International Journal of Current Science Research and Review
ISSN: 2581-8341
Volume 08 Issue 06 June 2025
DOI: 10.47191/ijcsrr/V8-i6-50, Impact Factor: 8.048
IJCSRR @ 2025
www.ijcsrr.org

Efficacy study of *Curcuma longa* l. and *Terminalia chebula* retz. against dncb-induced atopic dermatitis in mice

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ABSTRACT: Atopic dermatitis (AD) presents formidable challenges in Veterinary Dermatology due to its persistent nature and diverse clinical manifestations, including itching, redness, swelling, and skin lesions. Despite advancements in therapeutic interventions like topical corticosteroids and immunomodulatory agents, a significant portion of AD patients continue to struggle with persistent symptoms and frequent flare-ups.

This study aimed to explore the effectiveness of *Curcuma longa* L. (CL) and *Terminalia chebula* Retz. (TC) alone and in combination in alleviating AD-like symptoms. A total of 54 Swiss albino mice (6 normal; 48 DNCB-treated) were divided into nine groups. Group A served as the normal control, while Group B had AD-like symptoms induced through repeated application of 2,4-dinitrochlorobenzene (DNCB) on the ear and dorsal skin (positive control). From the 14th day onwards, Group C received standard Dexamethasone treatment at 3mg, Group D was administered *Curcuma longa* L. extract at 100mg, and Group E received *Terminalia chebula* Retz. Extract at 100mg. Groups F through H received varying concentrations (50mg, 100mg, and 200mg) of both extracts in combination, while the vehicle Carbopol was topically applied in Group I for two weeks to assess their anti-pruritic effects.

Results indicated that both *Curcuma Longa* and *Terminalia Chebula* extracts, either individually or combined, alleviated DNCB-induced AD-like symptoms, demonstrated by improvements in body weight gain, reductions in skin lesions, dermatitis scores, ear thickness, and total leukocyte count values. Histopathological analysis revealed that the combination of *Curcuma Longa* and *Terminalia Chebula* extracts at 100mg and 200mg doses reduced inflammatory cell infiltration into DNCB-induced skin lesions. Moreover, this combination also decreased the DNCB-induced elevation of Th2 cytokines interleukin (IL)-4 and Th1 cytokines IFN-γ. These findings suggest that combining *Curcuma Longa* and *Terminalia Chebula* extracts holds promise as a therapeutic approach for managing atopic dermatitis.

KEY WORDS: Atopic dermatitis, *Terminalia chebula, curcuma longa*, mice, DNCB, curcumin, haritaki

INTRODUCTION: Dermatological issues frequently challenge small animal practitioners and include conditions such as otitis, pyoderma, anal sac problems, flea allergies, and atopic dermatitis (AD). AD, affecting about 10% of dogs, is the second most common cause of itching and allergic skin diseases (Lund et al., 1999; Hillier and Griffin, 2001; Scott et al., 2001). This chronic inflammatory skin condition is marked by persistent itching and various skin lesions. The most affected areas typically include the groin, abdomen, neck, and muzzle (Brar et al., 2017), though lesion distribution may vary by breed (Nuttall et al., 2014). Treatment options for AD consist of emollients, corticosteroids, antimicrobials, and immunomodulating agents (Leung et al., 2007). While corticosteroids are effective, they may also lead to side effects like metabolism issues and increased infection risk (Hon et al., 2011). Many people rely on traditional medicine and herbal remedies, especially in developing regions (World Health Organization, 2022; Evans et al., 2002). Two notable plants are Terminalia chebula and Curcuma longa. Terminalia chebula has shown anti-inflammatory effects and can help alleviate symptoms of atopic dermatitis (Kim et al., 2022). Curcuma longa contains curcumin, which modulates inflammatory responses (Agrawal et al., 2017). Most studies have focused on single-plant treatments for AD, while research on the effects of combined plant extracts remains limited. This study aims to evaluate the efficacy of a combination of Curcuma longa and Terminalia chebula for managing atopic dermatitis.

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MATERIAL AND METHODS

Collection and Processing: Curcuma longa rhizomes and Terminalia chebula fruits were purchased from the local market in Mumbai. Following steaming, the rhizomes of Curcuma longa were finely sliced and dried in a hot air oven at 50°C for approximately 6 hours until fully dried (Surojanametakul et al., 2010). Meanwhile, Terminalia chebula fruits were cut and left to room dry Rangsriwong et al., (2009). The dried Curcuma longa rhizomes and Terminalia chebula fruits were meticulously ground into a fine powder utilizing a high-speed blender. To ensure uniformity in particle size, the ground powder was then passed through a sieve with a mesh size of 80, resulting in particles of approximately 0.18 mm in size (Sahne et al., 2016). The dry, ground Curcuma longa and Terminalia chebula powder were securely packed in separate plastic bags, sealed to prevent moisture ingress, and subsequently stored in a refrigerator at 5°C until further use to maintain its quality.

Extraction of *Curcuma longa rhizomes and Terminalia chebula fruits*: Crude extracts of Curcuma longa rhizomes and Terminalia chebula fruits were prepared with slight modifications in the methods of extraction (Khusro et al., 2013; Mukhtar and Ghori, 2012). Initially, 100 grams of ground turmeric powder were accurately weighed and placed into a thimble, while an equivalent amount of Terminalia chebula was processed in a separate thimble. Acetone served as the extraction solvent for both materials. The apparatus was meticulously assembled, witha water-cooled condenser connected to the extraction flask and the siphon tube. The extraction process was conducted at 60°C for 8 hours, with heat applied to the extraction flask to induce solvent boiling. The resulting vapor condensed in the condenser and dripped back into the extraction flask, facilitating continuous extraction of soluble compounds. This cyclic process persisted as long as heat was applied, ensuring an iterative and efficient extraction process for both Curcuma longa and Terminalia chebula extracts. (Sahne et al., 2016, Surojanametakul et al., 2010, Negi et al., 1999, Singh et al. 2014).

Preparation of Topical Formulation of *Curcuma longa L and Terminalia chebula* Retz.For topical preparation of Curcuma longa, 200 mg of Carbopol was dispersed in 2.5 mL of distilled water, while 200 mg of Curcuma longa was dissolved in 2 mL of ethanol. Subsequently, the ethanolic dispersion of Curcuma longa and an appropriate volume of ethanol were incorporated into the aqueous dispersion of Carbopol. A mixture comprising 1.25 mL of methanol and 1 mL of ethanol was prepared and added to the Curcuma longa and Carbopol dispersion, followed by gradual stirring to allow Carbopol to hydrate for 2 hours. Triethanolamine was employed to neutralize the Carbopol solution and facilitate gelation, after which the pH was adjusted to 6.8. for preparation of the topical formulation of Terminalia chebula Retz., the same procedure was followed as for the Curcuma longa topical formulation.(Kim et al. 2016).

Experimental animals: Fifty-four (54) Adult Female Swiss Albino Mice (18-22g) were obtained from the Central Laboratory Animal House (CLAHF), Department of Veterinary Pharmacology and Toxicology at Mumbai Veterinary College (MVC), Mumbai, India. The procurement adhered to ethical standards, as the laboratory facility is approved by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CCSEA) and the Institutional Animal Ethics Committee (IAEC). The experimental protocol was approved by the Institutional Animal Ethical Committee (IAEC) with the reference MVC/IAEC/51/09//2023.

At the end of the study on the 30 th day, all animals were sacrificed, and ear and skin were collected for histopathological examination, score, and Cytokine estimation. Blood was collected on day 0 and day 30, from the retro-orbital plexus using a capillary tube, following the method outlined by Sorg and Buckner (1964), preceding the animals' sacrifice. The collected blood was preserved in K3-EDTA anticoagulant vials for subsequent hematological analysis, with processing completed within 8 hours of collection. Additionally, a thin blood smear was prepared to facilitate the differential leukocyte count.

Induction of Atopic Dermatitis by DNCB: The 2,4-dinitrochlorobenzene (DNCB) solutions, prepared by dissolving the compound in a 1:3 mixture of acetone/olive oil to achieve concentrations of 1% (w/v) and 0.5% (w/v), were administered for the induction of atopic dermatitis. The sensitization phase involved cleaning and shaving the dorsal skin using a depilatory cream. For three consecutive days, mice, except Group A, received daily administration of a 200 µL solution containing DNCB 0.5% (3:1, v/v) on a designated 1 cm×1 cm area. Subsequently, on days 14, 17, 20, 23, 26, and 29, re-exposure was administered, involving the application of 20 µL DNCB 1% (3:1, v/v) on both ears and 100 µL DNCB 1% on the dorsal skin, excluding Group A from this re-exposure regimen (Suryawatiet al., 2022).

Statistical analysis: The data generated during the experiment were statistically analyzed by using a One-way analysis of variance (ANOVA). The comparisons between the groups and between the days were tested by the Tukey test. Values are represented as

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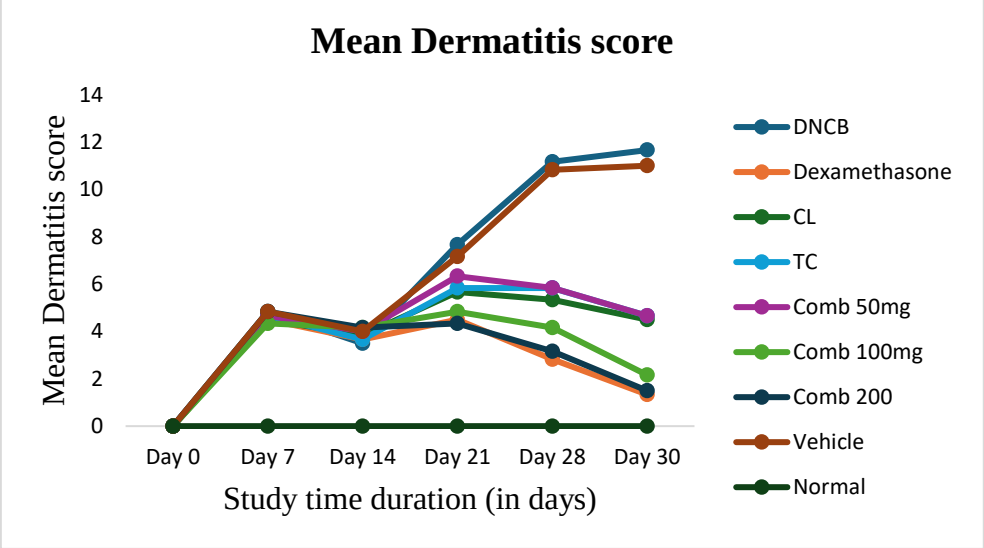
mean, standard error mean and (P < 0.05) considered as significant. Mean values and standard error of mean were calculated and all the values were expressed as mean ± SEM (Graph Pad Prism 5 Software, 2007).

RESULT

Extractability Percentage: The 50gm powder leaves were subjected to extraction in Soxhlet extraction apparatus for *Curcuma Longa* Linn & *Terminalia chebula* with 1.5 lit ethanol separately. After extraction *Curcuma Longa* Linn & *Terminalia chebula* with ethanol 6.8gm & 5.2gm extracts were obtained and percent extractability was found to be 13.6 & 10.2 percent respectively.

Effect of *Curcuma longa* and *Terminalia chebula* against DNCB-induced atopic dermatitis on body weight: The body weights of all mice were recorded every three days using an electronic balance after treatment with an acetone–olive oil suspension with or without DNCB. The scratching frequency was measured after all treatments. First, the mice were allowed to acclimate within the cage for 1 h, and then the scratching frequency was measured and recorded for 10 min. Three researchers were randomly assigned to groups and measured it three times. To distinguish it from grooming, we counted only the scratching behaviour of the right ear

Effect of *Curcuma longa L* and *Terminalia chebula* extract alone & in combination on Dermatitis score in DNCB-induced atopic dermatitis in different groups



Group A- Normal control, , Group B- DNCB alone,, Group C- DNCB + Dexamethasone,
Group D- DNCB + *Curcuma longa* (100mg),, Group E- DNCB +*Terminalia chebula* (100mg),
Group F- DNCB + CL + TC (50mg),, Group G- DNCB + CL + TC (100mg), , Group H- DNCB + CL + TC (200mg), Group I- DNCB + Carbopol

The mean dermatitis scores for the different mouse groups were calculated and presented in Table 10, with changes illustrated in Figure 5. On Day 0, all groups (A to I) had a mean score of 0, indicating no dermatitis symptoms, and statistical analysis showed no significant differences ($P \geq 0.05$). By Day 7, scores were as follows: A: 0, B: 4.83 ± 0.307, C: 4.5 ± 0.223, D: 4.5 ± 0.562, E: 4.66 ± 0.494, F: 4.67 ± 0.210, G: 4.33 ± 0.494, H: 4.83 ± 0.307, I: 4.83 ± 0.307, with all groups except A showing significant increases ($P \leq 0.001$). On Day 21, the scores were A: 0, B: 7.66 ± 0.558, C: 4.5 ± 0.341, D: 5.66 ± 0.210, E: 5.83 ± 0.401, F: 6.33 ± 0.210, G: 4.83 ± 0.542, H: 4.33 ± 0.494, I: 7.16 ± 0.600, with significant increases in groups B and I ($P \leq 0.001$). On Day 30, scores were A: 0, B: 11.66 ± 0.333, C: 1.33 ± 0.494, D: 4.5 ± 0.223, E: 4.66 ± 0.614, F: 4.66 ± 0.421, G: 2.16 ± 0.477, H: 1.5 ± 0.428, I: 11 ± 0.365. Significant increases were noted for groups B and I, while groups C and H showed reductions, followed by groups D and E.

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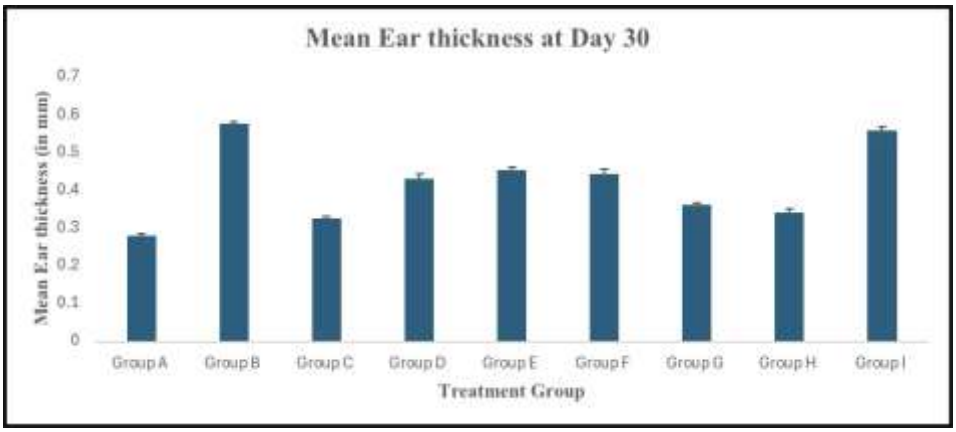
Table 3. The effect of *Curcuma longa* L. and *Terminalia chebula* Retz. Extract alone & in combination with ear thickness in DNCB-induced atopic dermatitis in different groups

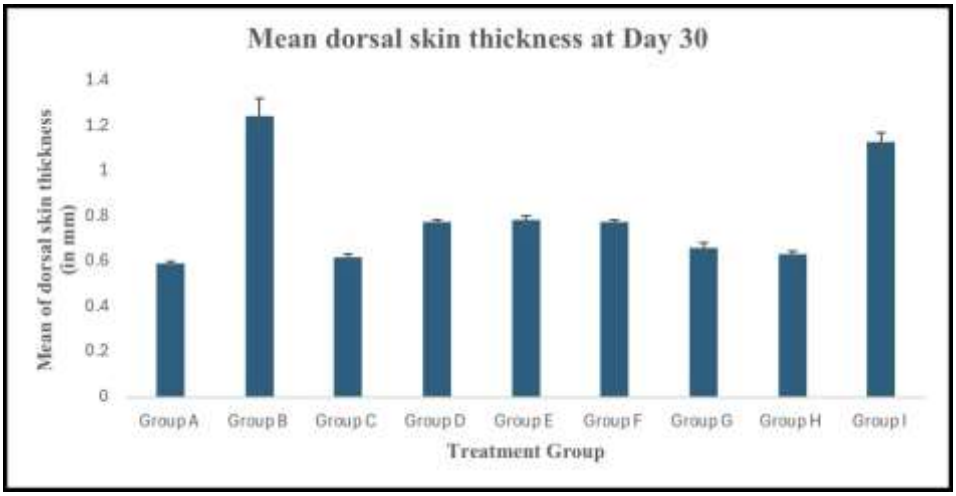
Body



Group A- Normal control, Group B- DNCB alone, Group C- DNCB + Dexamethasone,
Group D- DNCB + *Curcuma longa* (100mg), Group E- DNCB +*Terminalia chebula* (100mg),
Group F- DNCB + CL + TC (50mg), Group G- DNCB + CL + TC (100mg), Group H- DNCB + CL + TC (200mg), Group I- DNCB + Carbopol

The average ear thickness of mice recorded in different groups on Days 14th, 22nd, and 30th are summarized in Table 6 and Figure 3. The pattern of change in ear thickness of mice having atopic dermatitis of different treatment groups and the normal group has also been recorded to assess the efficacy of *Curcuma longa* L. and *Terminalia chebula* Retz. and their combination in comparison with Dexamethasone. Administration of *Curcuma longa* L. and *Terminalia chebula* Retz. In combination showed a significant effect in comparison to DNCB control and Carbopol group. A significant decrease in ear thickness was observed in all three combination groups. Presence of polyphenolic compound Curcumin in *Curcuma longa* L. and chebulic acid, gallic acid, corilagin, chebulanin, choragic acid, ellagic acid, and chebulinic acid in *Terminalia chebula* Retz. responsible for the ear's thickness due to anti-inflammatory effects.

Table No. 8: Effect of *Curcuma longa* L. and *Terminalia chebula* Retz. Extract alone & in combination on skin thickness in DNCB-induced atopic dermatitis in different groups



Group A- Normal control, Group B- DNCB alone,\ Group C- DNCB + Dexamethasone,
Group D- DNCB + *Curcuma longa* (100mg), Group E- DNCB +*Terminalia chebula* (100mg),
Group F- DNCB + CL + TC (50mg), Group G- DNCB + CL + TC (100mg), Group H- DNCB + CL + TC (200mg), Group I- DNCB + Carbopol

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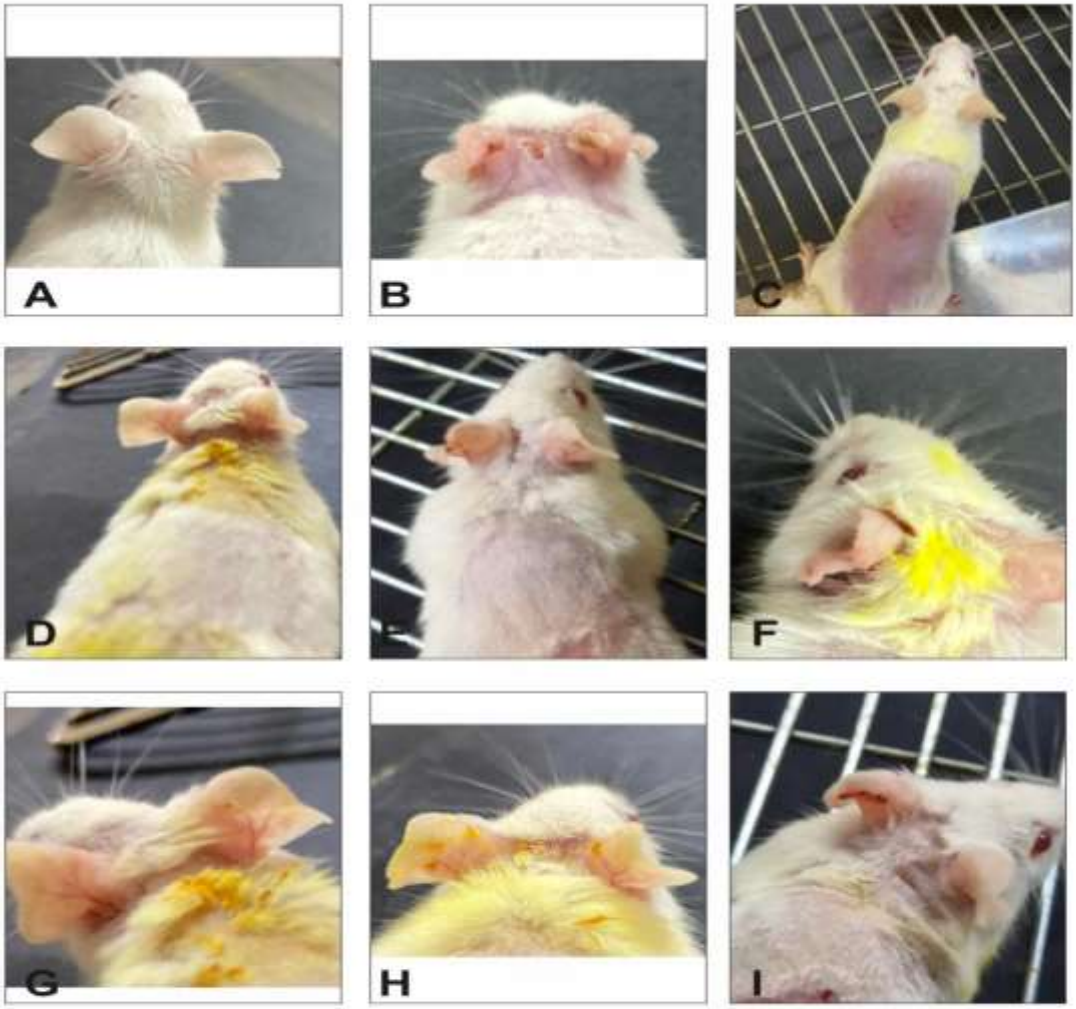

Plate 6 DNCB induced AD Ear lesion in different group at Day 30

Group A- Normal control, Group B- DNCB alone, Group C- DNCB+Dexamethasone,
Group D- DNCB+Curcuma longa (100mg), Group E- DNCB+Terminalia chebula (100mg),
Group F- DNCB+CL+TC(50mg), Group G- DNCB+CL+TC(100mg),
Group H- DNCB+CL+TC(200mg), Group I- DNCB+Carbopol
Group H- DNCB+CL+TC(200mg), Group I- DNCB+Carbopol

On day 30, the average thickness of the dorsal skin (mean ± S.E.) for groups A, B, C, D, E, F, G, H, and I were 0.595 ± 0.008, 1.248 ± 0.08, 0.62 ± 0.014, 0.777 ± 0.01, 0.788 ± 0.018, 0.777 ± 0.011, 0.665 ± 0.021, 0.635 ± 0.014, and 1.133 ± 0.0415, respectively, as shown in Table 8 and Figure 4. The combination of Curcuma longa L. and Terminalia chebula Retz. resulted in a significant decrease in ear thickness compared to the DNCB control and Carbopol group. This effect is linked to curcumin in Curcuma longa and various polyphenolic compounds in Terminalia chebula. Sharma et al. (2019) found that curcumin led to notable improvements in patients with atopic dermatitis, including reduced epidermal thickness and inflammatory cell infiltration. Jami et al. (2014) noted that the fruit of Terminalia chebula contains compounds that inhibit inflammatory pathways, decreasing skin thickness. Additionally, Cronin (2003) stated that Curcuma longa reduces swelling by inhibiting inflammatory prostaglandins.

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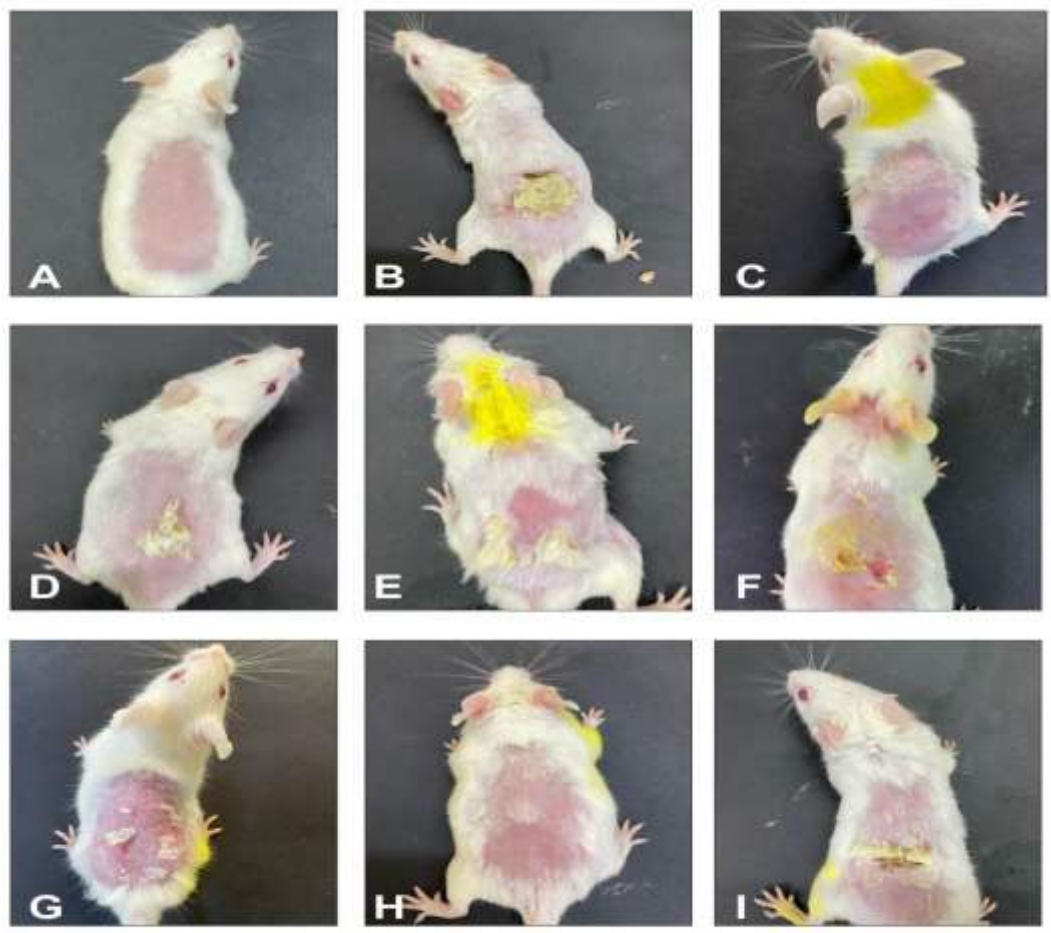

Plate 5 DNCB induced AD Skin lesion in different group at Day 30

Group A- Normal control, Group B- DNCB alone, Group C- DNCB+Dexamethasone,
Group D- DNCB+Curcuma longa (100mg), Group E- DNCB+Terminalia chebula (100mg),
Group F- DNCB+CL+TC(50mg), Group G- DNCB+CL+TC(100mg),
Group H- DNCB+CL+TC(200mg), Group I- DNCB+Carbopol
Group H- DNCB+CL+TC(200mg), Group I- DNCB+Carbopol

Effect of *Curcuma longa* L and *Terminalia chebula* Retz. extract alone & in combination on IL-4 in DNCB-induced atopic dermatitis in different groups On day 30, the mean IL-4 concentration values (mean ± S.E.) for groups A through I were as follows: 18.365 ± 1.47 (A), 50.175 ± 1.60 (B), 23.428 ± 1.40 (C), 34.508 ± 2.50 (D), 38.015 ± 2.35 (E), 37.235 ± 2.15 (F), 26.908 ± 1.27 (G), 26.107 ± 1.47 (H), and 48.782 ± 1.24 (I), as shown in Table 14 and Figure 8. Groups B, D, E, F, G, H, and I had significantly higher IL-4 values compared to groups A and C ($P \leq 0.001$). Groups B and I also had significantly higher levels than groups D, E, F, G, and H. There were no significant differences in IL-4 concentrations among groups C, G, and H, or among groups D, E, and F. The DNCB-induced atopic dermatitis group showed significantly higher IL-4 values than the healthy control

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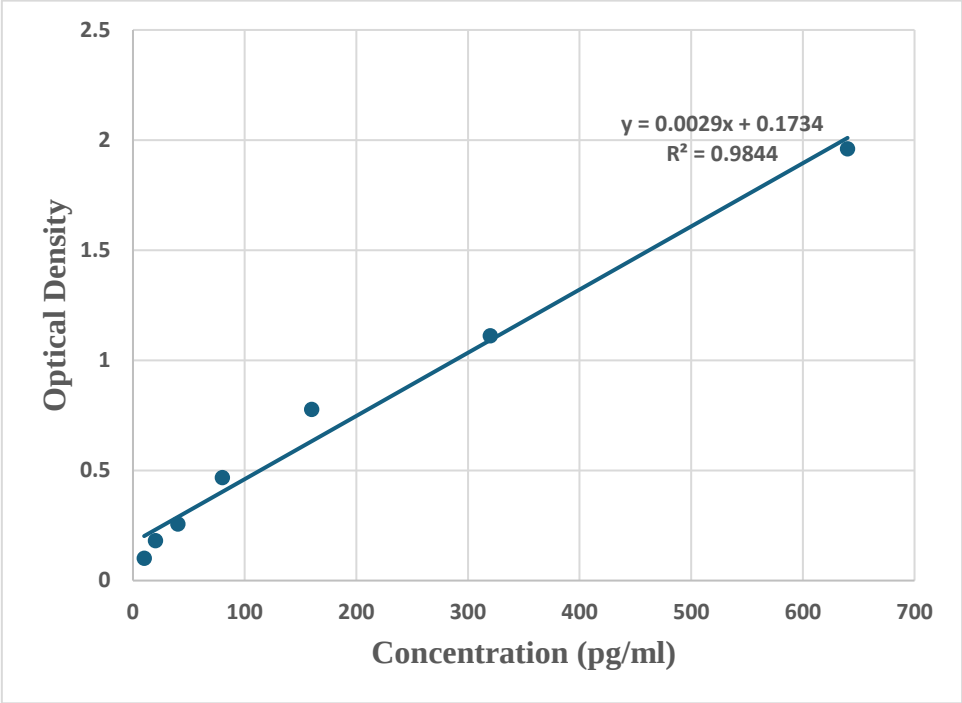
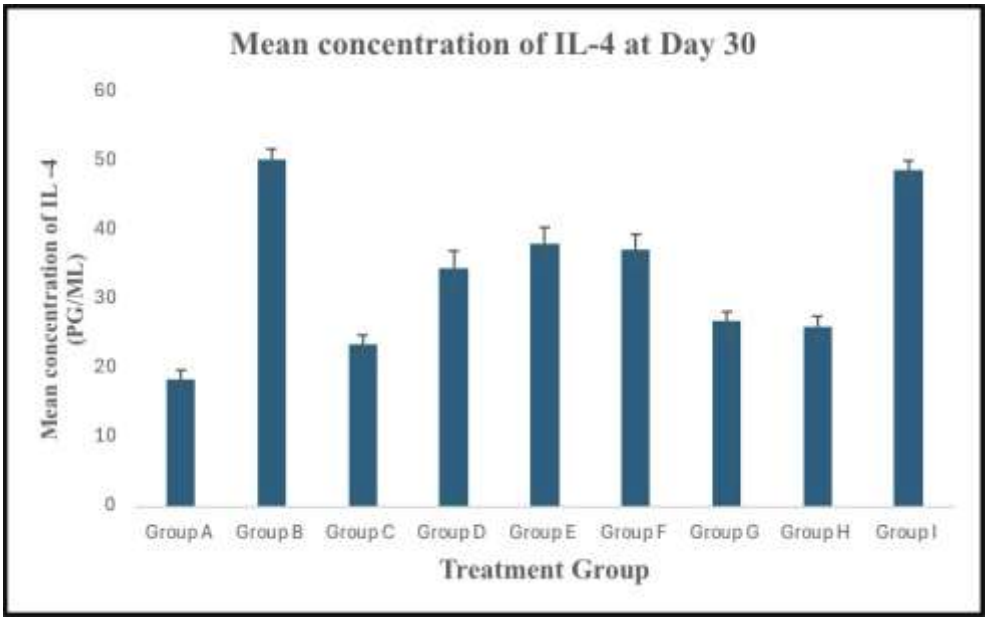
group. Treatment groups exhibited a significant reduction in IL-4 levels compared to the DNCB-induced group.





Group A- Normal control, , Group B- DNCB alone,, Group C- DNCB + Dexamethasone, Group D- DNCB + *Curcuma longa* (100mg), Group E- DNCB +*Terminalia chebula* (100mg), Group F- DNCB + CL + TC (50mg), Group G- DNCB + CL + TC (100mg), Group H- DNCB + CL + TC (200mg), Group I- DNCB + Carbopol

Effect of *Curcuma longa L* and *Terminalia chebula* extract alone & in combination on IFN-γ concentration in DNCB-induced atopic dermatitis in different groups

The mean concentration of IFN-γ (mean ± S.E.) on day 30 varied significantly across groups: Group A had 69.75 ± 1.52, while Group B reached an impressive 308.48 ± 2.53. Other values included Group C - 81.76 ± 1.95, Group D - 154.25 ± 1.88, Group E -

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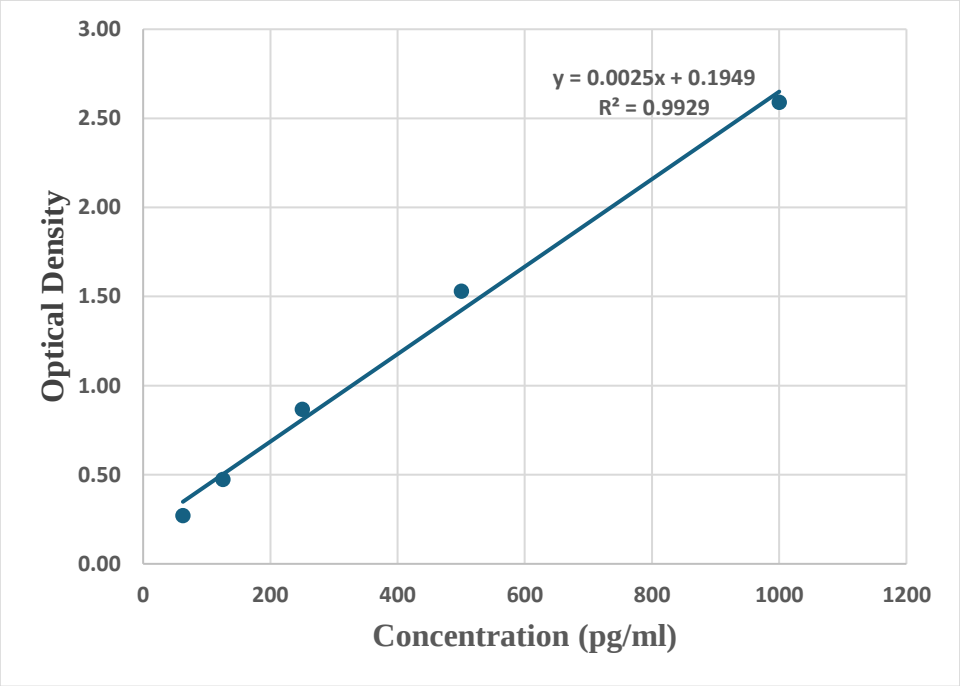
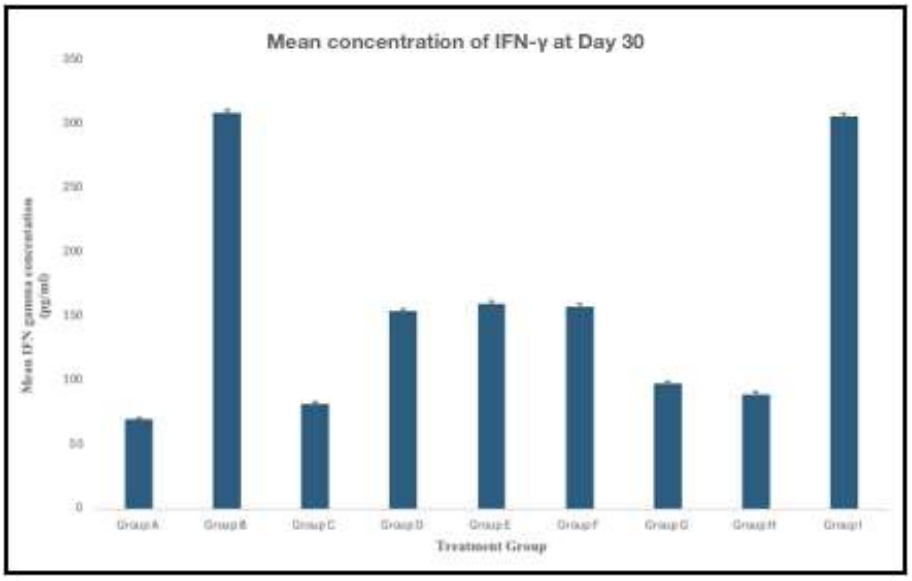
160.17 ± 2.11, Group F - 157.40 ± 2.38, Group G - 97.41 ± 2.06, Group H - 89.05 ± 2.41, and Group I - 305.85 ± 2.36, as shown in Table 16 and Figure 10. Groups B, C, D, E, F, G, H, and I all had significantly higher IFN-γ levels than Group A ($P \leq 0.001$), with Group B having the highest levels compared to the others. Notably, Groups C, G, and H showed lower IFN-γ levels than Group B (DNCB), while Groups D, E, and F showed no significant differences ($P \geq 0.05$). This study clearly demonstrates a significant increase in mean IFN-γ levels in the DNCB-induced atopic dermatitis group compared to the healthy control group, emphasizing IFN-γ's important role in atopic dermatitis.





Group A- Normal control, , Group B- DNCB alone,, Group C- DNCB + Dexamethasone,
Group D- DNCB + *Curcuma longa* (100mg),, Group E- DNCB +*Terminalia chebula* (100mg),
Group F- DNCB + CL + TC (50mg), Group G- DNCB + CL + TC (100mg), Group H- DNCB + CL + TC (200mg), Group I- DNCB + Carbopol

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Histopathology: The study concluded with the sacrifice of the animals on the 30th day. Their ears and dorsal skin were excised and fixed in 10% formalin for 24 hours, then dehydrated in an ascending series of alcohol and embedded in paraffin wax. Sections of 5 microns were cut and stained with hematoxylin and eosin (H&E) for microscopic examination. In Group A (normal control), the dermal and epidermal layers showed no pathological changes (see Plates 5 and 6). In contrast, Group B (positive control for atopic dermatitis treated with DNCB, see Plates 7-10) and Group I (Carbopol vehicle, see Plates 23 and 24) displayed significant skin alterations, including epidermal thickening, hyperkeratosis, and inflammatory cell infiltration, along with moderate spongiosis, edema, congestion, and hemorrhage. Groups C (dexamethasone at 3 mg, see Plates 11 and 12) and H (Curcuma longa and Terminalia chebula at 200 mg, see Plates 21 and 22) displayed a near-normal skin histology but still showed minimal signs of hyperkeratosis and edema. Groups D (Curcuma longa, see Plates 13 and 14), E (Terminalia chebula, see Plates 15 and 16), and F (Curcuma longa and Terminalia chebula at 50 mg, see Plates 17 and 18) exhibited moderate changes, including hyperkeratosis and mild edema in both the epidermis and dermis. Lastly, Group G (Curcuma longa and Terminalia chebula at 100 mg, see Plates 19 and 20) showed milder alterations. Zhang et al. (2009) reported similar findings, highlighting inflammatory cell infiltration and vascular congestion in the dermis.

DISCUSSION

The results of this study align with those of Sahne et al. (2016), who extracted 50 g of powdered *Curcuma longa* rhizome in a Soxhlet apparatus, yielding an extractability percentage of 5.9%. Similarly, Takuathung et al. (2023) used ethanol to extract 55 g of *Terminalia chebula* fruit at 52°C for 12 hours, achieving a yield of 4.5 g (9.1% extractability). Wang et al. (2022) found that mice with atopic dermatitis exhibited severe symptoms, including itching and edema, which improved significantly with treatment using bisdemethoxycurcumin. Kim et al. (2022) reported similar findings with *Terminalia chebula* Retz, attributing the improvements to inhibited inflammatory cytokine expression. Suryawati et al. (2022) also noted decreased ear thickness across combination treatment groups, linking this to the anti-inflammatory properties of compounds in both *Curcuma longa* and *Terminalia chebula*. Contrasting these improvements, Zhang et al. (2009) and others noted increased ear thickness and dermatitis scores in control groups. Additionally, Nam et al. (2011) found that DNFB-treated animals experienced significant ear thickness increases, which were inhibited by *Terminalia chebula* extract. Chen et al. (2004) suggested that atopic dermatitis onset is influenced by Th2 cytokines, with Kim et al. (2022) confirming reductions in key inflammatory cytokines following treatment. Lee et al. (2016) highlighted that treatment with p-hydroxycinnamic acid from *Curcuma longa* reduced cytokine levels. However, Zhang et al. (2009) and others reported increased IFN-γ levels after DNCB treatment, emphasizing the complex roles of IFN-γ in inflammation and immune responses. Overall, Lee et al. (2016), Sharma et al. (2019), and Wang et al. (2022) demonstrated that curcumin effectively inhibits atopic dermatitis induced by DNCB in female BALB/c mice, leading to significant improvements compared to the control group.

CONCLUSION

In conclusion**,** the present study evaluated the efficacy of *Curcuma longa* L. and *Terminalia chebula* Retz, against DNCB-induced atopic dermatitis. Using a BALB/c mouse model, atopic dermatitis was induced via DNCB, with positive control mice exhibiting symptoms such as decreased body weight gain, increased ear and skin thickness, elevated dermatitis scores, cytokine levels, and histopathological changes characteristic of atopic dermatitis. Treatment groups showed significant improvements across all parameters, particularly in ear and skin thickness, dermatitis scores,, and inflammatory markers upon administration of glucocorticoids and a combination of *Curcuma longa* L. and *Terminalia chebula* Retz. Histopathological analysis further supported the beneficial effects of the treatment combination. Additionally, the treatment combination led to a notable reduction in cytokine levels, specifically IL-4 and IFN-γ, indicating its potential to mitigate inflammation associated with atopic dermatitis.

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International Journal of Current Science Research and Review
ISSN: 2581-8341
Volume 08 Issue 06 June 2025
DOI: 10.47191/ijcsrr/V8-i6-50, Impact Factor: 8.048
IJCSRR @ 2025
www.ijcsrr.org

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International Journal of Current Science Research and Review
ISSN: 2581-8341
Volume 08 Issue 06 June 2025
DOI: 10.47191/ijcsrr/V8-i6-50, Impact Factor: 8.048
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Cite this Article: Joshi, P., Ghadigaonkar, S., Sole, S., Dalvi, S.H., Sawale, G.K., Thorat,V.D., Jadhav, S.S., Mote, V.(2025). Efficacy study of Curcuma longa l. and Terminalia chebula retz. against dncb-induced atopic dermatitis in mice. International Journal of Current Science Research and Review, 8(6), pp. 3139-3149. DOI: https://doi.org/10.47191/ijcsrr/V8-i6-50