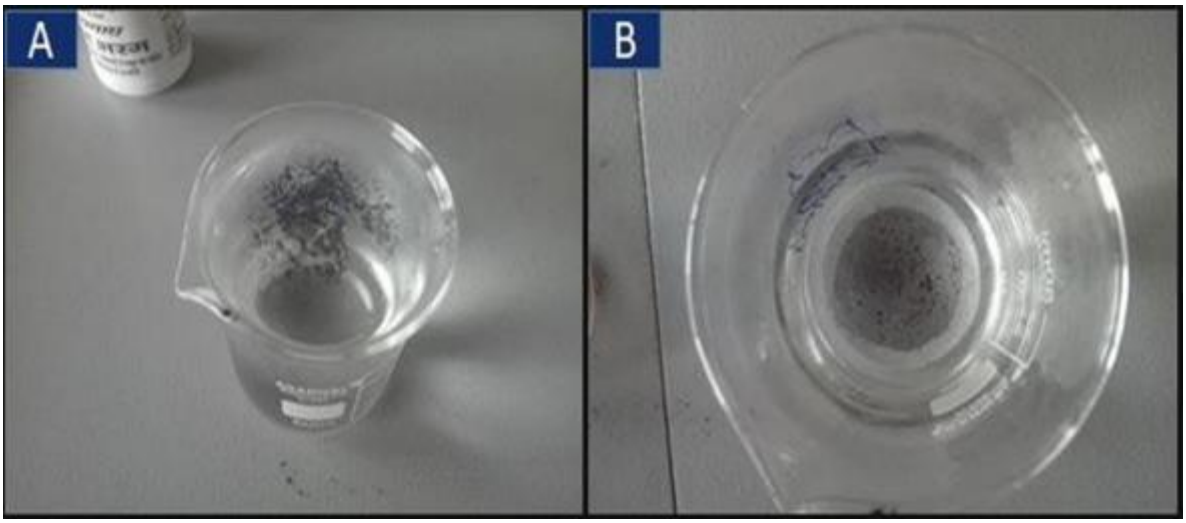


Figure 3. Varitaratavam of studied bhasmas. Figure 3A = Tamra bhasma (TB), Figure 3B = Lauha bhasma (LB)

Table 4 Effect of Tamra bhasma (TB) and Lauha bhasma (LB) on Carrageenaninduced paw edema

Groups	**Paw volume (ml)**					
	0 hr	1 hr	2 hr	3 hr	4 hr	5 hr
Control (Gum acacia+ water) 2 ml/kg	0.98 ±0.06	1.48 ±0.03	1.94 ±0.21	2.36 ±0.20	2.53 ±0.16	2.63 ±0.13
Standard (Diclofenac)40 mg/kg	0.85 ±0.03	1.09 ±0.07 **	0.93 ±0.08 ***	0.88 ±0.04 **	0.78 ±0.07	0.72 ±0.05 *
Tamra bhasma (TB) 2.75 mg/kg (TED/2)	0.98 ± 0.06	1.38 ± 0.1	1.32 ± 0.17 *	1.27 ± 0.40 **	1.23 ± 0.31 ***	1.21 ± 0.10 ***
Tamra bhasma (TB) 5.5 mg/kg (TED)	0.87 ±0.02	1.22 ±0.07 **	1.19 ±0.06 **	1.15 ±0.05 **	1.06 ±0.20 ***	0.91 0.07 ***

Tamra bhasma (TB) 11 mg/kg (TEDx2)	0.99 ±0.06	1.20 ±0.06 **	1.17 ±0.05 **	1.07 ±0.19 **	0.89 ±0.15 ***	0.83 ±0.10 ***
Lauha bhasma (LB) 5.5 mg/kg (TED/2)	0.962 ± 0.04	1.373 ±0.08	1.306 ±0.15 *	1.179 ±0.20 *	1.030 ±0.16	1.036 ± 0.05 *
Lauha bhasma (LB) 11 mg/kg (TED)	0.922 ± 0.03	1.212 ±0.09	1.221 ±0.13 **	1.094 ±0.24 *	0.907 ± 0.08 *	0.817 ± 0.10 *
Lauha bhasma (LB) 22 mg/kg (TEDx2)	0.972 ± 0.06	1.212 ±0.04 *	1.268 ±0.11 **	1.012 ±0.17 *	0.811 ± 0.14 *	0.717 ± 0.08 *

Values represents mean ± SEM., ANOVA: Dunnett's multiple comparison test. **P<0.01;*** P<0.001; *P<0.05 versus the control group.

Table 5 % inhibition of paw edema by Tamra bhasma (TB) and Lauha bhasma (LB)

Groups	**% inhibition of paw edema**					
	0 hr	1 hr	2 hr	3 hr	4 hr	5 hr
Control(Gum acacia+ water)2 ml/kg	-	-	-	-	-	-
Standard (Diclofenac) 40 mg/kg	-	27	50	61	8	73
Tamra bhasma (TB)2.75 mg/kg (TED/2)	-	10	25	44	52	54
Tamra bhasma (TB)5.5 mg/kg (TED)	-	17	37	50	56	65
Tamra bhasma (TB)11 mg/kg (TEDx2)	-	20	39	55	64	69
Lauha bhasma (LB)	-	55	33	50	59	61
5.5 mg/kg (TED/2)						
Lauha bhasma (LB)11 mg/kg (TED)	-	66	37	54	64	69
Lauha bhasma (LB)22 mg/kg (TEDx2)	-	66	35	57	68	73

Table 6 Inhibition of dry and wet weight of granuloma by Tamra bhasma (TB) and Lauha bhasma (LB)

Groups	**Wet weight of cotton pellet(mg)**	**Dry weight of cottonpellet (mg)**
Control (Gum acacia+ water)2 ml/kg	101.4 ± 5.1	59.03 ± 2.24
Standard (Diclofenac) 40 mg/kg	55.14 ± 6.4 ***	20.37 ± 2.27 **
Tamra bhasma (TB) 2.75 mg/kg (TED/2)	97.94 ± 2.5	50.27 ± 4.07
Tamra bhasma (TB) 5.5 mg/kg (TED)	88.60 ± 3.2	43.67 ± 5.53 *
Tamra bhasma (TB) 11 mg/kg (TEDx2)	77.42 ± 3 **	30.60 ± 4.31 **

Lauha bhasma (LB) 5.5 mg/kg (TED/2)	99.42 ± 3	47.24 ± 5.03
Lauha bhasma (LB) 11 mg/kg (TED)	85.99 ± 2.3	42.08 ± 2.14
Lauha bhasma (LB) 22 mg/kg (TEDx2)	75.51 ± 3.6 **	32.68 ± 2.59 *

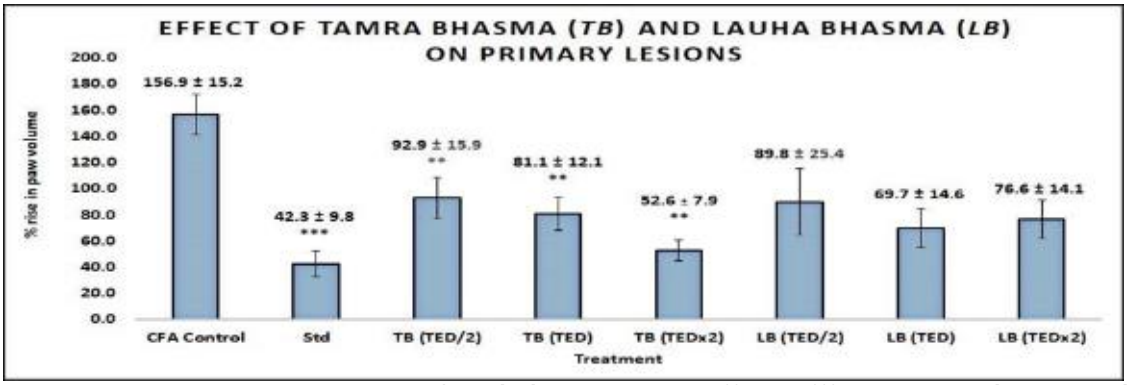
Values represents mean ± SEM., ANOVA: Dunnett's multiple comparison test. **P<0.01;*** P<0.001; *P<0.05 versus the control group. Dry and wet weight of cotton pellets were measured with sensitive electronic balance..

Table 7 Percent inhibition of dry and wet weight of granuloma by Tamra bhasma (TB) and Lauha bhasma (LB)

Groups	**% inhibition of wet weight granuloma**	**% inhibition of dry weight granuloma**
Control(Gum acacia+ water)2 ml/kg	-	-
Standard (Diclofenac)40 mg/kg	45.60	65.5
Tamra bhasma (TB) 2.75 mg/kg (TED/2)	3.38	14.8
Tamra bhasma (TB) 5.5 mg/kg (TED)	12.59	26.0
Tamra bhasma (TB) 11 mg/kg (TEDx2)	23.63	48.16
Lauha bhasma (LB) 5.5 mg/kg (TED/2)	1.93	19.96
Lauha bhasma (LB) 11 mg/kg (TED)	15.17	28.7
Lauha bhasma (LB) 22 mg/kg (TEDx2)	25.51	44.6

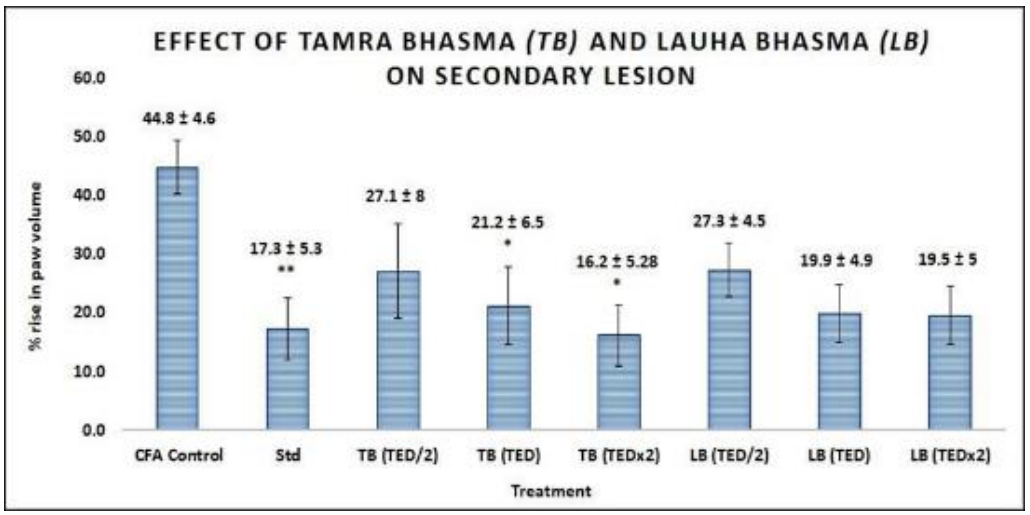
% inhibition of dry and wet weight of granuloma were calculated as indicated in Table 17. The results indicated that diclofenac produced maximum % inhibition of granuloma of dry (65.5%) and wet (45.6%) cotton pellets. Amongst both studied drugs, wet weight inhibition was highest at Lauha bhasma (LB) 22 mg/kg (25.5%), while dry weight inhibition was highest at Tamra bhasma (TB) 11 mg/kg (48.16%).

Obtained results clearly indicated that anti-granuloma activity and its anti- inflammatory activity of both studied Tamra bhasma (TB) and Lauha bhasma (LB).



Values represents mean ± SEM., ANOVA: Dunnett's multiple comparison test.**P<0.01;*** P<0.001 versus the CFA control group.

Figure 4 Effect of Tamra bhasma (TB) and Lauha bhasma (LB) on primary pawlesions. (Standard- Diclofenac sodium 10 mg/kg; TB (TED/2) - 2.75 mg/kg; TB (TED) –5.5 mg/kg; TB (TEDx2) - 11 mg/kg; LB (TED/2) – 5.5 mg/kg; LB (TED) - 11 mg/kg; LB (TEDx2) - 22 mg/kg.



Values represents mean ± SEM., ANOVA: Dunnett's multiple comparison test. **P<0.01;* P<0.05 versus the CFA control group.

Figure 5 Effect of Tamra bhasma (TB) and Lauha bhasma (LB) on secondary pawlesions. (Standard- Diclofenac sodium 10 mg/kg; TB (TED/2) - 2.75 mg/kg; TB (TED) – 5.5 mg/kg; TB (TEDx2) - 11 mg/kg; LB (TED/2) – 5.5 mg/kg; LB (TED) - 11 mg/kg; LB(TEDx2) - 22 mg/kg.

Table 8 Body weight gain by treatment of Tamra bhasma (TB) and Lauha bhasma (LB)

Groups	**Average body weight gain(g)**
Control (Gum acacia+ water) 2 ml/kg	17 ± 0.8
Standard (Diclofenac)40 mg/kg	40 ± 1.95 ***
Tamra bhasma (TB)2.75 mg/kg (TED/2)	21 ± 1.1
Tamra bhasma (TB)5.5 mg/kg (TED)	29 ± 1.8 ***
Tamra bhasma (TB)11 mg/kg (TEDx2)	38 ± 2.3 ***
Lauha bhasma (LB)5.5 mg/kg (TED/2)	19 ± 1.23
Lauha bhasma (LB)11 mg/kg (TED)	20 ± 0.87 *
Lauha bhasma (LB)22 mg/kg (TEDx2)	28 ± 1.98 **

Values represents mean ± SEM., ANOVA: Dunnett's multiple comparison test. *P<0.05;**P<0.01;*** P<0.001 versus the CFA control group. Body weight gain was calculated.

Table 9 Hematological alterations by treatment of Tamra bhasma (TB)and Lauha bhasma (LB)

Groups	**RBC count** 10^6 **3 (X 10 /mm)**	**WBC count** 10^3 **3 (X 10 /mm)**	**Hb count(gm/dl)**	**ESR (mm/hr)**
Control (Gum acacia+ water)2 ml/kg	7 ± 0.63	15 ± 0.21	13 ± 0.44	16 ± 0.91
Standard (Diclofenac) 40 mg/kg	9 ± 0.73 **	7 ± 0.28 ***	16 ± 0.24 ***	10 ± 0.39 ***
Tamra bhasma (TB) 2.75 mg/kg (TED/2)	8 ± 0.11	12 ± 0.92 **	13 ± 0.44	15 ± 0.55
Tamra bhasma (TB) 5.5 mg/kg (TED)	9 ± 0.12 *	10 ± 2 **	14 ± 0.26 *	13 ± 0.61 **
Tamra bhasma (TB) 11 mg/kg (TEDx2)	10 ± 0.48 **	9 ± 1 **	15 ± 0.64 **	11 ± 0.33 **
Lauha bhasma (LB) 5.5 mg/kg (TED/2)	7 ± 0.22	14 ± 0.43	13 ± 0.22	16 ± 0.52
Lauha bhasma (LB) 11 mg/kg (TED)	9 ± 0.64 *	12 ± 1.4	14 ± 0.41	15 ± 1.1
Lauha bhasma (LB) 22 mg/kg (TEDx2)	9 ± 0.51 *	11 ± 0.57 **	15 ± 0.71 **	14 ± 1.1 **

Values represents mean ± SEM., ANOVA: Dunnett's multiple comparison test. *P<0.05;**P<0.01;*** P<0.001 versus the CFA control group. Hematological alterations were observed.

RBC count: Treatment of Tamra bhasma (TB) in rats were found to have reversed effect on RBC levels. The count of RBC was significantly (P<0.05, P<0.01) elevated by two doses i.e. 5.5 and 11 mg/kg compared to CFA control group. It was interestingly noted that, RBC count shown by Tamra bhasma (TB) 5.5 mg/kg treated rats was comparable to count of standard drug, even count was found to be greater than standard drug by

treatment with 11 mg/kg dose. Similarly, Lauha bhasma (LB) treated rats showed to raise RBC count when compared to CFA control group. This effect was significant (P<0.05) at 11 and 22 mg/kg. A dose of 5.5 mg/kg of Lauha bhasma (LB) showed non-alteration in RBC count.

Hemoglobin count: Treatment of Tamra bhasma (TB) in rats were found to have reversedeffect on Hb levels. The count of hemoglobin was significantly (P<0.05, P<0.01) elevated by two doses i.e. 5.5, 11 mg/kg compared to CFA control group. But the dose of 2.75 mg/kg of Tamra bhasma (TB) was found to have similar values like CFA control group indicating non-alteration. Similarly, Lauha bhasma (LB) treated rats showed to raisehemoglobin count as compared to CFA control group. This effect was only significant (P<0.01) at 22 mg/kg.

WBC count: CFA treated rats showed increased WBC count indicates arthritic condition. (Table 20). The treatment of rats with Tamra bhasma (TB) and standard drug werereduced the count when compared to CFA control group. The changes produced were significant (P<0.01). Likewise, the alterations done by Lauha bhasma (LB) only found to be significant at highest dose tested. Amongst both the bhasmas treated group, highest dose (11mg/kg) of Tamra bhasma (TB) showed maximum reduction in WBC count.

ESR count: CFA treated rats showed increased ESR count which were reduced by drug treatments (Table 20). The treatment of rats with Tamra bhasma (TB) 5.5 and 11 mg/kg and standard drug were found to decrease the ESR count when compared to CFA control group. The changes produced were significant (P<0.01, P<0.001). Likewise, the alterations done by Lauha bhasma (LB) only found to be significant at highest dose tested. Amongst both the bhasmas treated group, highest dose (11mg/kg) of Tamra bhasma (TB) showed maximum reduction in ESR count which was 11mm/hr.

3.4. Effect on organ weights

Table 10 Changes in organ weights by treatment of Tamra bhasma (TB) and Lauhabhasma (LB)

Groups	Spleen weight (mg)	Thymus weight (mg)
Control (Gum acacia+ water) 2 ml/kg	192 ± 10.8	123 ± 4.1
Standard (Diclofenac) 40 mg/kg	95 ± 7.6 **	94 ± 2.8 **
Tamra bhasma (TB) 2.75 mg/kg (TED/2)	159 ± 5.1 *	111 ± 7.6
Tamra bhasma (TB) 5.5 mg/kg (TED)	142 ± 6.9 **	102 ± 6.2 *
Tamra bhasma (TB) 11 mg/kg (TEDx2)	120 ± 11.4 **	96 ± 4.0 *
Lauha bhasma (LB) 5.5 mg/kg (TED/2)	162 ± 6.8	115 ± 2.8
Lauha bhasma (LB) 11 mg/kg (TED)	159 ± 5.2 *	109 ± 3.3 *
Lauha bhasma (LB) 22 mg/kg (TEDx2)	148 ± 7.7 *	104 ± 4.2 **

Values represents mean ± SEM., ANOVA: Dunnett's multiple comparison test. *P<0.05;**P<0.01 versus the CFA control group. Organ weights were measured.

Table 11 Biochemical alterations by treatment of Tamra bhasma (TB) and Lauha bhasma (LB)

Groups	ALT(U/L)	AST(U/L)	Creatinine(mg/dl)	Urea (mg/dl)
Control (Gum acacia+ water) 2 ml/kg	86.1± 2.1	89.33±4.4	2.47±0.1	62.2 ±2.4
Standard (Diclofenac) 40 mg/kg	54.4±4.3*	58.2±5.7*	1.33±0.2**	32.0±2.2*
Tamra bhasma (TB) 2.75 mg/kg (TED/2)	91.7±5.3	81.48±5	2.38±0.17	54.9±2.3
Tamra bhasma (TB) 5.5 mg/kg (TED)	69.5±3.3*	68.96±5.9*	1.95±0.24	44.3±0.8 **
Tamra bhasma (TB) 11 mg/kg (TEDx2)	59.0±4.3*	63.14±2 **	1.53±0.16 **	38.4±1.8 **
Lauha bhasma (LB) 5.5 mg/kg (TED/2)	90.5±11.9	86.13±12	2.7±0.73	62.2±2
Lauha bhasma (LB) 11 mg/kg (TED)	70.1±7.5	78.57±5.1	2.1±0.36	54.7±1.4
Lauha bhasma (LB) 22 mg/kg (TEDx2)	64.8±9.9	73.33±8.3	2.0±0.81	50.1±3**

Values represents mean ± SEM., ANOVA: Dunnett's multiple comparison test. *P<0.05;**P<0.01 versus the CFA control group. Organ weights were measured.

Table 12 Changes in inflammatory and arthritic parameters by treatment of Tamra bhasma (TB) and Lauha bhasma (LB)

Groups	CRP(mg/dl)	RF(mg/dl)	TNF-αPg/ml	IL-1βPg/ml
Control(Gum acacia+ water)2 ml/kg	8.1 ±1.5	75 ±11	53.8 ±2.9	417 ±27.4
Standard (Diclofenac)40 mg/kg	1.9±0.4*	27.1±5*	33.7±2.4*	181±27.1*
Tamra bhasma (TB)2.75 mg/kg (TED/2)	4.7±0.75*	41.8±13.5	48.0±3.7	298±31.3
Tamra bhasma (TB)5.5 mg/kg (TED)	3.4±0.89*	37.5±5.5*	40.7±2.6*	260±21.7*
Tamra bhasma (TB)11 mg/kg (TEDx2)	2.4±1.1**	27.1±5**	36.7±1.9*	206±23.3*
Lauha bhasma (LB)5.5 mg/kg (TED/2)	6.6±0.93	41.7±5.2	54.0±2.9	417±19.4
Lauha bhasma (LB)11 mg/kg (TED)	4.7±9.4	39.6±6.7*	52.2±5.9	410.±28.3
Lauha bhasma (LB)22 mg/kg (TEDx2)	3.3±0.96 *	37.5±5.5*	46.8±5.2*	390±8.7*

Values represents mean ± SEM., ANOVA: Dunnett's multiple comparision test. *P<0.05;**P<0.01 versus the CFA control group. The cytokines and other inflammatory markers were measured with the aid of commercial kits.

3.5. Effect on arthritic joint by X-ray analysis

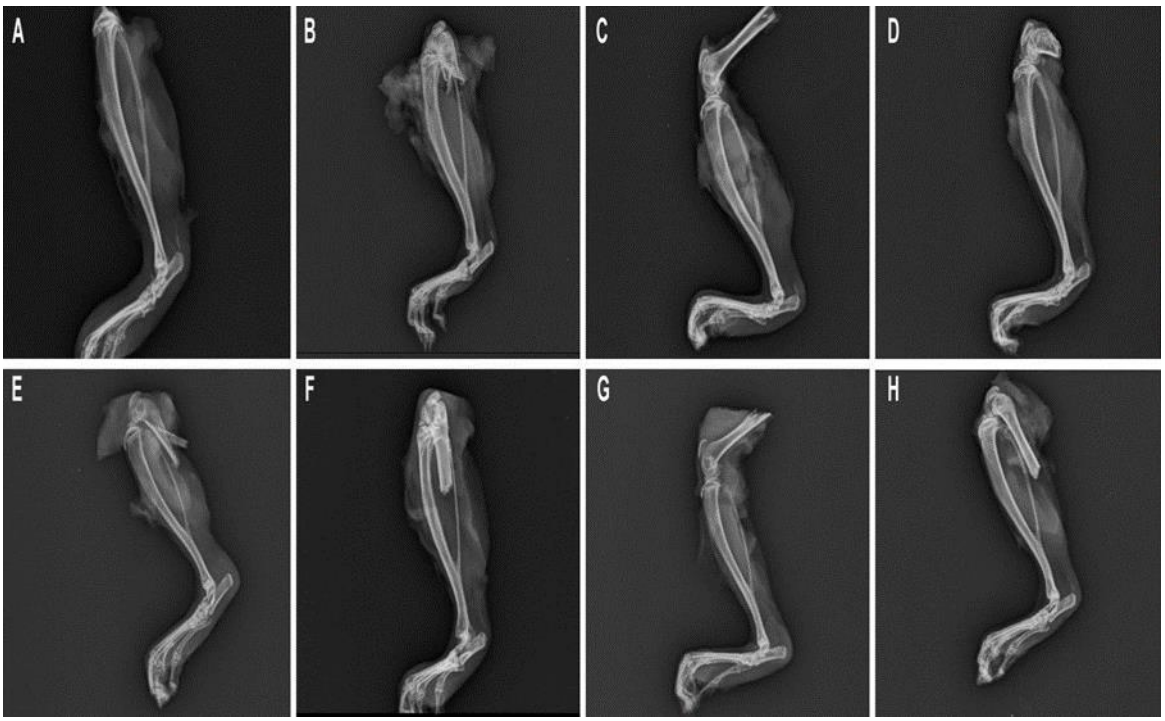


Figure 6 Effect of Tamra bhasma (TB) and Lauha bhasma (LB) on rat paw swelling by X-ray analysis. A = CFA control; B = (Standard- Diclofenac sodium 10 mg/kg; C = TB (TED/2) - 2.75 mg/kg; D = TB (TED) – 5.5 mg/kg; E = TB (TEDx2) - 11 mg/kg; F = LB (TED/2) – 5.5 mg/kg; G = LB (TED) - 11 mg/kg; H = LB (TEDx2) - 22 mg/kg. X-ray analysis was performed at X-ray center.

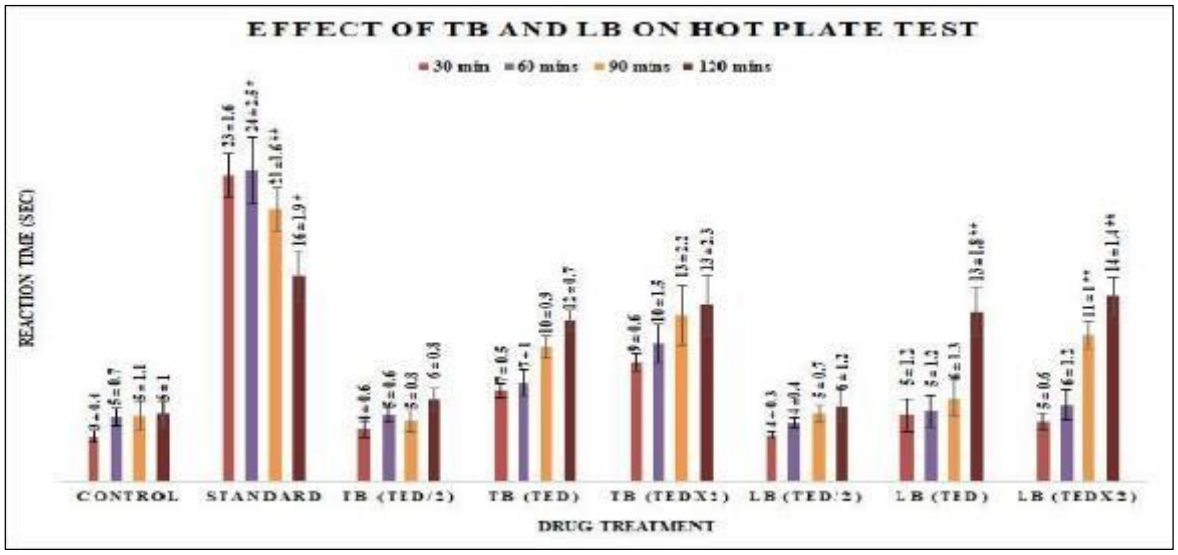
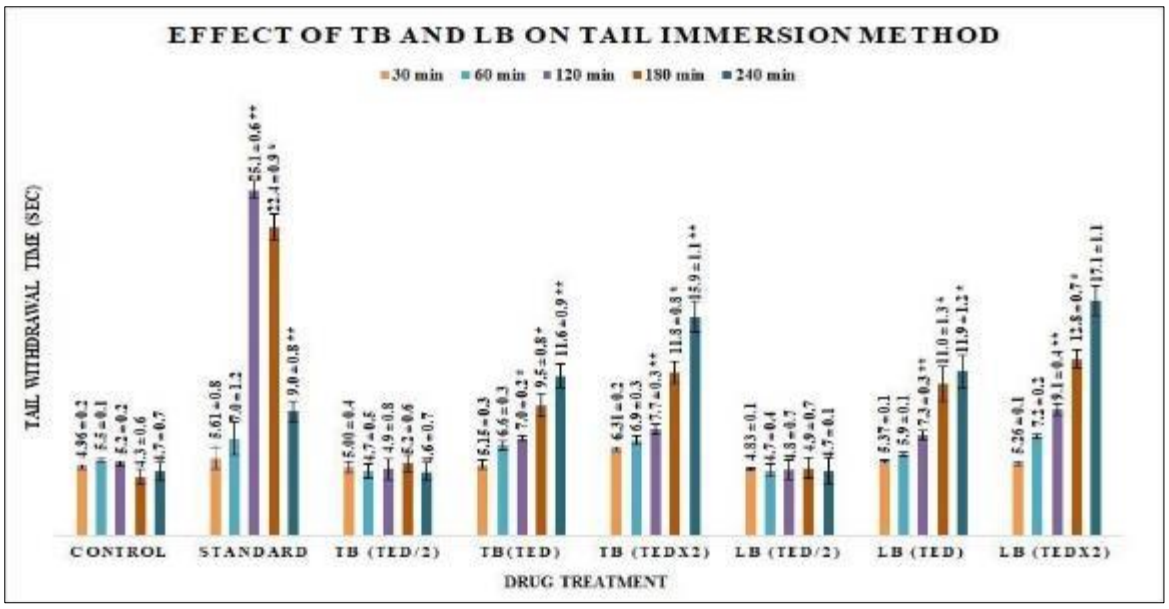


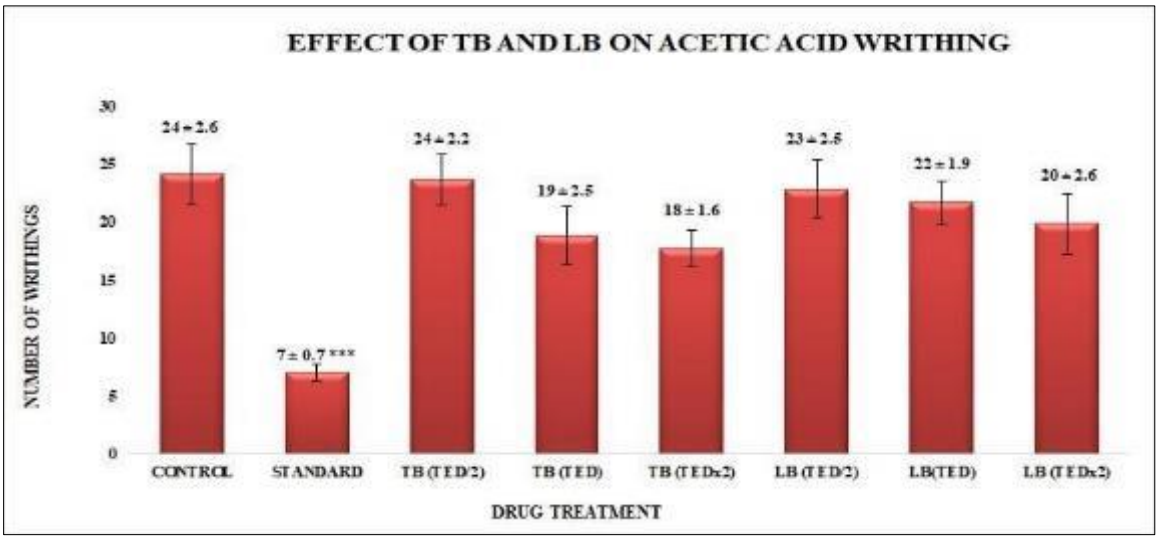
Figure 7 Effect of Tamra bhasma (TB) and Lauha bhasma (LB) on hot plate inducedthermal algesia. (Standard- Indomethacin sodium 10 mg/kg; TB (TED/2) - 2.75 mg/kg; TB (TED) – 5.5 mg/kg; TB (TEDx2) - 11 mg/kg; LB (TED/2) – 5.5 mg/kg; LB (TED) - 11 mg/kg; LB (TEDx2) - 22 mg/kg.

Values represents mean ± SEM., ANOVA: Dunnett's multiple comparision test. **P<0.05;** P<0.01 versus the control group.



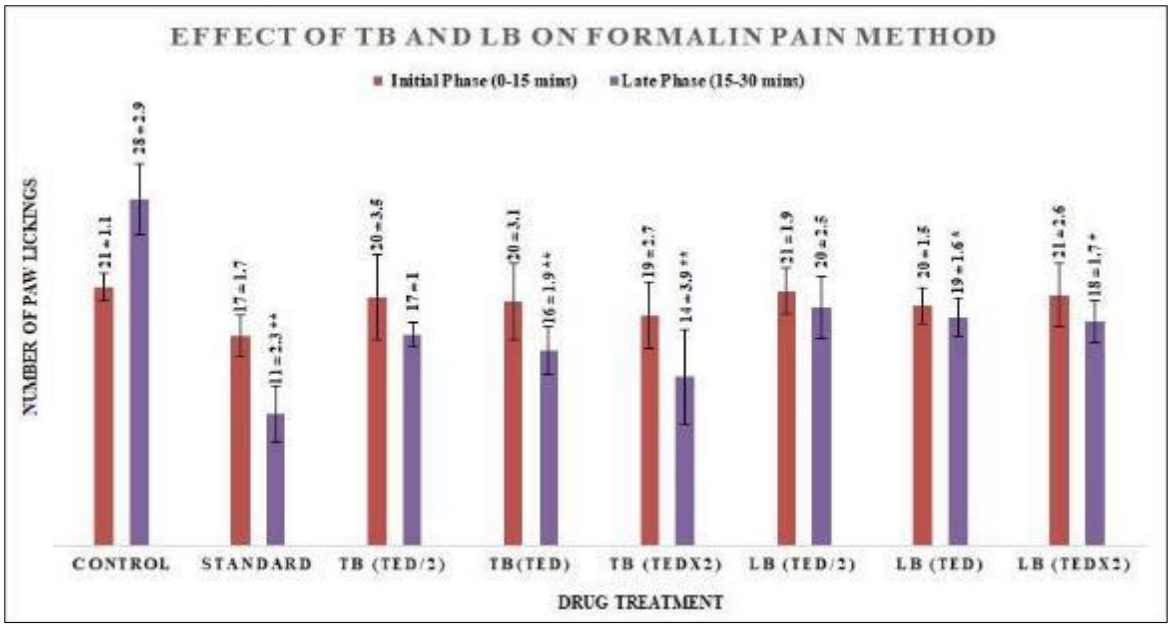
Values represents mean ± SEM., ANOVA: Dunnett's multiple comparision test *P<0.05;** P<0.01 versus the control group.

Figure 8 Effect of Tamra bhasma (TB) and Lauha bhasma (LB) on tail immersion method. (Standard- Indomethacin sodium 10 mg/kg; TB (TED/2) - 2.75 mg/kg; TB(TED) – 5.5 mg/kg; TB (TEDx2) - 11 mg/kg; LB (TED/2) – 5.5 mg/kg; LB (TED) – 11 mg/kg; LB (TEDx2) - 22 mg/kg.



Values represents mean ± SEM., ANOVA: Dunnett's multiple comparision test. ***P<0.001

Figure 9 Effect of Tamra bhasma (TB) and Lauha bhasma (LB) on acetic acid writhing method. (Standard- Aspirin 5 mg/kg; TB (TED/2) - 2.75 mg/kg; TB (TED) – 5.5mg/kg; TB (TEDx2) - 11 mg/kg; LB (TED/2) – 5.5 mg/kg; LB (TED) - 11 mg/kg; LB (TEDx2) - 22 mg/kg.



Values represents mean ± SEM., ANOVA: Dunnett's multiple comparision test. *P<0.05;**P<0.01

Figure 10 Effect of Tamra bhasma (TB) and Lauha bhasma (LB) on formalin inducedpain. (Standard- Indomethacin sodium 10 mg/kg; TB (TED/2) - 2.75 mg/kg; TB (TED) –5.5 mg/kg; TB (TEDx2) - 11 mg/kg; LB (TED/2) – 5.5 mg/kg; LB (TED) - 11 mg/kg; LB(TEDx2) - 22 mg/kg.

4. Conclusion

Physicochemical characterization by traditional parameters of selected Tamra and Lauha bhasma was revealed their genuine quality. While acute toxicity studies of both the bhasmas showed no mortality at 2000 mg/kg.

The Scanning electron microscopy of both Tamra and Lauha bhasma indicated the nanomaterial characteristics which justifies claim of Ayurvedic bhasmas as ancientnanomaterials.

Tamra and Lauha bhasma were found to be effective as anti-inflammatory agent inacute models of inflammation. It inhibits all phases of inflammation induced by carrageenan.

Tamra and Lauha bhasma were found to be effective as anti-inflammatory agent inchronic model of inflammation. It significantly inhibits wet as well as dry weightof cotton pellet granuloma at highest doses only.

Analgesic activity of both Tamra and Lauha bhasma in hot plate and tail immersion models were found to be significant at 120-240 mins.

Analgesic activity of both Tamra and Lauha bhasma in acetic acid models were found to be non-significant in nature, while found to be significant at late phase in formalin induced model.

Tamra and Lauha bhasma also were found to have significant anti-arthritic activityin animal study.

The whole pharmacological investigations were indicated that activity was only significant at therapeutic and more than therapeutic dose but nonsignificant at doselower than therapeutic doses.

The obtained results indicates the anti-inflammatory and analgesic potential of both these bhasmas probably by inhibiting inflammatory mediators.

Compliance with ethical standards

Acknowledgments

We are grateful to SIMS College of Pharmacy for the support in everything.

Disclosure of conflict of interest

No conflict of interest to be disclosed.

References

[1] Nagarajan S, Krishnaswamy S, Pemiah B, Rajan KS, Krishnan U, Sethuraman S. (2014). Scientific insights in the preparation and characterisation of a lead-based naga bhasma. Indian journal of pharmaceutical sciences, 76(1):38.

[2] Virupaksha GK, Kumar N (2012). Characterization of Tarakeshwara rasa: An Ayurvedic herbomineral formulation. Ayu, 33(3):406.

[3] Rasheed A, Naik M, Haneefa M, Pillanayil K, Kumar A, Pillai R, Azeem AK (2014). Formulation, characterization and comparative evaluation of Trivanga bhasma: a herbo-mineral Indian traditional medicine. Pakistan journal ofpharmaceutical sciences, 27(4): 793-800.

[4] Kumar A, Nair AG, Reddy AV, Garg AN (2006). Availability of essential elements in Bhasmas: Analysis of Ayurvedic metallic preparations by INAA. J Radioanalytical Nuclear Chem, 270:173–80.

[5] Singh A, Dubey SD, Patney S, Kumar V (2010). Acute and subchronic toxicity study of calcium based Ayurvedic 'Bhasmas' and a 'Pishti'prepared from marine animals. Journal of Herbal Medicine and Toxicology, 4(1):35-47

[6] P K Sarkar, P K Prajapati, A K Choudhary, V J Shukla, B Ravishankar (2012).

[7] Haematinic evaluation of Lauha Bhasmas and Mandura Bhasmas onHgCl2induced-anaemia in rats. Indian J Pharm Sci, 69:91-795.Pirmohamed M, James S, Meakin S et.al(2014). Adverse drug reactions as causeof admission to hospital: prospective analysis of 18 820 patients. BMJ, 329:15–19.

[8] Cannon GW, Holden WL, Juhaeri J, Dai W, Scarazzini L, Stang P (2014). Adverseevents with disease modifying antirheumatic drugs (DMARD): a cohort study of leflunomide compared with other DMARD. The Journal of rheumatology, 31(10):1906-11.

[9] Schäcke H, Döcke WD, Asadullah K (2002). Mechanisms involved in the side effects of glucocorticoids. Pharmacol. Ther, 96(1):23-43.

[10] Jagtap CY, Prajapati P, Patgiri B, Shukla VJ (2012). Quality control parameters forTamra (copper) Bhasma. Anc Sci Life, 31(4):164–170. doi:10.4103/0257- 7941.107348.

[11] Wadekar MP, Rode CV, Bendale YN, Patil KR, Prabhune AA (2005). Preparation and characterization of a copper based Indian traditional drug: Tamra bhasma. Journal of pharmaceutical and biomedical analysis, 39(5):951-5.

[12] Sutar H, Tembhurne M, Bhave A, Shrikhande B, Sathaye S, Gaikwad A, Patil K, Kulkarni S, Bhavan BA (2015). Systematic study of Tamra (Copper) Bhasma prepared by traditional Ayurveda method. IJPSR, 6(8):3511-20.

[13] Chaudhari SY, Ruknuddin G, Biswajyoti JP, Kumar PP (2014). Effect of tamra bhasma (calcined copper) on ponderal and biochemical parameters. Toxicology international, 21(2):156.

[14] Pedernera, A.M., Guardia, T., Calderón, C.G., Rotelli, A.E., de la Rocha, N.E., Di Genaro, S., Pelzer, L.E (2006). Anti-ulcerogenic and anti-inflammatory activity of the methanolic extract of Larrea divaricata Cav. in rat. Journal of ethnopharmacology, 105:415-420.