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Research Article

ACUTE DERMAL TOXICITY ASSESSMENT OF *ARK TAILA* (AN AYURVEDIC HERBAL OIL) IN WISTAR ALBINO RATS

Mamata Pravin Adhao[1,2]*, Mamata V. Narvekar[3]

[1]Ph.D Scholar, [3]Associate Professor, PhD (Guide), Dept. of Agadtantra and Vidhi Vaidyak, YMT Ayurvedic Medical College and Hospital and P.G. Institute, Kharghar, Navi Mumbai, Maharashtra, India.

*[2]Professor, P.G. Guide, Ph D Guide, Dept. of Agadtantra and Vidhi Vaidyak, Bhausaheb Mulak Ayurved Mahavidyalay and Medical Science and Research Hospital, Nandanvan, Nagpur, Maharashtra, India.

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ABSTRACT

Arka Taila is a classical Ayurvedic medicated oil Prepared by *Swarasa Siddhi* method mainly composed of *Arka Patra Swarasa* (juice of Calotropis leaves), *Haridra Kalka* (paste of Curcuma longa) and *Sarshapa Taila* (Mustard oil). It has traditionally been used in managing various skin disorders. Though its therapeutic benefits are proved by various researches scientific evidence regarding its dermal safety is limited. **Objective:** The objective of the present study was to evaluate the acute dermal toxicity and safety profile of *Arka Taila* in Wistar albino rats as per OECD Guideline 402**. Methodology:** *Arka Taila* was prepared classically and applied topically to the shaved dorsal skin of healthy female rats (n=3 per group) at three dose levels: the limit dose of 2000mg/kg, 1000mg/kg, and 200mg/kg. Animals were observed daily for 14 days for mortality, toxicity, and behavioural changes. Local dermal reactions (erythema and edema) were scored. Body weights were recorded and a gross necropsy followed by histopathological examination of the vital organs was conducted at the end of the study. **Result:** a) No mortality or significant clinical and neurobehavioral signs of systemic toxicity were observed in any dose group throughout the period of 14 days. b) All the animals showed normal, steady body weight gain. c) Dermal examination revealed no edema at any of the doses. Only very slight, transient erythema was noted in few animals at 1000 and 2000mg/kg dose, which resolved spontaneously. d) Gross necropsy and histopathological analysis of the organs like skin, liver, kidney, and heart did not reveal any pathological lesions or toxicity to organs. **Conclusion:** Based on the outcomes of this toxicity study, it may be concluded that *Arka Taila* is non-irritant and safe upon acute dermal exposure to the limit dose of 2000 mg/kg body weight. This observation supports the traditional topical use of *Arka Taila* in dermatological practice.

INTRODUCTION

Ayurveda has long utilized plant-based formulations for the management of skin disorders. Among these is *Arka Taila*, a medicated oil preparation described in classical Ayurvedic texts. The formulation is made from the leaves of *Arka* (Calotropis spp.), classified as one of the *Upavisha* (semi-toxic herbs). [1] It is frequently recommended for topical use in *Kustha* (skin disorders) such as *Vicharchika* (eczema*), Pama* (Scabies) and *Kandu* (pruritus). The formulation is primarily composed of *Arka Patra Swarasa* (juice of *Calotropis procera /gigantea*), *Haridra Kalka* (paste of Curcuma longa) and *Sarshapa Taila* (mustard oil). The formulation and therapeutic use of *Ark Tail* are well-documented in multiple authoritative Ayurvedic texts such as *Sharangdhar Samhita,*[2] *Bharat Bhaisajya Ratnakar (Brihad Nighantu Ratnakar),*[3] *Vangasen Samhita*, [4] *Yog Ratnakar.*[5] *Rasaratna Samuchchaya - Karna-Nasa-Mukharoga Chikitsa Adhyaya* - mentions *Arkpatra Swaras Siddha Taila* for *Karna Shool* (Earache).[6] These classical references confirm the therapeutic utility of *Ark Taila* specially in the

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management of *Kustha* (skin disorders), *Karna Shool* (earache) and other related conditions.

The unique healing potential of *Arka Taila* can be attributed to the synergistic action of its constituents. *Calotropis procera* has been recognized for its anti-inflammatory and antipruritic activities. Curcuma longa provides antioxidant and healing benefits through its active compound curcumin and mustard oil facilitates dermal penetration and contributes additional antimicrobial properties. This classical oil is prepared using the traditional *Swarasa Siddhi* method, improving the bioavailability of the phytochemicals through precise heating. Anti-inflammatory, antipruritic, antioxidant, wound-healing, antimicrobial, and bioavailability-enhancing activities exhibited by *Arka Taila* contribute to the efficacy of treatment in a variety of skin and inflammatory conditions. [7-9]

Although *Arka Taila* is widely used in Ayurvedic dermatology, scientific research data regarding its safety profile, in particular dermal exposure, is scant. As herbal preparations take centre stage in integrative health, there is an increasing need for toxicological evaluation to ensure their safe application, especially in higher dosage and for extended periods, onto the skin. In view of the therapeutic claims and growing use of *Arka Taila*, its dermal safety needs to be established using standardized toxicological methods. Acute dermal toxicity testing is a crucial component of the preclinical testing of topically applied agents.[10]

The efforts have been taken by this study to evaluate the acute dermal toxicity of *Arka Taila* in Wistar albino rats with the aim of generating essential preclinical safety data to facilitate its further development and potential clinical translation in dermatological applications.

OBJECTIVE: The objective of the study was to assess the acute dermal toxicity and safety profile of *Arka Taila* - A medicated oil preparation in Wistar albino rats as per OECD Guideline 402.

MATERIAL AND METHODS

Acute dermal toxicity study of *Arka Taila* was conducted as per following Organization for Economic Co-operation and Development (OECD)-TG 402 guidelines using the Fixed Dose Procedure, following the 3R principles to ensure ethical and regulatory compliance. [11]

Preparation of Test Drug *Ark Tail* - 1. Collection & Authentication of Raw Materials:

Fresh leaves of *Arka* (Calotropis spp.) and *Haridra* rhizomes (Curcuma longa) were collected from Herbal Garden, of parent institute while ingredients of *Sarshapa Taila Murchana* (*Amalaki, Mustaka, Bilva, Dadima*, etc.) were procured from an authenticated Ayurveda pharmacy. All the raw materials were authenticated by the Department of *Dravyaguna Vigyan*. 2. *Arka Taila* was prepared as per the classical method described in *Sharangdhar Samhita*. The mustard oil was subjected to purification by *Murchana* as described in *Bhaishajya Ratnavali*. Fresh leaves of *Arka* were processed for *Swarasa* and *Haridra* rhizomes were made into *Kalka*. The formulation was prepared by taking *Haridra Kalka*, *Murchita Sarshapa Taila* and *Arka Patra Swarasa* in the ratio of 1:4:16 i.e., 500g:2000ml8000ml, respectively, by maintaining uniform heat and continuous stirring until the completion of classical *Taila Paka*. Analytical and chromatographic assessments for the standardisation of *Arka Taila* were conducted in a government-recognized analytical laboratory. The results indicated that the formulation is safe and effective, thus proving its therapeutic potential.[12]

Animal Experimentation

Animal experimentation was performed at Smt. Kishoritai Bhoyar College of Pharmacy, Kamptee, Nagpur, India, after getting prior approval from IAEC with Reg. No. 853/PO/Re/S/04/CCSEA; Protocol No. 853/IAEC/2023-24/26 as per the guidelines of CPCSEA.

Experimental Animals

Inclusion Criteria: Healthy Female Wistar albino rats with intact skin, 8–10 weeks old, Body Weight Range: 170–210g, Within ±20% of the mean weight of the group. Normal behaviour with normal activity, feeding and grooming patterns were used for the study.

Exclusion Criteria: Disease or ill health, abnormal physiology. Pregnant or lactating females. Animals showing unusual clinical signs or in acute stress. Rats whose body weight deviates >20% from group mean. Animals that have received any drug, vaccine or experimental treatment within the past 2-4 weeks. Rats that display excessive aggression or cannot be handled safely. Rats not acclimatizing to laboratory conditions or exhibiting abnormal stress responses during acclimatization. Animals that show extreme lethargy or failure to feed normally were excluded from the study.

Housing and Diet of Animals: The animals were acclimatized for one week under environmentally controlled conditions (22±3°C, 30–70% RH, 12 h light/dark cycle). Animals were kept in their individual polypropylene cages with clean bedding. They were given a standard Nutrivet rat/mice pellet diet provided by Nutrivet Lifesciences, Pune, along with potable water ad libitum.

Preparation for Dosing: Animals were acclimatized to laboratory conditions for five days, assigned to the group in a random manner and individually marked.

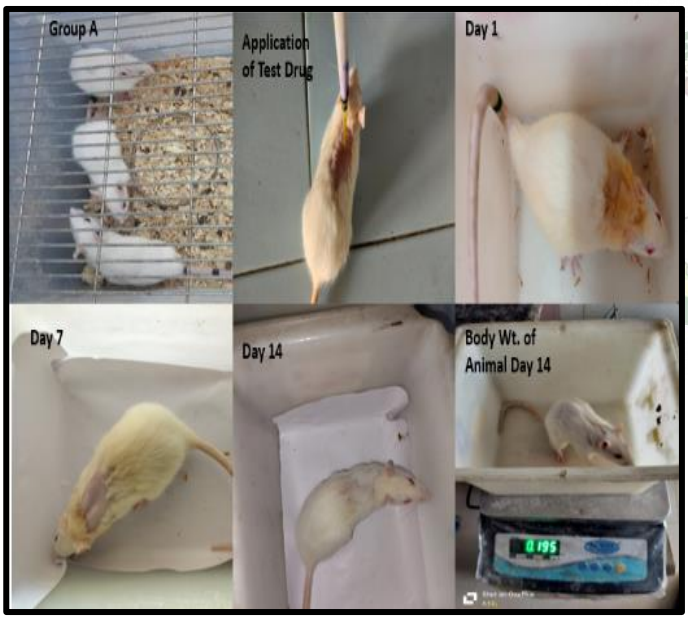
Approximately 10% of the dorsal surface area of animals was shaved 24 h before dosing with a hair clipper under mild anesthesia (Ketamine 50mg/kg and Xylazine 5mg/kg) as per OECD Guideline 402 without causing abrasion or irritation to the skin.

Experimental Design

Acute dermal toxicity of *Ark Taila* was performed in female rats as per the OECD Guideline 402 - Acute Dermal Toxicity: Fixed Dose Procedure. A total of nine healthy adult female rats were used in this study. The animals were randomly assigned into three groups (n = 3 per group) based on the targeted dose levels of the test substance.

- Group A: *Ark Taila* 2000mg/kg (limit dose)
- Group B: *Ark Taila* 1000mg/kg
- Group C: *Ark Taila* 200mg/kg

The test substance *Ark Tail* was applied uniformly over the 10% of the total body surface area which was shaved previously to exposed skin area. At the end of the 24-hour contact period, the residual test substance was carefully removed using a sterile gauze pad moistened with distilled water. [13,14,15]



OBSERVATIONS AND RESULT

The animals were observed individually for any signs of toxicity or adverse reactions Immediately after dosing after once during the first 30 minutes, periodically after 2 hrs, 4 hours during the first 24, 48, and 72 hours and thereafter once daily for a total observation period of 14 days. Clinical signs, behavioural changes and mortality were recorded throughout the study. Body weights were measured on Days 0, 7 and 14. At the end of the 14-day observation period, one animal from each group were euthanized and a gross necropsy was performed as per ethical consideration by IEAC. All major organs and tissues were examined for gross pathological changes. [16]

Acute Dermal Toxicity Observation

All animals were individually observed for a period of 14 days following dermal application of Ark Taila. No mortality and no clinical signs of toxicity were evident in any of the groups (200, 1000, and 2000mg/kg). All the animals were alert, active, and without visible signs of distress during the first 30 minutes, at 2 hours, and for the entire first 24 hours post-dosing. Daily observation throughout the 14-day post-exposure showed no signs of toxic insult due to the test formulation.

Body Weight Changes

Individual body weights of animals were recorded just before the application of the test item on day 0 and then once on day 7 and day 14 of the observation period. Mean body weight values are given in Table 1.

Table 1: (Mean ± SD*)* Body weight data of Wistar Rats exposed to *Ark Taila*

S.No	Group	Dose (mg/kg)	Day 0 (g)	Day 7 (g)	Day 14 (g)
1	A	2000	171.33 ± 1.53	182.67 ± 3.06	197.00 ± 2.65
2	B	1000	171.33 ± 1.53	182.67 ± 3.06	197.00 ± 2.65
3	C	200	171.33 ± 1.53	182.67 ± 3.06	197.00 ± 2.65

Clinical Observations

Following dermal application of the test formulation *Ark Taila* at 200, 1000, and 2000mg/kg body weight, all experimental animals were observed for a period of 14 days. No mortality or abnormal clinical signs of toxicity were recorded during the entire study period. All animals were found to be normal, active, and healthy, with no changes noticed in their behavioural and neurological patterns.

Cage-Side and Functional Observations

Cage-side observations were performed daily to monitor the general health status, appearance and behaviour of the animals. Parameters included changes in skin and fur, eyes and mucous membranes, respiratory, circulatory, autonomic and central nervous systems, and somatomotor activity.

During the course of the study, all treated animals of Groups A, B, and C maintained their normal posture, gait, and handling response. No skin and fur texture changes were observed and the appearance of mucous membranes was normal. No animal showed any evidence of hypoactivity, hyperactivity or ataxia. Respiratory pattern was undisturbed without findings of bradypnoea, tachypnoea or laboured breathing. Circulatory and autonomic functions like salivation, lacrimation, piloerection, urination, and defecation were apparently unaffected.

FOB assessments - Showed no autonomic or central nervous system disturbances, including changes in pupil size, body temperature and grip strength. Stereotypical or bizarre behaviours, such as excessive grooming, circling, self-mutilation or walking backward, were not exhibited. Moreover, no clonic or tonic seizures were found to occur in any animal for the entire duration of observations.

Table 2: Tabulated Clinical Observation Summary

Observation Parameter	Group A (2000 mg/kg)	Group B (1000 mg/kg)	Group C (200 mg/kg)
Changes in skin and fur	–	–	–
Somatomotor activity	–	–	–
Eyes and mucous membranes	–	–	–
Respiratory signs (bradypnea, tachypnea, distress)	–	–	–
Autonomic responses (salivation, lacrimation, piloerection, urination, defecation)	–	–	–
Circulatory changes	–	–	–
CNS responses	–	–	–
Stereotypical behaviors	–	–	–
Clonic/Tonic seizures	–	–	–

Note: "–" = absent; "+" = present.

All the parameters recorded were within normal limits on all days of observation, 1–14. Absence of any "+" entries confirms that *Ark Taila* failed to produce either localized dermal irritation or systemic neurobehavioral changes at up to 2000mg/kg. [17]

Erythema and Eschar Formation

After removing the test formulation, the application sites were carefully examined for erythema and eschar formation in accordance with OECD Guideline 404. The erythema scores are summarized in Table 3.

Table 3. Erythema and Eschar Formation Scores

Group	Dose (mg/kg)	Animals	Scores (A1–A3)	Mean ± SD	Observation
Group A - *Ark Tail*	2000	A1–A3	0, 1, 1	0.33 ± 0.58	Two animals exhibited very slight erythema; no eschar formation observed.
Group B - *Ark Tail*	1000	A1–A3	1, 0, 1	0.33 ± 0.58	Two animals exhibited very slight erythema. no eschar formation observed.
Group C - *Ark Tail*	200	A1–A3	0, 0, 0	0.00 ± 0.00	No erythema or eschar formation observed.

A very slight erythema (score 1) was noted in two animals of Group A and two animals of Group B. No well-defined or severe erythema and no eschar formation were noted in the treated animals. The reactions noted were transient and resolved spontaneously within the observation period.

Edema Formation

Edema formation was scored in parallel with erythema and the results are reported in Table 4.

Table 4: Edema Formation Scores

Group	Dose (mg/kg)	Animals	Scores (A1–A3)	Mean ± SD	Observation
Group A – *Ark Tail*	2000	A1–A3	0, 0, 0	0.00 ± 0.00	No edema observed
Group B – *Ark Tail*	1000	A1–A3	0, 0, 0	0.00 ± 0.00	No edema observed

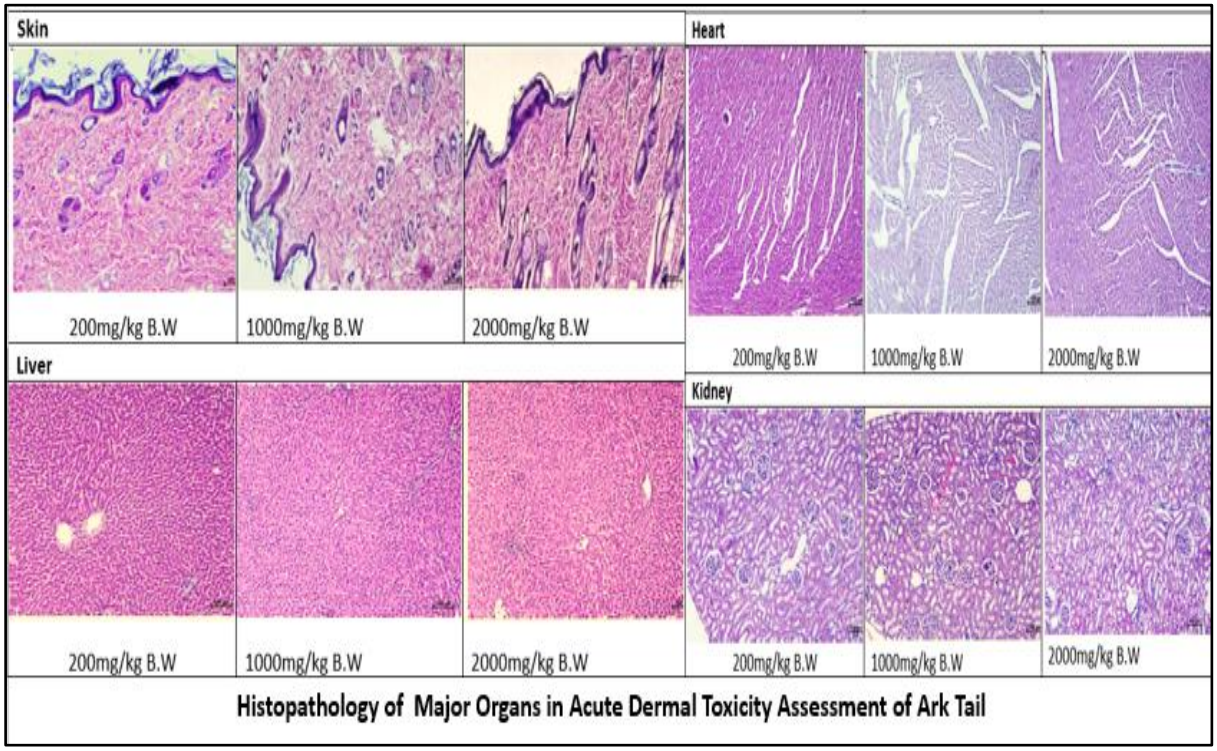
Group C – *Ark Tail*	200	A1–A3	0, 0, 0	0.00 ± 0.00	No edema observed

No animal of any group has shown edema formation. The mean edema score in all groups was 0.00 ± 0.00, denoting the absence of dermal irritation.[18]

Gross Necropsy Findings

At the end of the 14-day observation period, one animal from each dose group (200, 1000 and 2000 mg/kg body weight) was sacrificed under deep anaesthesia. The study ended with a detailed gross necropsy in accordance with IAEC and CPCSEA guidelines. The necropsy included examination of the external body surface, cranial cavity, thoracic and abdominal cavities along with all major organs such as heart, liver, kidneys and skin. [19]

No gross pathological abnormalities or visible lesions were observed in any treated animals. All the organs appeared normal regarding their size, shape, color, and consistency. No signs of haemorrhage, necrosis, congestion or tissue discoloration were observed across all groups. This suggests no macroscopic toxicity or organ damage was evident after dermal administration with *Ark Taila*.



Histopathology of Major Organs in Acute Dermal Toxicity Assessment of Ark Tail

Statistical Interpretation

Changes in body weight are a critical indicator of the overall health and physiological reaction of experimental animals to a test compound.

A gradual increase in mean body weight was observed in all treatment groups throughout the duration of the 14-day observation period. No statistically significant differences ($p > 0.05$) were seen between groups at any time point studied, as confirmed by one-way ANOVA followed by Tukey's post hoc test. The above finding of consistent body weight gain reflects normal growth without systemic toxicity or any other adverse effects due to *Ark Taila* at all levels of tested doses.

The gradual weight increase indicated that *Ark Taila* did not impede metabolic processes, nutritional absorption or the state of health in general.

Data from clinical observations were analyzed using descriptive statistics and categorical findings were compared across groups by the Chi-square test for incidence data and one-way ANOVA for any continuous parameters, such as body temperature and body weight. There were no statistically significant differences (p-value > 0.05) among treatment groups for any measured endpoint.

Absence of adverse clinical signs uniformly across the dose levels indicates that the dermal exposure to *Ark Taila* was greater than 2000mg/kg body weight. Consequently, the formulation will, therefore, be considered nontoxic by GHS classification via dermal route (Category 5 or unclassified).

Descriptive statistical analysis was done for erythema and edema scores. Mean ± SD values were calculated for each group. The data were analyzed by one-way ANOVA followed by Dunnett's multiple comparison test to compare treatment groups with control.

No significant difference ($p>0.05$) was demonstrated across groups for either erythema or

edema scores, further establishing the absence of dose-dependent dermal irritation. All dose formulations had Primary Irritation Index (PII) values less than 0.5, identifying them as non-irritants by the Draize classification system.

Descriptive statistical analysis was performed to evaluate any dose-related histopathological changes. Quantitative ANOVA comparison was not necessary because all treated animals exhibited normal morphology and histology of the organs. For interpretation, a semi-quantitative scoring system (0 = normal, 1 = mild change, 2 = moderate change, 3 = severe change) was employed to confirm homogeneity among groups.

Table 5: Statistical Interpretation of histopathological examination of Major organs

Organ	Mean ± SD (200 mg/kg)	Mean ± SD (1000 mg/kg)	Mean ± SD (2000 mg/kg)	Statistical Inference
Skin	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	No lesion or inflammation ($p > 0.05$)
Kidney	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	Normal histology ($p > 0.05$)
Heart	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	No cardiac pathology ($p > 0.05$)
Liver	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	Normal hepatic histo architecture ($p > 0.05$)

No statistically significant differences ($p > 0.05$) in any organ were found between the dose groups, confirming the absence of dose-dependent pathological changes. All groups showed an unchanged mean pathology index (MPI) of 0.00, indicating complete histological normalcy.

The preserved architecture of epidermis, dermis, and subcutis, in concert with normal hepatocytes, glomeruli, renal tubules, and myocardial fibers, indicates that the test formulation did not evoke either local irritation or systemic organ toxicity. Results are consistent with the non-irritant category according to OECD 404, since degenerative, necrotic, or inflammatory responses were not present. [20, 21]

DISCUSSION

The present study was designed to evaluate the acute dermal toxicity of *Arka Taila*, a widely used Ayurvedic medicated oil, in various dermatological disorders. While its therapeutic applicability in conditions like eczema, pruritus, and scabies finds mention in classical literature [22], modern dermal safety data regarding the same is rare. Since the primary ingredient Calotropis procera has been traditionally categorized as *Upavisha* and is reported to possess biologically potent latex constituents [23], structured safety evaluation is imperative. The present investigation was therefore conducted in accordance with OECD Guideline 402 and represents the first structured assessment of its acute dermal toxicity profile.

No mortality and no sign of systemic toxicity was exhibited at any dose, including the limit dose of 2000 mg/kg, suggesting a wide dermal safety margin. Lack of behavioural, autonomic, and neurological changes further corroborates earlier reports that controlled topical exposure to Calotropis spp. usually does not lead to systemic toxicity Normal and steady body weight increase during the course of study further establishes that the formulation did not affect physiological growth or metabolic status as evidenced from routine toxicological indices of health.

Dermal testing showed only slight, transient erythema at higher doses, without any edema. The Primary Irritation Index (<0.5) categorized the formulation as non-irritant per Draize . This slight erythema might be explained by the rubefacient action of mustard oil, which induces temporary vasodilation without actual irritation. Histopathological examination showed normal cyto architecture of all organs examined without any inflammatory, degenerative or necrotic lesions. This would suggest that dermal penetration of Calotropis constituents with potential toxicity was negligible or fully neutralized by the traditional processing. Various Ayurvedic pharmaceutical transformations, such as *Murchana* and *Swarasa Siddhi*, reportedly detoxify potent herbs while enriching therapeutic bioavailability. This supports the long-held Ayurvedic view that processing *Upavisha* substances within oil matrices reduces toxicity. [24,25]

The lack of dose-related toxicity at any tested level defines a NOAEL above 2000 mg/kg, making *Arka Taila* fall into the GHS Dermal Category 5 or Unclassified category. Overall, the present study has comprehensively established that *Arka Taila* is nontoxic and nonirritant upon acute dermal exposure. These findings support its traditional use and lay a scientific basis for its further evaluation in clinical dermatology. Further studies on repeated-dose dermal toxicity, dermal penetration kinetics and safety assessment on diseased skin may give additional information on long-term use and therapeutic optimization.

CONCLUSION

This preclinical study was conducted to evaluate acute dermal toxicity of *Arka Taila*, a classical Ayurvedic formulation, in Wistar albino female rats according to OECD Guideline 402. Application of the

formulation was done topically at dose levels of 200 mg/kg, 1000 mg/kg and 2000 mg/kg body weight using the fixed-dose procedure.

Throughout the entire 14-day observation period, no mortality or significant clinical signs of toxicity were noted in any of the dose groups. Cage-side observation of autonomic, central nervous system, behavioural, and physiological parameters did not reveal any abnormalities. Very slight erythema occurred among a few animals at higher doses (1000mg/kg and 2000mg/kg) and resolved spontaneously without intervention. No edema was recorded for any group.

Body weight for all animals studied showed a steady increase, with no signs of adverse effect on growth or general health. Gross necropsy and histopathology examination of the vital organs including the skin, liver, kidney, and heart did not indicate any pathological lesions, active inflammation, or organ toxicity.

Overall, these findings suggest that *Arka Taila* is safe for topical application, even at high doses of up to 2000 mg/kg and exhibits no evidence of acute dermal toxicity in Wistar albino rats under the test conditions.

Thus, the present study provides preclinical evidence supporting the dermal safety of *Arka Taila*, justifying its continued use in Ayurvedic dermatological practice. Further sub-chronic and chronic toxicity studies may be warranted to support its long-term safety profile.

ABREVETIONS

1. **(OECD)-TG**: (OECD)-Organisation for Economic Co-operation and Development, **TG -**Test Guidelines.
2. **The 3R principle for animal experiments stands for:** Replacement, Reduction, and Refinement. These principles are a framework for humane animal research, aiming to replace animal use where possible, minimize the number of animals used, and minimize pain and distress for those that are used.
3. **IEAC**: Institutional Animal Ethics Committee.
4. FOB- A Functional Observational Battery (FOB) in animals, is a document describing a systematic assessment of a laboratory animal's nervous system function.
5. **GHS classification** - Globally Harmonized System of Classification and Labelling of Chemicals.

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***Address for correspondence**
Dr. Mamata Pravin Adhao
Professor,
P.G. Guide, Ph D Guide,
Dept. of Agadtantra and Vidhi Vaidyak
Bhausaheb Mulak Ayurved Mahavidyalay and Medical Science and Research Hospital Nandanvan, Nagpur, Maharashtra, India.
Email: mamata.adhao@gmail.com