International Journal of Veterinary Sciences and Animal Husbandry

**ISSN:** 2456-2912
VET 2024; 9(3): 259-263

www.veterinarypaper.com
Received: 17-02-2024
Accepted: 21-03-2024

**Dr. Akshay G Shinde**
Research Scholar, Department of Veterinary Pharmacology and Toxicology, College of Veterinary and Animal Sciences, Parbhani, Maharashtra, India

**Dr. GD Ranvir**
Associate Professor, Department of Veterinary Pharmacology and Toxicology, College of Veterinary and Animal Sciences, Parbhani, Maharashtra, India

**Dr. DP Patil**
Assistant Professor, Department of Veterinary Pharmacology and Toxicology, College of Veterinary and Animal Sciences, Parbhani, Maharashtra, India

**Dr. GR Gangane**
Associate Professor, Department of Veterinary Pathology, College of Veterinary and Animal Sciences, Parbhani, Maharashtra, India

**Dr. Amol P Upase**
Research Scholar, Department of Veterinary Pharmacology and Toxicology, College of Veterinary and Animal Sciences, Parbhani, Maharashtra, India

**Corresponding Author:**
**Dr. Akshay G Shinde**
Research Scholar, Department of Veterinary Pharmacology and Toxicology, College of Veterinary and Animal Sciences, Parbhani, Maharashtra, India

# Acute dermal toxicity of *Lawsonia inermis* ethanolic leaves extract gel formulation in wistar rats

**Dr. Akshay G Shinde, Dr. GD Ranvir, Dr. DP Patil, Dr. GR Gangane and Dr. Amol P Upase**

**Abstract**
The objective of the present study was to evaluate the acute dermal toxicity of the *Lawsonia inermis* ethanolic leaves extract gel formulation on Wistar rats as per OECD guideline no. 402.
10% *Lawsonia inermis* ethanolic leaves extract was prepared by mixing dried leaves powder in 70% ethanol and obtained filtrate was evaporated. The 10% (w/v) gel was prepared by using extract, carbopol and tri-ethanolamine. Acute dermal toxicity study of *Lawsonia inermis* ethanolic leaves extract gel was conducted as per OECD guideline no. 402. The animals were observed immediately after dosing and 24, 48 & 72 hrs after exposure period and daily thereafter for 14 days of experimental period. Weekly feed consumption, body weight, the skin reactions were observed and graded by using Draize scoring criteria and necropsy for histopathological examinations of skins and different vital organs of exposed animals were carried out on 14$^{th}$ day of experimental period. There were neither any mortality nor any abnormal reactions on gross and microscopic examination of the drug applied skin of exposed rats were observed. There were no significant changes in the weekly feed consumption and body weights from drug applied rats were noticed.
From the results of this study, it is concluded that the *Lawsonia inermis* ethanolic leaves extract gel formulation is safe to apply on the skin.

**Keywords:** *Lawsonia inermis* Linn., ethanolic leaves extraction, hydrogel formulation, acute dermal toxicity evaluation, histopathology

## Introduction
According to estimates, up to 25% of all medications are made up of plants in industrialized nations like the United States, whereas up to 80% are found in rapidly emerging nations like China and India. On Earth, there should be between 250,000 and 500,000 different types of plants (Aejazuddin, Q., 2016) [1].
Herbal therapy is becoming more and more common among medical professionals and patients, and it is frequently used to treat a variety of illnesses, including skin disorders. In Europe and Asia, herbal remedies have been effectively treating dermatological conditions for thousands of years. Herbal remedies that have been utilized for generations in Asia are currently the subject of scientific research. Dermatologists who treat both humans and animals should be aware of this possibility of side effects. Almost all herbal medicines have the potential to trigger allergic responses, and some may even lead to photosensitization. Certain herbal therapies, especially Ayurvedic ones, such aloe vera, eucalyptus, camphor, and henna, can have adverse effects on the skin (Ernst, E., 2000) [4].
The widely branched, glabrous shrub or small tree known as *Lawsonia inermis* L. (Henna) is grown mostly for its leaves, however traditional medicine has also made use of the stem bark, roots, flowers, and seeds. Alkaloids, terpenoids, quinones, coumarins, xanthones, proteins, carbohydrates, flavonoids, tannins, and phenolic chemicals are all said to be present in the plant. The plant is said to possess the following benefits: anti-inflammatory, antibacterial, antimicrobial, antifungal, antiviral, antitrypanosomal, antidermatophytic, antioxidant, antifertility, tuberculostatic, hepatoprotective, immunostimulant, and anticancer. It is today regarded as a valuable source of distinctive natural goods for the creation of industrial products as well as medications to treat a variety of illnesses (Chaudhary *et al*., 2010) [2].

Also, the hydrogel prepared from ethanolic leaves extract of *Lawsonia inermis* possess potential antimicrobial, anti-inflammatory, anti-oxidant and wound healing activities (Khan, B. *et al*., 2021; Dixit, K. *et al*., 2022 and Moutawalli, A. *et al*., 2023) [6, 3, 7].

These properties of the gel contributed to its use for treatment of various dermatological conditions. So, this necessitates the dermal toxicity evaluation of *Lawsonia inermis* ethanolic leaves extract gel formulation.

The objective of this research work is to evaluate the dermal safety of the *Lawsonia inermis* ethanolic leaves extract gel.

## Materials and Methods

**Plant material:** The plant *Lawsonia inermis* was identified by taking help of botanist from VNMKV, Parbhani. Fresh leaves of *Lawsonia inermis* were collected.

**Chemicals:** Ethanol (70%): used as a solvent for extraction; Carbopol – 940 and Tri-ethanolamine: As a gelling agent.

**Miscellaneous:** Butter paper, cotton and adhesive tapes for preparations of patch for application and porous gauze for holding the patch in position.

**Preparation of extract:** After being carefully cleaned with clean water, the freshly harvested henna leaves were dried at room temperature in a shady area to prevent the phytoconstituents from being destroyed by direct sunlight. The leaves were ground into a powder using an electric grinder after drying. In order to create the extract from this powder, 50 grams of powdered dry leaves were combined with 70% ethanol. This resulted in a final volume of 500 millilitres of solution. After that, the produced solution was stirred intermittently for 24 hours. After that, it was filtered through muslin cloth and filter paper, and the resulting extract was allowed to evaporate at room temperature in order to concentrate it.

**Preparation of extract hydrogel:** Entire process of gel formulation was done under the guidance of Dr. S. R. Rajurkar (Head Department of Veterinary Pharmacology and Toxicology) in departmental experimental laboratory as hereby: 2 gm of Carbopol – 940 powder was added in the 80 ml of distilled water and allowed 8-10 hrs. to solubilize it. The accurately weighed 10 gm ethanolic extract was poured into the hydrated Carbopol 940 dispersion with constant mixing followed by 4-5 drops of Tri-ethanolamine was added and the final volume of 100 ml was achieved by adding remaining quantity distilled water.

**Experimental Animals:** 8-10 weeks old female Wistar rats with body weight ranging from 200 to 300 gm were purchased from LACSMI Biofarms Pvt. Ltd., Pune. The study was started after approval by the Institutional Animal Ethical Committee (IAEC), which met the national guidelines as per the guidelines of Committee for The Purpose of Control and Supervision of Experiments on Animals (CPCSEA) vide Resolution No. IAEC/125/23 dated on 15/12/2023.

Upon arrival, the rats were weighed and assigned randomly in rat cages, where one rat was placed in each cage and housed in an animal room with controlled conditions involving these parameters; Temperature (22±3 °C), Relative humidity (55±10%) and lighting (12 hrs. light and dark) in the animal house at the laboratory animal house, College of Veterinary and Animal Sciences, Parbhani.

**Skin preparation for dermal toxicity study:** Skin at the dorsal neck and trunk area of the rats was clipped using an electric clipper, followed by manual shaving using razor blade. Based on OECD guidelines 402, not less than 10% of the body surface area was cleared for application of the test substance (Image No. 1 to 4).

**Image 1:** Experimental animals used for acute dermal toxicity study and their weighing

**Image 2:** Preparation of site for dermal application of formulation

**Image 3:** Dermal application of formulation

**Image 4:** Animal after application of formulation

**Image 5:** Necropsy of sacrificed rat

**Image 6:** Targeted organs taken for histopathological examinations

**Experimental Design**

**Range finding study**

The range finding study of *Lawsonia inermis* ethanolic leaves extract gel formulation was initiated in the 2 Wistar rats. The dose of 2000 mg/kg body weight was selected and applied on the prepared site on skin. During 24 hrs. of exposure period behavioural patterns were studied.

Reconfirmation of the range finding study was conducted on two more Wistar rats with the same dose rate of 2000 mg/kg body weight.

**Main study**

Based on the outcome in range finding study, the main study was conducted on two more Wistar rats to confirm the classification outcome, following the procedure outlined in OECD guideline 402.

All the gel applied rats were kept isolated one rat in each cage till exposure period of 24 hrs. to avoid ingestion of patch by other rats after removal of patch the rats were allowed to be with the other rats in the cage. The duration of this study was 14 days. Each rat in the main study group underwent application of the extract once at day 1 and sacrificed at day 14 of the experimental period.

**Necropsy:** All the gel applied rats were humanely scarified under anaesthesia (Image No. 5).

**Gross pathology:** complete gross examination was conducted to detect any gross changes, especially skin necrosis.

**Histopathology:** Skin, Liver, Kidney, Lung, Spleen & Heart samples were collected and fixed in 10% formalin and samples taken were dispatched to the histopathology labs for slide processing. The prepare histological slides were observed and read under the guidance of veterinary pathologist in detail using a light microscope at 10x, 40x and 100x magnifications (Image No. 6).

**Statistical analysis**

The data obtained was statistically analysed by using Web Agri Stat Package - 2.0. The values were expressed as Mean±S. E. for different parameters.

**Result and Discussion**

**Mortality:** No mortality was observed in the *Lawsonia inermis* ethanolic leaves extract gel applied rats in both range finding and main study.

**General signs and behaviour of the rats:** No toxic signs or mortality were observed in any *Lawsonia inermis* ethanolic leaves extract gel applied animals, which survived up to 14 days after applying of the extracts once on the first day at single dose level of 2000 mg/kg body weight. Following application of extracts, animal behaviour patterns were monitored for the first 6 hours and 14 hours. The gel-treated animals were normal and did not display significant changes in their behaviour, body condition, body contour, breathing patterns, coat change, impairment in food intake and water consumption.

Draize scoring for skin reactions observed in gel treated rats for range finding and main study groups were given in the Table No. 1 & 2 respectively.

**Table 1:** Scoring pattern for Erythema & Oedema for Range finding study

| Animals | Erythema | | | Oedema | | |
|---|---|---|---|---|---|---|
| | 24 hr. | 48 hr. | 72 hr. | 24 hr. | 48 hr. | 72 hr. |
| Rat 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| Rat 2 | 0 | 0 | 0 | 0 | 0 | 0 |

**Table 2:** Scoring pattern for Erythema & Oedema for Main study

| Animals | Erythema | | | Oedema | | |
|---|---|---|---|---|---|---|
| | 24 hr. | 48 hr. | 72 hr. | 24 hr. | 48 hr. | 72 hr. |
| Rat 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| Rat 2 | 0 | 0 | 0 | 0 | 0 | 0 |

The above scoring pattern showed that prepared gel neither produced erythema nor oedema to the skin of treated rats.

**Weekly Body weight (gm):** Mean ± S.E. values of Weekly body weight of the control and main study group is shown in Table No. 3 & Fig No. 1, respectively. All rats from both control and main study group showed normal increment in the body weight, which was statistically non-significant between both the groups.

**Table 3:** Mean ± S.E. values of body weight (in gm) of Control & Main study groups

| Control Group | | | Main Study | | | F cal |
|---|---|---|---|---|---|---|
| $0^{th}$ Day | $7^{th}$ Day | $14^{th}$ Day | $0^{th}$ Day | $7^{th}$ Day | $14^{th}$ Day | |
| $215^{de}$±5 | $232.5^{bc}$±2.5 | $245^{a}$±0 | $205^{e}$±5 | $222.5^{cd}$±2.5 | $237.5^{ab}$±2.5 | 19.364 |
| COV = 2.116 | | | | | | |
| Treatments found significant at 1% and 5% level<br>CD (0.01) = 17.746 & CD (0.05) = 11.714 | | | | | | |

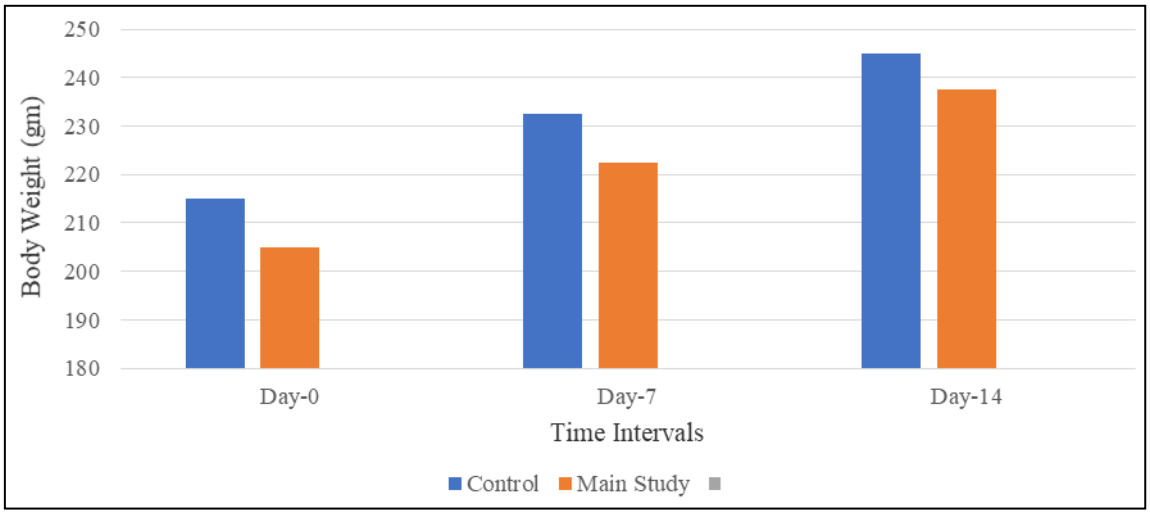


**Fig 1:** Mean values of body weight (gm)

**Weekly Feed consumption (gm):** Increased body weight of both control and main study group rats were statistically non-significant between the groups. Weekly Mean ± S.E. values of Feed consumption (in gm) in Control and Main study group Table No. 4 & Fig No. 2.

**Table 4:** Weekly Mean ± S.E. values of Feed consumption (in gm) in Control and Main study group

| Control | | Main Study | | F cal |
|---|---|---|---|---|
| $1^{st}$ week | $2^{nd}$ week | $1^{st}$ week | $2^{nd}$ week | |
| 92±2 | 96.5±0.5 | 91.5±0.5 | 95.5±1.5 | 3.691 |
| COV = 1.957 | | | | |
| Treatments found to be non-significant | | | | |

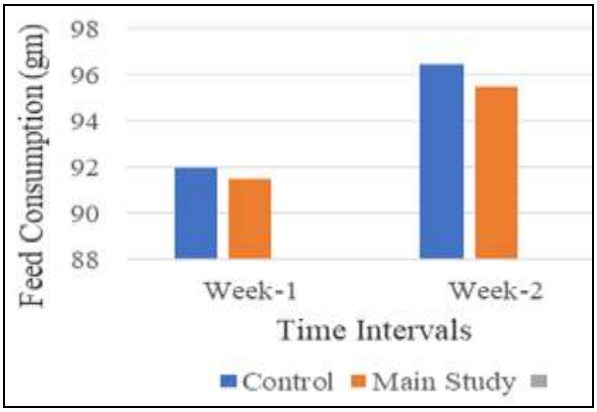


**Fig 2:** Mean values of weekly feed consumption (gm)

**Histopathological examinations (Image No. 7-12)**

The acute dermal toxicity of *Lawsonia inermis* ethanolic leaves extract gel formulation was evaluated through conduct of histopathological alterations in Skin, Liver, Kidney, Lung, Spleen & Heart. On exposure of targeted organs for histoarchitectural studies, none of the targeted organ revealed any toxicity related histopathological alterations. However, few sections of kidneys and lungs showed minimal degeneration and congestion respectively as incidental changes (Image No. 7-12).

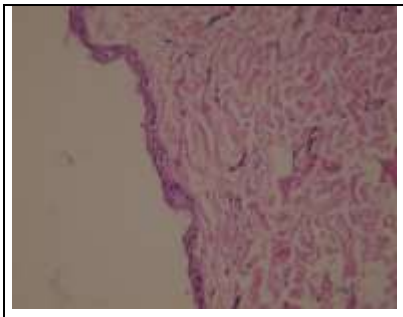

**Image 7:** Section of skin showing normal histological features (H & E Stain, ×400 magnification)

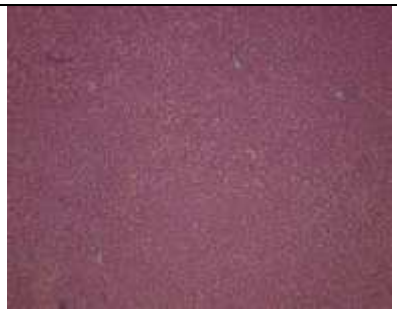

**Image 8:** Section of liver showing normal histoarchitecture (H & E Stain, ×100 magnification)

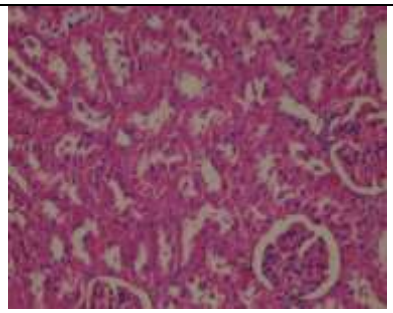

**Image 9:** Section of kidney showing minimal focal degenerative changes (H & E Stain, ×400 magnification)

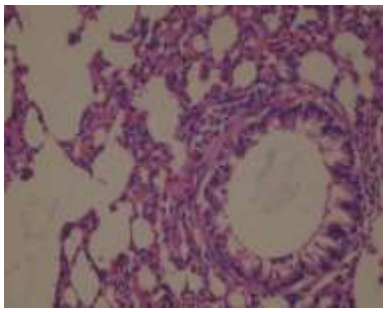

**Image 10:** Section of lung with normal histoarchitecture (H & E Stain, ×400 magnification)

**Image 11:** Microphotograph of spleen showing normal histoarchitecture (H & E Stain, ×100 magnification)

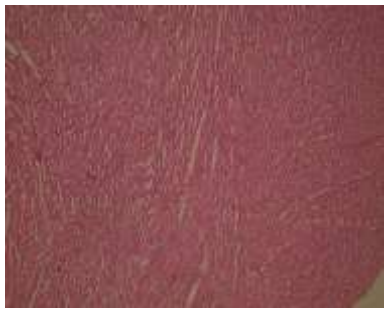

**Image 12:** Microphotograph of heart within histological limit (H & E Stain, ×100 magnification)

**Plate 1-6:** Histopathological sections of targeted organs such as Skin, Liver, Kidney, Lung, Spleen & Heart

Similar results of these parameters were also observed by the Kaur, M. *et al*., (2014) and Zahi, A. *et al*., (2016).

## Conclusion

From the results of this toxicity study, it is concluded that, the hydrogel prepared from the *Lawsonia inermis* ethanolic leaves extract which has potential medicinal properties is safe to apply over skin.

## Conflict of Interest

Not available


## Financial Support

Not available


## References


1. Aejazuddin Q. Herbal Medicine: A Comprehensive Review. Int. J Pharm. Res. 2016;8:01-05.
2. Chaudhary G, Goyal S, Poonia P. *Lawsonia inermis* Linnaeus: a phytopharmacological review. Int J Pharm Sci Drug Res. 2010;2(2):91-98.
3. Dixit K, Kohina, Mohapatra D, Senapati P, Panda R, Sahu A. Formulation Development and Evaluation of *Lawsonia inermis* Extract Loaded Hydrogel for Wound Dressing Application. Indian J Pharm Sci. 2022;84. doi:10.36468/pharmaceutical-sciences.980.
4. Ernst E. Adverse effects of herbal drugs in dermatology. Br J Dermatol. 2000;143(5):923–929. https://doi.org/10.1046/j.1365-2133.2000.03822.x
5. Kaur M, Dangi CB, Singhai A, Singh M, Kosta S, Singh H, *et al*. Toxicity profile of ethanolic extract of *Lawsonia inermis* leaves in albino Wistar rats. WJPPS. 2014;3:835-848.
6. Khan BA, Khan A, Khan MK, Braga VA. Preparation and properties of High sheared Poly (Vinyl Alcohol)/Chitosan blended Hydrogels films with *Lawsonia inermis* extract as wound dressing. J Drug Deliv Sci Technol. 2021;61:102227.
7. Moutawalli A, Benkhouili F, Doukkali A, Benzeid H, Zahidi A. The biological and pharmacologic actions of *Lawsonia inermis* L. Phytomed Plus. 2023;3:100468. doi:10.1016/j.phyplu.2023.100468.
8. Zahi AK, Hamzah H, Shaari MR, Widodo RT, Johnny L, Noordin MM, Sithambaram S. Evaluation of acute and sub-acute dermal toxicity studies of ethanolic leaf extract of *Lawsonia inermis* in rats. J Adv. Biotechnol. 2016;6(1):835-847.



**How to Cite This Article**

Shinde AG, Ranvir GD, Patil DP, Gangane GR, Upase AP. Acute dermal toxicity of *Lawsonia inermis* ethanolic leaves extract gel formulation in wistar rats. International Journal of Veterinary Sciences and Animal Husbandry. 2024; 9(3): xx-xx.