



### Research Article

## Toxicological Evaluation of *Aargwadhadi Taila* and its Ointment Formulation: An OECD-Guided Dermal Safety Study in Rats

Premadevi Kalmegh<sup>1\*</sup>, Bharat Rathi<sup>2</sup>

1. Associate Professor, Dept. of Rasashastra and Bhaishajya Kalpana, Dr. R.K. Kambe Ayurved College, Murtizapur; Ph.D. Scholar, Dept. of Rasashastra and Bhaishajya Kalpana, Mahatma Gandhi Ayurved College Hospital and Research Center (MGACHRC), Salod(H), Sawangi (M), Wardha. Datta Meghe Institute of Higher Education and Research (Deemed to be University), Wardha, Maharashtra. India.
2. Professor and HOD, Dept. of Rasashastra and Bhaishajya Kalpana, MGACHRC, Salod(H), Sawangi (M), Wardha. India.

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### Abstract

**Background:** *Aargwadhadi Taila* is a classical Ayurvedic medicated oil traditionally indicated for skin disorders such as *Kushtha* (skin diseases), *Vicharchika* (eczema), and *Pama* (scabies). Despite its widespread use, systematic evaluation of its dermal safety in accordance with modern toxicological protocols has been limited. **Aim and Objective:** To assess the acute and subacute dermal toxicity of *Aargwadhadi Taila* and its ointment formulation in Albino Wistar Rats following OECD guidelines. **Methods:** Acute dermal toxicity was evaluated as per OECD 402 and sub-acute (28-day) dermal toxicity as per OECD 410. Healthy Albino Wistar rats (n = 10 per group) received topical applications of *Aargwadhadi Taila*, a 2% ointment formulation, or vehicle. Clinical signs, mortality, body weight, haematological and biochemical parameters, and histopathology of the skin and vital organs (liver, kidney, heart, lungs, and spleen) were examined. **Results:** No mortality or toxicity signs were recorded. Body weight, haematological, and Biochemical values remained within normal limits (p > 0.05). Histopathology of skin and organs revealed no pathological alterations. **Conclusion:** *Aargwadhadi Taila* and its ointment formulation were well tolerated and showed no acute or subacute dermal toxicity in Albino Wistar rats. These findings scientifically validate its traditional therapeutic safety and support its potential in clinical and cosmeceutical applications.

**Keywords:** *Aargwadhadi Taila*, Dermal safety, Albino Wistar rats, OECD guidelines, Toxicity evaluation

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## Introduction

The burgeoning global demand for natural and Ayurvedic cosmetic product has spotlighted the safety and regulatory evaluation of classical herbal formulations intended for topical application(1). In this context, *Aargwadhadi Taila* is a classical Ayurvedic formulation mentioned in *chakradutta* for the treatment of *Kuṣṭha* (skin disorders), particularly *Śvitra* (vitiligo)(2). It contains potent herbs such as *Aragvadha* (*Cassia fistula*), *Haritala* (orpiment), and *Manashila* (realgar), which are known for their *Krmighna*, *Kuṣṭhaghna*, and *Svitraghna* properties. These ingredients have shown potential antimicrobial, DE pigmentation, and anti-inflammatory activities, making the formulation effective in managing chronic skin diseases. However, its classical *taila* (oil) form has several limitations for dermal application, including

stickiness, difficulty in absorption, and potential for staining clothes, and low patient compliance, especially in humid climates (3, 4). Therefore, to enhance ease of application, stability, aesthetic appeal, and bioavailability, converting *Aargwadhadi Taila* into an ointment (*malahara*) form becomes essential. Ointments provide a non-greasy base that enhances drug penetration through the skin, increases contact time with affected areas, and reduces the likelihood of allergic reactions or irritation compared to raw oils(5). Modern pharmaceutical techniques allow the transformation of oil-based formulations into more acceptable dosage forms without compromising their therapeutic efficacy (6). Hence, developing a modified dosage form like *Aargwadhadi* ointment is crucial for improving therapeutic outcomes and patient adherence.

*Aargwadhadi Talia*, a classical Ayurvedic formulation, includes potent ingredients like *Hartala* (orpiment) and *Manashila* (realgar), both classified as *Upaviṣha* (sub-toxic minerals). Although these are therapeutically valuable in conditions like *shvitra* (vitiligo), they must be handled with caution due to their inherent toxicity (7). The inclusion of such ingredients necessitates toxicological assessment to ensure the formulation's

### \* Corresponding Author:

#### Premadevi Kalmegh

Associate Professor, Department of Rasashastra and Bhaishajya Kalpana, Dr. R.K. Kambe Ayurved College, Murtizapur India.

Email Id: [dr.premadevichoudhari@gmail.com](mailto:dr.premadevichoudhari@gmail.com)

safety for topical application. Exposure to toxic substances may lead to sub-acute or chronic toxicity, particularly when used over extended periods without safety profiling (8,9).

Importantly, very few published studies have conducted a comprehensive OECD compliant acute and sub-acute dermal toxicity investigation of Aargwadhadi Taila or its ointment, leaving a significant evidence gap that presents a barrier to its regulatory acceptance and safe commercialization. Thus the main Objective of this Pre-clinical trial aims to fill that gap by evaluating the acute (OECD 402) and sub-acute (OECD 410) dermal toxicity of Aargwadhadi Taila and its ointment formulation in Wistar rats, thereby the present study was conducted to evaluate dermal toxicity of Aargwadhadi Tail and its ointment preparation

in rats, which could be used to establish a safe and optimal dose to treat skin problems providing a scientifically validated safety profile to support its future therapeutic and cosmetic applications (10, 11).

## Materials and Methods

### Test Drug preparation: Aargwadhadi oil preparation

Procurement of raw drug Aargwadhadi oil was done from from local region of Nagpur/wardha in August – September Varsha and Sharad Ritu<sup>12</sup> Identification and Authentication of all the raw drugs was done by Taxonomist from Department of Dravyaguna and FRLHT Bangalore

**Table No 1: Table Showing Ingredients of Aargwadhadi Tail**

| Sr. no. | Drug Name             | Latin name/                                                            | English Name     | Part used  | Proportion |
|---------|-----------------------|------------------------------------------------------------------------|------------------|------------|------------|
| 1       | Aargwadh (13)         | <i>Cassia fistula L.</i>                                               | Purging cassia   | Phal majja | 1 part     |
| 2       | Dhav (14)             | <i>Anogeissus latifolia(DC)</i>                                        | Axle wood tree   | Bark       |            |
| 3       | Kushta (14)           | <i>Saussurea lappa C.B. Clerk)</i>                                     | Costus           | Root       |            |
| 4       | Haridra (15)          | <i>Curcuma longa Linn</i>                                              | Turmeric         | Rhizome    |            |
| 5       | Daruharidra (16)      | <i>Berberies Aristata DC</i>                                           | Indian Berberies | Stem       |            |
| 6       | Shodhit Hartala (17)  | <i>Yellow arsenic As<sub>2</sub>S<sub>3</sub> (Arsenic trisulfide)</i> | Orpiment         | -----      |            |
| 7       | Shodhit Manshila (17) | <i>Red arsenic As<sub>2</sub>S<sub>2</sub> (Arsenic disulfide)</i>     | Realgar          | -          |            |
| 8       | Tila Tail (14)        | <i>Sesamum Indicum L.</i>                                              |                  | -          | 4 parts    |
| 9       | Water                 |                                                                        |                  | -          | 16 parts   |

To prepare Aargwadhadi oil Hartala and Manashila were detoxified as per Ayurved text by swedan in churnodak and nimaanjan in churnodak respectively (18,19). Oil is prepared via classical Sneha Paka methods. One part of Kalka (a fine paste consisting of equal proportions of Aaragvadh, Dhava, Kushta, Haridra, Daruharidra, and purified Hartala and Manashila) is combined with 4 parts of Tila Taila (sesame oil) and 16 parts of water. The mixture is processed over a mild fire (Mandagni). It is kept at a gentle, continuous boil with regular stirring until the liquid content is completely evaporated, leaving behind the therapeutically potent, infused oil (20).

### Aargwadhadi ointment preparation

**Table 2: Table Showing Ingredients of Aargwadhadi ointment**

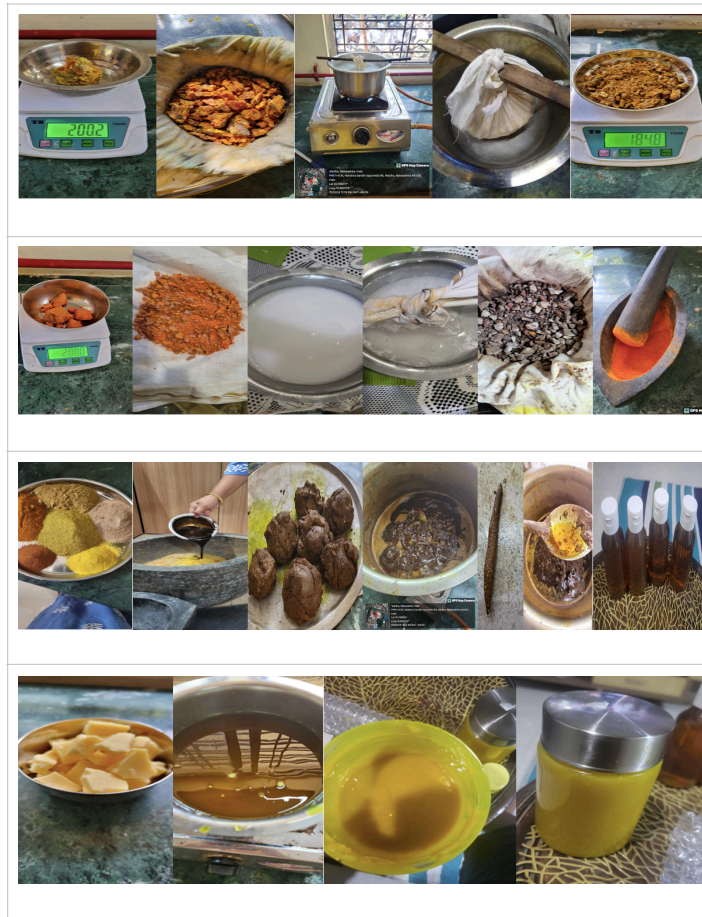
| Sr. no | Ingredients      | Proportion             | Quantity |
|--------|------------------|------------------------|----------|
| 1      | Aargwadhadi tail | 1 part                 | 1liter   |
| 2      | White wax        | 1\5 <sup>th</sup> part | 200gm    |

Aargwadhadi ointment is prepared as per malhar kalpana, taking 1 part of Aargwadhadi oil and 1\5<sup>th</sup> part of white wax (21,22).

### Experimental Animals

Healthy Wistar albino rats were procured from a CPCSEA-registered animal house. Animals were housed in polypropylene cages with husk bedding under controlled conditions ( $22 \pm 2^\circ\text{C}$ , 12 h light/dark cycle, 50–60% relative humidity) and fed a standard pellet diet with water ad libitum (11). Animal Selection: Healthy, nulliparous, and non-pregnant female young adult rats (8–10 weeks old, 200–300 g) with intact skin were used. Rats were randomly assigned, individually marked, and weighed. The day before dosing, fur was removed from the dorsal/flank region (minimum 10% of total body surface area). Dosing: The test chemical was applied uniformly over the shaved area

**Figure 1: Purification of Harital, Manahshil and the preparation of Aargwadhadi ointment**



## Ethical Approval

The study protocol was approved by the Institutional Animal Ethics Committee (IAEC) in accordance with CPCSEA guidelines (Approval No: XXX/IAEC/2024).

## Acute Dermal Toxicity Study (OECD Guideline 402)

Acute dermal toxicity were determined via a single topical application using a limit test design according to OECD Guideline 402. Following local application on Day 1, rats were monitored continuously for the first 6 hours, at 24 hours, and then daily for

14 days. Animals were observed for mortality, behavioural changes, skin reactions, and systemic signs of toxicity immediately after dosing.

## Group design

Thirty Albino Rats had been selected in this animal model and divided in three groups each group containing Ten Rats (Five male and Five Female) Respectively. The study period was 14 days.

- Group 1 - Control Group (5M, 5F)
- Group 2 - Experimental Group (5M, 5F)
- Group 3 - Experimental Group (5M, 5F)

**Table 3: Group design**

| Group    | Drug                        | Dose       | No. of Animals | Duration | Route   |
|----------|-----------------------------|------------|----------------|----------|---------|
| Group I  | Control group No Medicine   | -          | 10 (5 M +5 F)  | 14 days  | -       |
| Group II | <i>Aargwadhadi Tail</i>     | 2000 mg/kg | 10 (5 M +5 F)  | 14 days  | Topical |
| Group II | <i>Aargwadhadi ointment</i> | 2000 mg/kg | 10 (5 M +5 F)  | 14 days  | Topical |

## Sub-acute Dermal Toxicity Study (OECD Guideline 410)

To evaluate dermal toxicity of *Aargwadhadi* oil and ointment in different dosage form, 28-days experimental study was structured using 70 animals. The animals were uniformly divided into seven distinct groups (n = 10 per group, with an equal sex distribution of 5 males and 5 females. Both formulations were administered via the topical route. The experimental groups were organized as follows: Group I (Control): Served as the baseline control group

and received no medical intervention. *Aargwadhadi Tail* (Oil) (Groups II–IV): Evaluated the oil formulation across three escalating dose levels: a low dose of 500 mg/kg (Group II), a mid-dose of 1000 mg/kg (Group III), and a high dose of 2000 mg/kg (Group IV). *Aargwadhadi Ointment Cohort* (Groups V–VII): Evaluated the ointment formulation mirroring the same escalating dose levels: 500 mg/kg (Group V), 1000 mg/kg (Group VI), and 2000 mg/kg (Group VII).

**Table 4: Table showing Grouping of animals for Sub-acute dermal toxicity study (n=10)**

| Group     | Drug                        | Dose       | No. of Animals | Duration | Route   |
|-----------|-----------------------------|------------|----------------|----------|---------|
| Group I   | Control group No Medicine   | -          | 10 (5 M +5 F)  | 28 Days  | -       |
| Group II  | <i>Aargwadhadi tail</i>     | 500 mg/kg  | 10 (5 M +5 F)  | 28 Days  | Topical |
| Group III | <i>Aargwadhadi tail</i>     | 1000 mg/kg | 10 (5 M +5 F)  | 28 Days  | Topical |
| Group IV  | <i>Aargwadhadi tail</i>     | 2000mg/kg  | 10 (5 M +5 F)  | 28 Days  | Topical |
| Group V   | <i>Aargwadhadi Ointment</i> | 500 mg/kg  | 10 (5 M +5 F)  | 28 Days  | Topical |
| Group VI  | <i>Aargwadhadi Ointment</i> | 1000 mg/kg | 10 (5 M +5 F)  | 28 Days  | Topical |
| Group VII | <i>Aargwadhadi Ointment</i> | 2000mg/kg  | 10 (5 M +5 F)  | 28 Days  | Topical |

**Figure 2: Images showing acute and sub-acute dermal toxicity study**



## Laboratory Investigations

At the end of treatment, blood samples were collected from retro-orbital plexus under mild anaesthesia for haematological and biochemical investigations (Packed cell Volume (PCV), Haemoglobin (HB) Red blood cell (RBC) count, Total white blood cell (WBC) count, Differential Leucocyte Count

Plasma Glucose (mg/dl), Creatinine (mg/dl), SGOT (U/L) and SGPT (U/L)) (14)

## Histopathology

One rat from control group and higher doses of *Aargwadhadi tail* and *Aargwadhadi ointment* was sacrificed after 14 days in acute dermal toxicity study and after 42 days in sub-acute dermal toxicity study by administering anaesthesia. Histological examination was performed on the preserved organs and tissues of all groups. The examination Liver, heart, kidney, stomach, brain skin were processed for Histopathological studies as per the prescribed procedures. (15).

## Statistical Analysis

A statistical method such as the ANOVA -test for observation weight Haematological parameters and Biochemical parameters is used to assess data for all groups the result was presented as mean, standard deviation, and standard Error of mean. A value of  $p < 0.05$  was considered statistically significant.

## Observations and Results

### Acute Dermal Toxicity

**Table 5.1: Showing observation of Behavior Patterns and Appearance of Wistar Rats**

| Signs                  | GROUP I        |               | GROUP II<br>Aargwadhadi tail 2000 mg/kg |               | GROUP III<br>Aargwadhadi Ointment2000mg/kg |               |
|------------------------|----------------|---------------|-----------------------------------------|---------------|--------------------------------------------|---------------|
|                        | After 24 hours | After 14 days | After 24 hours                          | After 14 days | After 24 hours                             | After 14 days |
| <b>Lethargy</b>        | Not found      | Not found     | Not found                               | Not found     | Not found                                  | Not found     |
| <b>Salivation</b>      | Normal         | Normal        | Normal                                  | Normal        | Normal                                     | Normal        |
| <b>Hunched posture</b> | No changes     | No changes    | No changes                              | No changes    | No changes                                 | No changes    |
| <b>Diarrhea</b>        | Not found      | Not found     | Not found                               | Not found     | Not found                                  | Not found     |
| <b>Eyes</b>            | No changes     | No changes    | No changes                              | No changes    | No changes                                 | No changes    |
| <b>Skin / fur</b>      | No changes     | No changes    | No changes                              | No changes    | No changes                                 | No changes    |
| <b>Tremors</b>         | Not found      | Not found     | Not found                               | Not found     | Not found                                  | Not found     |
| <b>Convulsions</b>     | Not found      | Not found     | Not found                               | Not found     | Not found                                  | Not found     |

**Table 5.2: Showing Evaluation of skin reaction By Draize scoring method**

| Group/Dose (mg/kg body weight)             | Observed signs |              |
|--------------------------------------------|----------------|--------------|
|                                            | Erythema       | Edema        |
| Group I                                    | Not observed   | Not observed |
| Group II (Aargwadhadi tail 2000 mg/kg )    | Not observed   | Not observed |
| Group III (Aargwadhadi ointment2000mg/kg ) | Not observed   | Not observed |

**Table 5.3: Showing Changes in Body weight [Mean  $\pm$ SD] in Wistar rats**

| Body weight recorded By time          | GROUP I          |                  | GROUP II (OIL GROUP) |                | GROUP III (OINTMENT GROUP) |               |
|---------------------------------------|------------------|------------------|----------------------|----------------|----------------------------|---------------|
|                                       | Male             | Female           | Male                 | Female         | Male                       | Female        |
| <b>On the day of dosing(day 1 )</b>   | 197.7 $\pm$ 8.9  | 199.4 $\pm$ 7.34 | 198.8 $\pm$ 5.61     | 185 $\pm$ 5.41 | 198 $\pm$ 6.62             | 196 $\pm$ 4.2 |
| <b>Day 7</b>                          | 199.6 $\pm$ 9.09 | 200 $\pm$ 7.8    | 201.67 $\pm$ 2.53    | 187 $\pm$ 6.2  | 200 $\pm$ 6.8              | 198 $\pm$ 7.2 |
| <b>Day14</b>                          | 201.5 $\pm$ 9.5  | 204 $\pm$ 6.8    | 204.83 $\pm$ 4.06    | 192 $\pm$ 5.4  | 203 $\pm$ 7.2              | 200 $\pm$ 8.2 |
| <b>On the day of sacrifice(day15)</b> | 201.8 $\pm$ 6.2  | 204 $\pm$ 6.4    | 204.2 $\pm$ 4.00     | 192 $\pm$ 6.8  | 203 $\pm$ 6.2              | 200 $\pm$ 8.2 |

**Table 5.4: Showing Hematological Parameters (Day14) Values are expressed as mean  $\pm$  SD (n= 5 for each group**

| Parameter          | Unit                      | GROUP I          |                  | GROUP II(OIL GROUP) |                  | GROUP III(OINTMENT GROUP) |                  |
|--------------------|---------------------------|------------------|------------------|---------------------|------------------|---------------------------|------------------|
|                    |                           | MALE             | FEMALE           | MALE                | FEMALE           | MALE                      | FEMALE           |
| <b>RBC</b>         | <b>X10<sup>12</sup>/L</b> | 8.27 $\pm$ 0.39  | 7.4 $\pm$ 4.6    | 7.2 $\pm$ 4.8       | 9.2 $\pm$ 0.21   | 7.8 $\pm$ 0.4             | 7.01 $\pm$ 0.14  |
| <b>Hb</b>          | <b>g/dL</b>               | 14.1 $\pm$ 0.20  | 12.4 $\pm$ 0.41  | 13.8 $\pm$ 0.20     | 12.2 $\pm$ 0.73  | 12.67 $\pm$ 2.62          | 13.2 $\pm$ 3.4   |
| <b>PCV</b>         | <b>%</b>                  | 44.2 $\pm$ 2.2   | 43.8 $\pm$ 2.2   | 42.83 $\pm$ 2.36    | 42.67 $\pm$ 1.97 | 46.28 $\pm$ 2.2           | 36.38 $\pm$ 2.6  |
| <b>MCV</b>         | <b>fl</b>                 | 54.6 $\pm$ 0.75  | 52.8 $\pm$ 0.68  | 52.6 $\pm$ 0.43     | 50.6 $\pm$ 2.16  | 50.6 $\pm$ 1.03           | 52.6 $\pm$ 1.75  |
| <b>MCHC</b>        | <b>g/dL</b>               | 32.8 $\pm$ 4.31  | 34.12 $\pm$ 6.74 | 30 $\pm$ 7.08       | 39 $\pm$ 4.56    | 34 $\pm$ 5.51             | 30.15 $\pm$ 5.74 |
| <b>MCH</b>         | <b>pg</b>                 | 12.67 $\pm$ 0.48 | 12.22 $\pm$ 0.61 | 14.8 $\pm$ 0.20     | 16.2 $\pm$ 0.63  | 14.67 $\pm$ 4.62          | 12.2 $\pm$ 3.4   |
| <b>WBC</b>         | <b>10<sup>9</sup>/L</b>   | 8.09 $\pm$ 4.32  | 8.21 $\pm$ 0.72  | 7.45 $\pm$ 0.86     | 7.70 $\pm$ 1.334 | 7.88 $\pm$ 1.26           | 8.22 $\pm$ 0.74  |
| <b>Neutrophils</b> | <b>10<sup>9</sup>/L</b>   | 1.80 $\pm$ 0.90  | 1.55 $\pm$ 0.75  | 1.50 $\pm$ 0.85     | 1.50 $\pm$ 1.35  | 1.60 $\pm$ 0.85           | 1.65 $\pm$ 0.85  |
| <b>Lymphocytes</b> | <b>10<sup>9</sup>/L</b>   | 5.43 $\pm$ 1.64  | 5.81 $\pm$ 1.26  | 6.21 $\pm$ 1.43     | 5.71 $\pm$ 1.71  | 6.36 $\pm$ 1.42           | 5.51 $\pm$ 1.20  |
| <b>Monocytes</b>   | <b>10<sup>9</sup>/L</b>   | 0.36 $\pm$ 0.29  | 0.34 $\pm$ 1.21  | 0.28 $\pm$ 0.21     | 0.26 $\pm$ 1.28  | 0.24 $\pm$ 1.06           | 0.30 $\pm$ 0.21  |
| <b>Eosinophil</b>  | <b>10<sup>9</sup>/L</b>   | 0.16 $\pm$ 0.61  | 0.14 $\pm$ 0.78  | 0.18 $\pm$ 1.12     | 0.16 $\pm$ 0.78  | 0.16 $\pm$ 1.26           | 0.19 $\pm$ 0.60  |
| <b>Platelets</b>   | <b>10<sup>9</sup>/L</b>   | 774 $\pm$ 54.9   | 972 $\pm$ 67.3   | 945 $\pm$ 68.5      | 767 $\pm$ 24.7   | 909 $\pm$ 28.2            | 865 $\pm$ 84.5   |

WBC: White Blood corpuscle; Hb: Haemoglobin; RBC: Read Blood corpuscle; HCT: Haematocrit; MCV: Mean corpuscular volume; MCH: Mean corpuscular haemoglobin; MCHC: Mean corpuscular haemoglobin concentration; PLT: Platelets;

(All values are calculated in Mean  $\pm$ SD)

**Table 5.5: Showing Bio-chemical Parameters in Albino Wistar Rats**

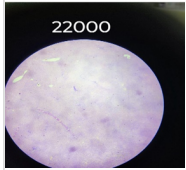
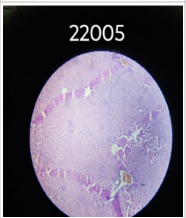
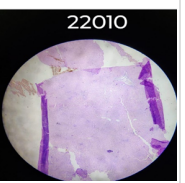
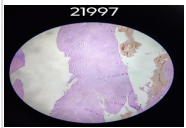
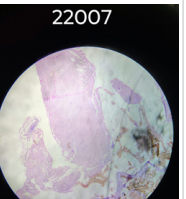
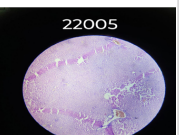
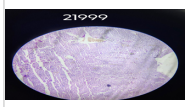
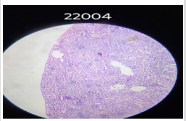
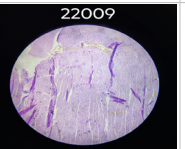
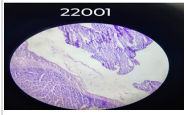
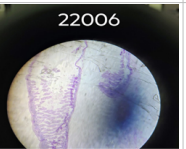
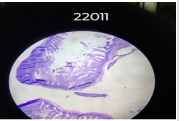
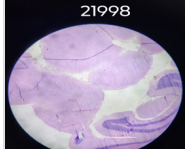
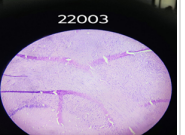
| Parameters              | GROUP I     |             | GROUP II (OIL GROUP) |             | GROUP III (OINTMENT GROUP) |              |
|-------------------------|-------------|-------------|----------------------|-------------|----------------------------|--------------|
|                         | Male        | Female      | Male                 | Female      | Male                       | Female       |
| <b>Blood</b>            | 68.63± 0.26 | 80.32± 0.38 | 72.01± 0.46          | 76.5± 0.28  | 63.32± 0.46                | 66.32± 0.386 |
| <b>Sr.Creatinine(mg</b> | 0.43± 0.02  | 0.38± 0.03  | 0.42± 0.06           | 0.34± 0.03  | 0.58± 0.04                 | 0.62± 0.06   |
| <b>SGOT(IU/L)</b>       | 124.06±12.4 | 128.05±08.4 | 116.46±20.4          | 118.20±16.4 | 129.62±10.4                | 125.6±14.4   |

Values are expressed as mean ± SD (n= 5 for each group)

**Histological changes in the Organs (14 days)****Table 5.6: Showing acute toxicity showing the results on different organs in group**

| Organs         | GROUP I                                                                    | GROUP II                                                                                        | GROUP III                                                                                           |
|----------------|----------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------|
| <b>Liver</b>   | Showed a normal structure of liver hepatocytes.                            | No significant alterations were noticed                                                         | No significant alterations were noticed                                                             |
| <b>Kidney</b>  | Kidney tissues showed a normal structure of glomeruli and Bowman's capsule | No significant alterations were observed                                                        | No significant alterations were observed                                                            |
| <b>Stomach</b> | Unremarkable gastric tissue                                                | Unremarkable gastric gland with alveoli unremarkable with mucosa, submucosa and mulcaris mucosa | Unremarkable gastric gland showing mucosa, submucosa, mulcularis mucosa, along with adipose tissue. |
| <b>BRAIN</b>   | Unremarkable nervous tissue                                                | Unremarkable nervous tissue                                                                     | Unremarkable nervous tissue                                                                         |
| <b>HEART</b>   | Unremarkable myocardial tissue                                             | Unremarkable Cardiac muscle tissue                                                              | Unremarkable cardiac muscle tissue                                                                  |

**Table 5.7: Table showing Histological images of organs in Different Groups**

| ORGAN  | GROUP I                                                                             | GROUP II                                                                            | GROUP III                                                                           | ORGAN     | GROUP I                                                                              | GROUP II                                                                              | GROUP III                                                                             |
|--------|-------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------|-----------|--------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------|
| LIVER  |   |   |   | HEART     |   |   |   |
|        |  |  |  |           |  |  |  |
|        |  |  |  |           |                                                                                      |                                                                                       |                                                                                       |
| KIDNEY |                                                                                     |                                                                                     |                                                                                     | INTESTINE |                                                                                      |                                                                                       |                                                                                       |
|        |                                                                                     |                                                                                     |                                                                                     |           |                                                                                      |                                                                                       |                                                                                       |
|        |                                                                                     |                                                                                     |                                                                                     |           |                                                                                      |                                                                                       |                                                                                       |
| BRAIN  |                                                                                     |                                                                                     |                                                                                     |           |                                                                                      |                                                                                       |                                                                                       |
|        |                                                                                     |                                                                                     |                                                                                     |           |                                                                                      |                                                                                       |                                                                                       |
|        |                                                                                     |                                                                                     |                                                                                     |           |                                                                                      |                                                                                       |                                                                                       |

**Sub –Acute Dermal toxicity study****Table 6.1: Observation of Behavior Patterns and Appearance of Wistar Rats**

| Signs                  | GROUP I    | Group II   | GROUP III  | GROUP IV   | GROUP V    | GROUP VI   | GROUP VII  |
|------------------------|------------|------------|------------|------------|------------|------------|------------|
| <b>Lethargy</b>        | Not found  | Not found  | Not found  | Not found  | Not found  | Not found  | Not found  |
| <b>Salivation</b>      | No         | No         | No         | No         | No         | No         | No         |
| <b>Hunched posture</b> | No changes | No changes | No changes | No changes | No changes | No changes | No changes |
| <b>Diarrhea</b>        | Not found  | Not found  | Not found  | Not found  | Not found  | Not found  | Not found  |
| <b>Eyes</b>            | No changes | No changes | No changes | No changes | No changes | No changes | No changes |
| <b>Skin / fur</b>      | No changes | No changes | No changes | No changes | No changes | No changes | No changes |
| <b>Tremors</b>         | Not found  | Not found  | Not found  | Not found  | Not found  | Not found  | Not found  |
| <b>Sleep</b>           | Normal     | Normal     | Normal     | Normal     | Normal     | Normal     | Normal     |
| <b>Convulsions</b>     | No         | No         | No         | No         | No         | No         | No         |
| <b>Coma</b>            | No         | No         | No         | No         | No         | No         | No         |

**Table 6.2: Table showing Evaluation of skin reaction by Draize scoring method**

| Group/Dose (mg/kg body weight) | Observed signs |              |
|--------------------------------|----------------|--------------|
|                                | Erythema       | Edema        |
| Group I                        | Not observed   | Not observed |
| Group II                       | Not observed   | Not observed |
| Group III                      | Not observed   | Not observed |
| Group IV                       | Not observed   | Not observed |
| Group V                        | Not observed   | Not observed |
| Group VI                       | Not observed   | Not observed |
| Group VII                      | Not observed   | Not observed |

**Table 6.3: Table Showing Changes in Body weight [Mean  $\pm$ SD] in Wistar rats**

| GROUPS    | DAY 1            | DAY 7            | DAY 14          | DAY 21          | DAY 28          |
|-----------|------------------|------------------|-----------------|-----------------|-----------------|
| GROUP I   | 195.2 $\pm$ 6.49 | 207.6 $\pm$ 9.09 | 212.5 $\pm$ 9.5 | 216.6 $\pm$ 8.5 | 218.5 $\pm$ 6.5 |
| GROUP II  | 198.7 $\pm$ 6.9  | 204.5 $\pm$ 6.39 | 207.4 $\pm$ 8.5 | 212.5 $\pm$ 9.5 | 215.5 $\pm$ 8.5 |
| GROUP III | 200.2 $\pm$ 7.9  | 201.6 $\pm$ 9.09 | 205.6 $\pm$ 9.5 | 206.4 $\pm$ 6.5 | 208.6 $\pm$ 4.5 |
| GROUP IV  | 196.6 $\pm$ 8.9  | 198.6 $\pm$ 6.49 | 201.4 $\pm$ 6.5 | 202.5 $\pm$ 7.5 | 205.6 $\pm$ 9.5 |
| GROUP V   | 197.2 $\pm$ 8.9  | 199.6 $\pm$ 7.89 | 200.4 $\pm$ 6.5 | 202.6 $\pm$ 6.5 | 204.6 $\pm$ 8.5 |
| GROUP VI  | 200.4 $\pm$ 8.9  | 201.4 $\pm$ 6.09 | 202.5 $\pm$ 9.5 | 204.8 $\pm$ 7.5 | 205.9 $\pm$ 8.5 |
| GROUP VII | 198.6 $\pm$ 8.9  | 199.6 $\pm$ 9.09 | 201.5 $\pm$ 4.5 | 203.5 $\pm$ 9.5 | 205.8 $\pm$ 6.5 |

**Table 6.4: Table showing Hematological Parameters (day 28)**

| Parameter   | Unit                 | G1               | G2               | G3               | G4               | G5               | G6               | G7               |
|-------------|----------------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|
| RBC         | X10 <sup>12</sup> /L | 7.37 $\pm$ 0.39  | 8.4 $\pm$ 4.2    | 7.2 $\pm$ 4.6    | 8.2 $\pm$ 0.21   | 7.8 $\pm$ 0.4    | 8.01 $\pm$ 0.4   | 6.8 $\pm$ 5.8    |
| Hb          | g/dL                 | 13.1 $\pm$ 0.28  | 14.4 $\pm$ 0.61  | 12.8 $\pm$ 0.60  | 11.2 $\pm$ 0.83  | 15.67 $\pm$ 2.62 | 11.2 $\pm$ 3.4   | 13.2 $\pm$ 2.4   |
| PCV         | %                    | 41.2 $\pm$ 2.2   | 43.8             | 41.53 $\pm$ 2.36 | 40.67 $\pm$ 1.97 | 42.38 $\pm$ 2.2  | 39.38 $\pm$ 2.6  | 40.46 $\pm$ 2.4  |
| MCV         | fl                   | 53.6 $\pm$ 1.75  | 51.8 $\pm$ 0.75  | 50.6 $\pm$ 1.03  | 52.6 $\pm$ 2.16  | 51.6 $\pm$ 1.03  | 53.6 $\pm$ 1.75  | 55.8 $\pm$ 0.75  |
| MCHC        | g/dL                 | 35.8 $\pm$ 7.31  | 36.15 $\pm$ 7.74 | 32 $\pm$ 7.08    | 37 $\pm$ 5.56    | 38 $\pm$ 5.51    | 34.15 $\pm$ 7.74 | 32.15 $\pm$ 7.74 |
| MCH         | pg                   | 15.67 $\pm$ 0.28 | 14.42 $\pm$ 0.61 | 16.8 $\pm$ 0.60  | 14.2 $\pm$ 0.83  | 15.67 $\pm$ 2.62 | 14.2 $\pm$ 3.4   | 16.2 $\pm$ 2.4   |
| WBC         | 10 <sup>9</sup> /L   | 7.89 $\pm$ 1.31  | 8.21 $\pm$ 0.74  | 7.85 $\pm$ 0.87  | 7.75 $\pm$ 1.33  | 7.68 $\pm$ 1.22  | 8.12 $\pm$ 0.74  | 7.86 $\pm$ 0.87  |
| Neutrophils | 10 <sup>9</sup> /L   | 1.92 $\pm$ 0.98  | 1.67 $\pm$ 0.75  | 1.49 $\pm$ 0.89  | 1.56 $\pm$ 1.36  | 1.68 $\pm$ 0.81  | 1.69 $\pm$ 0.89  | 1.86 $\pm$ 1.36  |
| Lymphocytes | 10 <sup>9</sup> /L   | 6.43 $\pm$ 1.54  | 4.81 $\pm$ 1.36  | 5.21 $\pm$ 1.03  | 6.71 $\pm$ 1.60  | 6.66 $\pm$ 1.32  | 5.21 $\pm$ 1.03  | 6.71 $\pm$ 1.60  |
| Monocytes   | 10 <sup>9</sup> /L   | 0.26 $\pm$ 1.09  | 0.24 $\pm$ 1.21  | 0.29 $\pm$ 0.51  | 0.29 $\pm$ 1.21  | 0.30 $\pm$ 1.26  | 0.28 $\pm$ 1.21  | 0.26 $\pm$ 0.51  |
| Eosinophil  | 10 <sup>9</sup> /L   | 0.16 $\pm$ 0.81  | 0.18 $\pm$ 0.75  | 0.17 $\pm$ 1.09  | 0.14 $\pm$ 0.75  | 0.14 $\pm$ 1.16  | 0.17 $\pm$ 0.75  | 0.16 $\pm$ 1.09  |
| Platelets   | 10 <sup>9</sup> /L   | 874 $\pm$ 44.9   | 872 $\pm$ 27.3   | 745 $\pm$ 69.5   | 867 $\pm$ 34.7   | 809 $\pm$ 38.2   | 765 $\pm$ 69.5   | 887 $\pm$ 34.7   |

**Table 6.5: Table showing Bio-chemical Parameters in Albino Wistar Rats.**

Values are expressed as mean  $\pm$  SD (n= 10 for each group)

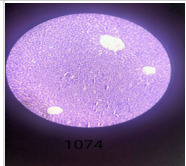
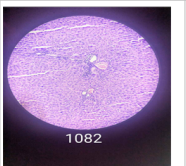
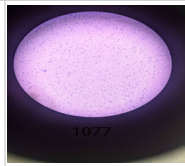
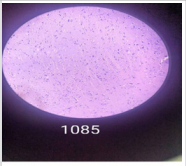
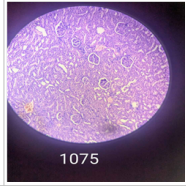
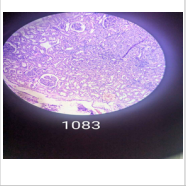
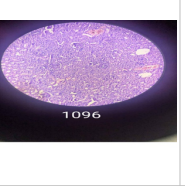
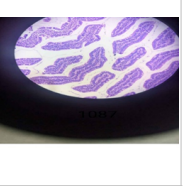
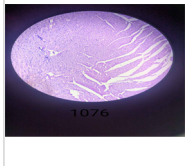
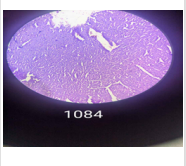
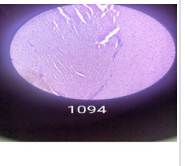
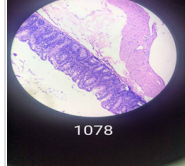
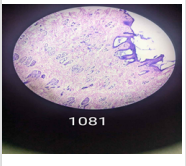
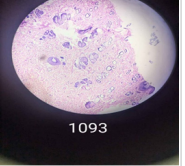
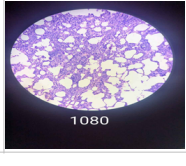
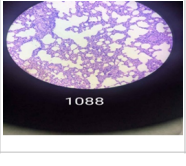
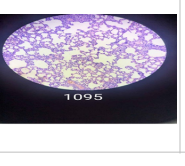
| PARAMETERS                           | Group I          | GROUP II         | GROUP III        | GROUP IV        | GROUP V          | GROUP VI         | GROUP VII                     |
|--------------------------------------|------------------|------------------|------------------|-----------------|------------------|------------------|-------------------------------|
| Blood glucose(mg/dl)<br>50-135mg/dl  | 86.63 $\pm$ 0.46 | 92.32 $\pm$ 0.48 | 82.01 $\pm$ 0.20 | 86.5 $\pm$ 0.03 | 93.32 $\pm$ 0.14 | 66.32 $\pm$ 0.18 | .01 $\pm$ 0.20                |
| Sr. Creatinine<br>(mg/dl)0.45to 1.07 | 0.63 $\pm$ 0.03  | 0.63 $\pm$ 0.03  | 0.58 $\pm$ 0.01  | 0.60 $\pm$ 0.02 | 0.61 $\pm$ 0.03  | 0.59 $\pm$ 0.03  | 0.60 $\pm$ 0.03<br>$\pm$ 0.03 |
| SGOT(IU/L)50-150                     | 139 $\pm$ 19.4   | 146 $\pm$ 12.4   | 147 $\pm$ 16.5   | 136 $\pm$ 19.4  | 132 $\pm$ 18.1   | 138 $\pm$ 13.5   | 132 $\pm$ 12.4                |

**Table 6.6: Histopathological observation of organs in different groups**

|         | GROUP I                                                                      | GROUP II                                                               | GROUP III                                                                                        |
|---------|------------------------------------------------------------------------------|------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------|
| Liver   | Showed a normal structure of liver hepatocytes.                              | no significant alterations were noticed                                | no significant alterations were noticed                                                          |
| Kidney  | kidney tissues t showed a normal structure of glomeruli and Bowman's capsule | no significant alterations were noticed                                | no significant alterations were observed                                                         |
| Stomach | Unremarkable gastric tissue                                                  | Unremarkable gastric gland with focal area of necrosis and hemorrhage. | Unremarkable gastric gland with alveoli unremarkable with mucosa, sub mucosa and mulcaris mucosa |

|              |                                            |                                            |                                            |
|--------------|--------------------------------------------|--------------------------------------------|--------------------------------------------|
| <b>Brain</b> | Unremarkable nervous tissue                | Unremarkable nervous tissue                | Unremarkable nervous tissue                |
| <b>Heart</b> | Unremarkable myocardial tissue             | Unremarkable myocardial tissue             | Unremarkable Cardiac muscle tissue         |
| <b>Skin</b>  | showing normal epidermis layer of the skin | showing normal epidermis layer of the skin | showing normal epidermis layer of the skin |

**Table 6.7 Showing histological images of organs in different groups**

| Organ  | Group I                                                                            | Group II                                                                           | Group III                                                                          | Organ   | Group I                                                                            | Group II                                                                            | Group III                                                                           |
|--------|------------------------------------------------------------------------------------|------------------------------------------------------------------------------------|------------------------------------------------------------------------------------|---------|------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------|
| Liver  |   |   |   | Stomach |  |  |  |
| Kidney |   |   |   | Brain   |  |  |  |
| Heart  |   |   |   | Skin    |  |  |  |
| Lungs  |  |  |  |         |                                                                                    |                                                                                     |                                                                                     |

## Discussion

### Acute dermal toxicity

Traditional medicine has maintained greater popularity all over developing world and the use is rapidly on the increase. Despite this, the safety of herbal medicine use has recently been questioned due to reports of illness and fatalities; hepatotoxicity and nephrotoxicity. Although there are many traditional herbal medicines available, only a few have been verified by clinical trials, their efficacy and safety are still questioned.

The acute dermal toxicity with the single highest test dose was conducted as per the OECD guideline 402. Acute toxicity test to find the dose that produces toxicity. Maximum tolerated dose would be calculated by employing OECD guidelines. This dose was applied locally only once at the first day of the study. Rats were monitored for the duration of 24 hours, with special attention given to the first 6 hours and once daily further for a period of 14 days.

Observation included the change in skin, fur, eyes and mucus membrane. Appearance of toxicity related to the central nervous system, cardiovascular system and Autonomic nervous system such as tremors, convulsions, behavior, salivation, diarrhea, sleep, coma and mortality were observed with special attention. The results revealed no treatment related death or signs of toxicity in the treated animals in all the doses throughout the study. Body weight gain of both male and female rat was also observed when compared with before and after treatment of the observed groups further, there were no gross pathological abnormalities which

prove the LD50 value was found to be greater than 2000mg/ kg b. wt.

There were no treatment-related toxicity signs and mortality observed in rats treated at 500mg, 1000mg and 2000 mg/kg locally for a period of 28 days. Body weight gain was observed between control and treated groups during the study. Food and water consumption of treated groups were found to be normal. Since there is no significance decrease in mean body weight and there is considerable increase in mean body weight of control and treatment groups.

Hematological profile such as Packed Cell Volume (PCV), Red Blood Cells (RBC) count, White Blood Cell Count (WBC), Platelet Count, Hemoglobin (Hb), Mean Cell Hemoglobin Concentration (MCHC), were found to be within the normal physiological limits for rodents.

Urea is formed in the liver from ammonia and glacial acetic acid, Ammonia is highly toxic, it is lungs and almost completely oxidized by tissues. Their detoxified through conversion to urea, which is work proposed that metabolic pathways for glacial nontoxic and water-soluble and is excreted through acetic acid is well known and involves the formation of urine by the kidneys. In higher concentration urea is ketone bodies. Main product of protein metabolism is urea and an increased level of urea in the blood is an indicator of renal impairment.

Creatinine is a breakdown product of creative phosphate in muscle, and is usually produced at a fairly constant rate by the body (depending on muscle mass) Serum creatinine (a blood measurement) is an important indicator of renal health because it

is an excreted unchanged by the kidneys. The present study showed no changes pertaining to renal parameters. SGOT & SGPT are largely used in the assessment of liver damage by drugs or any other hepatic. So, to elucidate the toxicity produced during liver metabolism of drug. SGOT and SGPT, which are the indicators of hepatocellular injury also did not show any alterations in the polyherbal formulation treated groups and control groups.

The Histopathological studies revealed no changes and normal architectural changes in the vital organs such as skin heart, brain, lungs, spleen, kidneys, liver, and stomach

#### Sub-acute dermal toxicity

After application of oil or ointment on skin, reaction was observed. Before the test, the rats will be selected randomly and assigned to the treatment and control groups, About 10% of the body surface area was cleared for the application of the test substance. Animals are observed for skin reactions and other signs immediately after dosing at least once during the first 30 minutes, periodically during the first 24 hours, with special attention given during the first 2 to 6 hours after the beginning of the exposure period, and daily thereafter, for a total of 28 days, Histopathological tests were done to compare the dermal toxicity of drugs.

#### Behavioural Changes

The Observations included the change in skin, fur, eyes and mucus membrane. Appearance of toxicity related to the central nervous system, cardiovascular system and Autonomic nervous system such as tremors, convulsions, behavior, salivation, diarrhea, sleep, coma and mortality were observed with special attention. The results revealed no treatment related death or signs of toxicity in the treated animals in all the doses throughout the study.

Body weight gain of both male and female rat was also observed when compared with before and after treatment of the observed groups. Further, there were no gross pathological abnormalities which prove the LD50 value was found to be greater than 2000mg/ kg b .wt.

There were no treatment-related toxicity signs and mortality observed in rats treated at 500mg, 1000mg and 2000 mg/kg locally for a period of 28 days. Body weight gain was observed between control and treated groups during the study. Food and water consumption of treated groups were found to be normal. Since there is no significance decrease in mean body weight and there is considerable increase in mean body weight of control and treatment groups.

#### Hematological Profile

Hematological profile such as Packed Cell Volume (PCV), Red Blood Cells (RBC) count, White Blood Cell Count (WBC), Platelet Count, Hemoglobin (Hb), Mean Cell Hemoglobin Concentration (MCHC), were found to be within the normal physiological limits for rodents.

Urea is formed in the liver from ammonia and glacial acetic acid, Ammonia is highly toxic, it is lungs and almost completely oxidized by tissues.<sup>16</sup> Their detoxified through conversion to urea, which is work proposed that metabolic pathways for glacial nontoxic and water-soluble and is excreted through acetic acid is well known and involves the formation of urine by the kidneys. In higher concentration urea is ketone bodies .main product of protein metabolism is urea and an increased level of urea in the

blood is an indicator of renal impairment. Creatinine, the product of creative phosphate in muscle, and is usually produced at a fairly constant rate by the body (depending on muscle mass) Serum creatinine (a blood measurement) is an important indicator of renal health because it is an excreted unchanged by the kidneys.

The present study showed no changes pertaining to renal parameters. SGOT & SGPT are largely used in the assessment of liver damage by drugs or any other hepatic problem. So, to elucidate the toxicity produced during liver metabolism of drug. SGOT and SGPT, which are the indicators of hepatocellular injury also did not show any alterations in the polyherbal formulation treated groups and control groups.

The Histopathological studies of the organs revealed no changes and normal architectural changes in the vital organs such as skin heart, brain, lungs, spleen, kidneys, liver, stomach etc.

#### Absence of Dermal Toxicity in Sub-Acute Study

Throughout the 28-day sub-acute toxicity study, animals showed:

- Normal grooming behavior,
- No lesions or scaling,
- No systemic signs of toxicity (e.g., change in body weight, feed intake, or behavior),
- Normal Histopathological architecture of the skin in biopsy samples

These findings support the classical Ayurvedic understanding that topically applied herbo-mineral formulations, when properly prepared, are safe for prolonged use. These results affirm that the topical application of *Hartala and Manashila in Shodhita* form is not associated with acute or sub-acute dermal toxicity when compounded properly in an oil or ointment base.

#### Regulatory and Safety Considerations

Despite the therapeutic benefits, the use of arsenicals in medicine is met with caution in modern regulatory systems. However, contextual use, dose control, and proper processing allow for safe application. Several government and institutional studies in India have supported the safe use of herbo-mineral preparations in external applications when processed per classical guidelines. Ongoing research and analytical standardization using HPLC, ICP-MS, or AAS can further substantiate the safety and define limits of arsenic content in formulations, ensuring they meet global safety

## Conclusion

#### Acute dermal Toxicity Study

The study assessed the acute dermal toxicity, hematological, biochemical, and Histopathological impact of the *Aargwadhadi tail*, and *Aargwadhadi* ointment in albino Wistar rats.

The following key points were observed: after 14 days of acute dermal toxicity study, all

- Hematological Parameters are within normal physiological range.
- Urea & Creatinine levels are normal; no renal impairment found.
- SGOT & SGPT are unchanged; no hepatotoxicity observed.
- In Histopathological findings, no pathological changes were observed; hence both were found safe at higher dose.

## Sub-acute dermal Toxicity Study

The study assessed the sub-acute dermal toxicity, haematological, biochemical, and Histopathological impact of the Aargwadhadi tail, and Aargwadhadi ointment in albino Wistar rats.

The following key points were observed after 28 days of acute dermal toxicity study,

- No signs of toxicity, abnormal behavior, or mortality at any dose (500, 1000, 2000 mg/kg).
- Weight gain observed in all treated groups
- Hematological Parameters were within normal physiological limits. Urea & Creatinine levels normal.
- Liver Enzymes: SGOT & SGPT unchanged, indicating no hepatic toxicity.
- In Histopathology Organs studied: No pathological changes found, thus Aargwadhadi oil and Aargwadhadi ointment exhibits no dermal toxicity at higher dose for long duration of allocation

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## Conflict of Interest

The authors declare no conflicts of interest

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