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QBD Based Formulation, Development and Evaluation of *In Vivo* Anti-Psoriatic Potentials of Polyherbal Gel and Winsoria Oil on Imiquimod-Induced Psoriasis

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KEYWORDS:

Wrightia tinctoria (Roxb.) R. Br., Rubia cordiofolia L., Hemidesmus indicus (L.) R. Br., Polyherbal Gel, Winsoria oil, Imiquimod, Psoriasis.

ABSTRACT:

BACKGROUND: Wrightia tinctoria (Roxb.) R. Br., Rubia cordiofolia L., Hemidesmus indicus (L.) R. Br. are traditionally used to treat various skin-related diseases. Conventionally, these plants are used in combination with or individually for treating and managing psoriasis-related symptoms. Winsoria oil is a formulation designed by Kerala Ayurveda Pvt. Ltd. is a blend of poly-herbal ingredients used in the treatment of psoriasis.

OBJECTIVE: In the present research, an attempt was made to develop a QbD-based formulation, evaluation and in vivo analysis of the anti-psoriatic potentials of Wrightia tinctoria (Roxb.) R. Br., Rubia cordiofolia L., Hemidesmus indicus (L.) R. Br. extracts loaded poly-herbal gel and Winsoria oil in Imiquimod-induced Psoriasis.

METHODS: The polyherbal gel was prepared and optimized using the quality by design (QbD) approach (Version 13, 32 factorial design) and evaluated for its anti-psoriatic potenstials using Imiquimoid induced psoriasis model.

RESULTS: The QbD based polyherbal gel was prepared and evaluated for its color, consistency, homogeneity, appearance, greasiness, washability, pH, spreadability and viscosity. The Imiquimod-induced Wistar rat model demonstrated significant anti-psoriatic efficacy for both formulated poly-herbal gel and Winsoria oil, as evident through the PASI index grading and Histopathological study.

Treatment with formulated poly-herbal gel and Winsoria oil confirmed a reduction in the severity of the psoriasis-associated symptoms in the treatment group, as there was a notable diminution in redness, inflammation, thickness and scaling of the skin.

CONCLUSION: The Wrightia tinctoria (Roxb.) R. Br., Rubia cordiofolia L., Hemidesmus indicus (L.) R. Br. Extracts loaded poly-herbal gel and Winsoria oil formulation showed significant results in the reduction of psoriasis-associated symptoms and can help treat and manage psoriasis. The current investigation scientifically validated the anti-psoriatic potential of the formulated poly-herbal gel and Winsoria oil.

INTRODUCTION:

Psoriasis is a chronic inflammatory disorder characterized by inflammation of the skin and scaling on the surface of the skin associated with erythema and edema. Psoriasis causes red, flaky and crusty patchy of skin covered with silvery scales, although

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the condition is not infectious and most people are affected in small patches on their body.[1-3]

People all over the world suffer with psoriasis, a widespread chronic skin condition. It affects between 100–125 million people worldwide, or 2-3% of the total population. Geographically, the prevalence ranges from 0.15% to 3.68% in adults, with wealthier nations reporting greater rates. The prevalence in India is believed to be between 0.44% and 2.8% of the population.

Although, *Wrightia tinctoria (Roxb.) R. Br., Rubia cordiofolia L.,*and *Hemidesmus indicus (L.) R. Br*. are all useful in treating psoriasis on their own, they have several drawbacks, including a narrow range of action, a delayed beginning of impact, and insufficient symptom relief. By offering synergistic activity (increased anti-inflammatory and immunomodulatory effects), a mixed (polyherbal) product gets over these restrictions. greater treatment coverage (addresses several psoriasis pathways). enhanced effectiveness with less irritation, itching, and scaling. less adverse effects as a result of the herbal composition's balance. Therefore, when these extracts are combined rather than used separately, psoriasis is more effectively and comprehensively managed.[8-11]

Winsoria oil is an Ayurvedic medication produced and marketed by Kerala Ayurveda Pvt. Ltd. is a blend of Ayurvedic ingredients that is used in the treatment and management of Psoriasis and associated symptoms, with promising results.[10-11]

In the present research, an attempt was made to develop a QbD-based formulation, evaluation and *in vivo* analysis of the anti-psoriatic potentials of *Wrightia tinctoria (Roxb.) R. Br., Rubia cordiofolia L., Hemidesmus indicus (L.) R. Br.*, extracts loaded poly-herbal gel and Winsoria oil in Imiquimod-induced Psoriasis.

MATERIALS AND METHODS:

The Psoriasis-inducing agent 5% w/w Imiquimod was obtained from Glenmark Pharmaceuticals, Mumbai, India. Winsoria oil was procured as a gift sample from Kerala Ayurveda Pvt. Ltd. All other chemicals and reagents used during the present research work were of good analytical grade.

PLANT MATERIAL AND EXTRACTION:

The Coarse powder of leaves of *Wrightia tinctoria (Roxb.) R. Br.,* Stem of *Rubia cordifolia L.,* and root of *Hemidesmus indicus (L.) R. Br*. were procured from the KLE Ayurveda pharmacy, Belagavi, Karnataka, India. The collected coarse powders were subjected to the ethanolic Soxhlet extraction individually, and the extracts were stored.

PHYTOCHEMICAL ANALYSIS:

Preliminary phytochemical analysis of the extracts of the Coarse powders of leaves of *Wrightia tinctoria (Roxb.) R. Br.,* Stems of *Rubia cordifolia L.,* and roots of *Hemidesmus indicus (L.) R. Br*. were performed as per standard procedures. The phytoconstituents present in the extracts were alkaloids, glycosides, flavonoids, tannin's, terpenoids and phenolic compounds.

FORMULATION OF POLYHERBAL GEL:

Precisely weighed Carbopol 934 was gradually dispersed in distilled water while stirring at 400 rpm and 37 °C for 30 minutes, until a viscous gel formed. A preservative solution was prepared by dissolving methyl and propyl-paraben in propylene glycol, while the extracts of *Wrightia tinctoria (Roxb.) R. Br.,* (leaf) *Rubia cordifolia L.* (stem), and *Hemidesmus indicus*

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(L.) R. Br. (root) were dissolved in ethanol. Both solutions were incorporated into the Carbopol dispersion under continuous stirring to yield a uniform, homogeneous gel.[7]

OPTIMISATION OF FORMULATED HERBAL GEL USING QbD APPROACH:

Initially, three batches were prepared, F1, F2 and F3, with the varying concentrations of Carbopol 934 and Triethanolamine. All three batches were evaluated for the parameters such as Color, Consistency, Homogeneity, Appearance, Greasiness, Washability, pH, Spreadability, and Viscosity. From the results of an evaluation of all the above parameters for all three batches, F2 batch was found to be optimized, which was further considered for the Quality by Design approach.

Using the design of expert, Version 13, 3^2 factorial design keeping Carbopol 934, Triethanolamine, Viscosity, pH, and Spreadability as dependent and independent variables optimized batch of the poly-herbal gel was prepared and evaluated.

EVALUATION OF FORMULATED HERBAL GEL:

Various evaluation parameters of the QbD suggested batch were evaluated, which include color, Consistency, Homogeneity, Appearance, Greasiness, Washability, pH, and Spreadability. The skin bio-compatibility of the formulated poly-herbal gel was assessed by measuring its pH at room temperature using a digital pH meter. Viscosity was evaluated using a Brookfield viscometer. To determine spreadability, 1g of the gel was placed between two glass slides (7.5 × 2.5 cm), which were pressed together and left undisturbed for one minute.[7]

The spreadability of the formulated poly-herbal gel was calculated by the formula,

$S = M.L / T$

Where,

S = Spreadability,

M = Weight tied to upper slide,

L = Length of glass slides,

T = Time taken to separate the slides from each other.

ACUTE SKIN IRRITANCY STUDIES:

Following established procedure, the formulated poly-herbal gel and Winsoria oil were examined for acute skin irritation. Rat's dorsal side. A 500mm^2 section of the rats dorsal hair was washed and shaved. Separate animal groups received 5% w/w of the gel formulation and Winsoria oil formulation, while the fourth group served as a control. After applying the topical gel formulation for 4 and 48 hours, the animal's skin was examined for erythema, inflammatory responses, and edema.

ANTI-PSORIATIC ACTIVITY:

IMIQUIMOD-INDUCED PSORIATIC MOUSE MODEL:

All the animal treatments were conducted in accordance with the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA, Government of India), with the reference no. 221/Po/Re/S/2000/CPCSEA., and the study protocols were approved by the Institutional Animal Ethics Committee of KLE College of Pharmacy, Belagavi, Karnataka, India.

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Rats were procured of 8-11 weeks old were used in the study. In each experimental group, animals were smoothly and clean-shaven on their backs.[4-7]

Psoriasis in a Wistar rats was induced using Imiquad cream; a clean-shaven dorsal area and ear pinna were applied with 5% w/w commercially available Imiquimod cream (Glenmark Pharmaceuticals Ltd.), with a dose of 12.5mg of Imiquimod was topically applied for 7 consecutive days.[4-7]

SCORING THE SEVERITY OF SKIN INFLAMMATION:

The anti-psoriatic activity of the formulated polyherbal gel containing extracts of *Wrightia tinctoria (Roxb.) R. Br., Rubia cordifolia L., and Hemidesmus indicus (L.) R. Br.,* along with Winsoria oil, was evaluated using the Psoriasis Area and Severity Index (PASI) scoring system (categorized as Zero, Slight, Moderate, and Very Marked), in conjunction with histopathological analysis of the skin.[4-7]

STATISTICAL ANALYSIS:

There was six animals in each group, and the experimental data are shown as mean ± SEM. One-way analysis of variance (ANOVA) was performed. The statistical significance between various groups was ascertained using the Newman-Keuls test for multiple comparisons.[7]

The Graph Pad Prism program was used for all statistical analysis. P-values less than 0.05 were deemed significant.

STABILITY STUDIES:

The stability of the medicated herbal gel containing extracts of *Wrightia tinctoria (Roxb.) R. Br., Rubia cordifolia L.,* and *Hemidesmus indicus (L.) R. Br*. was evaluated under accelerated conditions as per ICH guidelines. The formulation was stored at 40 ± 2 °C and 75 ± 5% relative humidity for a period of up to three months. Samples were analyzed at predetermined intervals for physical parameters such as color, appearance, homogeneity, pH, spreadability, and consistency. Any changes in these characteristics were recorded to assess the stability and integrity of the formulation over time.

RESULTS:

OPTIMIZATION OF FORMULATED POLYHERBAL GEL:

Table 1:
QbD based formulation of herbal gel.

	FACTOR 1	**FACTOR 2**	**RESPONSE 1**	**RESPONSE 2**	**RESPONSE 3**
Run	**A. Carbopol 934 (mg)**	**B. Triethanolamine (ml)**	**Viscosity (cps)**	**pH**	**Spreadability (g.cm/sec)**
1.	2.5	0.6	1760	8.2	4.78
2.	2.70711	0.4	1830	7.34	4.87
3.	1.5	0.2	1380	6.45	7.74
4.	2.5	0.2	1630	6.18	5.12

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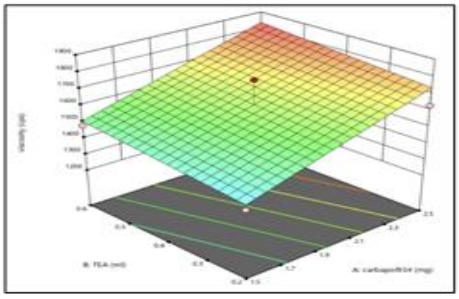
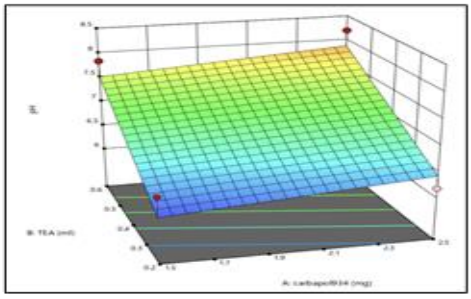
5.	2	0.117157	1678	6.02	5.36
6.	1.29289	0.4	1280	6.38	7.36
7.	2	0.4	1768	6.62	5.95
8.	2	0.682843	1794	7.72	5.68
9.	1.5	0.6	1480	7.84	7.18

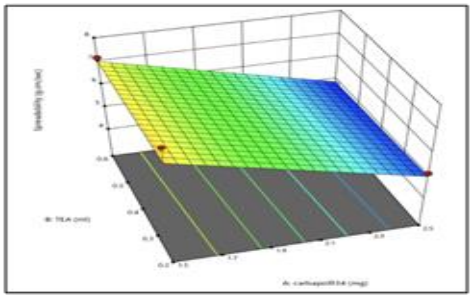
The formulated poly-herbal gel was evaluated for Color, Consistency and Appearance, and it was found to be greenish black, uniform, homogeneous and non-sticky in nature. The optimization of formulated herbal gel was performed using the design of expert, Version 13, 3^2 factorial design keeping Carbopol 934, Triethanolamine, Viscosity, pH, and Spreadability as dependent and independent variables and the results of optimization are as in Figure 1 and 2.



a. Viscosity = +1622.22 +163.48 A +49.26 B



b. pH = +6.97 +0.1810 A +0.7268 B



c. Spreadability = +6.00 -1.07 A -0.0559 B

Figure 1:
Optimization of formulated polyherbal gel using QbD approach. 3D Response surface on Viscosity, pH, and Spreadability.

- **In equation a factor A (Carbopol 934) shows positive response for Viscosity and factor B (Triethanolamine) shows slight increase in Viscosity.**
- **In equation b factor B (Triethanolamine) shows positive response for pH and factor A (Carbopol 934) shows slight increase in pH.**
- **In equation c factor A (Carbopol 934) shows positive response on Spreadability and factor B (Triethanolamine) shows negligible effect on Spreadability.**

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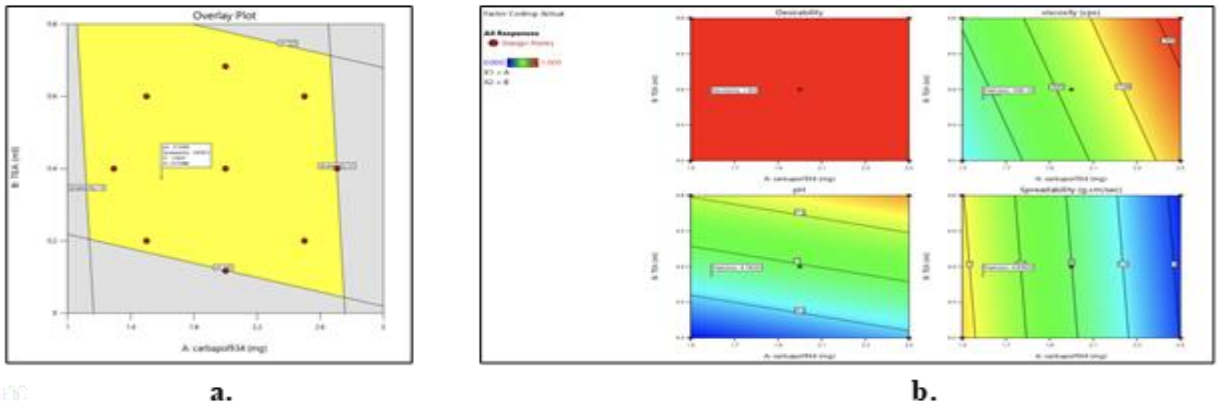

Figure 2:

Overlay plot obtained from Design of expert, Version 13, 3^2 factorial design keeping Carbopol 934, Triethanolamine, Viscosity, pH, and Spreadability as dependent and independent variables for optimization of the formulated polyherbal gel.

EVALUATION FORMULATED POLYHERBAL GEL:

The formulated poly-herbal gel, incorporating extracts of *Wrightia tinctoria* (Roxb.) R. Br., *Rubia cordifolia* L., and *Hemidesmus indicus* (L.) R. Br., exhibited a pH of 6.8, making it suitable for topical application. The gel demonstrated a Viscosity of 1530 cps and a Spreadability of 6.47g.cm/sec.

STABILITY STUDIES:

Accelerated stability testing was conducted in accordance with ICH guidelines by storing the gel at 40°C and 75% relative humidity for 90 days. Throughout the study period, no significant changes were observed in the gel's color, consistency, homogeneity, appearance, greasiness, washability, pH, or spreadability, indicating good stability.

ANTIPSORIATIC ACTIVITY:

IMIQUIMOD-INDUCED PSORIASIS MOUSE MODEL:

Rats were induced with psoriasis after seven days using Imiquimod. Each rat received a grade both before and after the test, in control, negative control and treatment control group animal's.

Figure 3 illustrates the effects of topical treatment with the formulated poly-herbal gel and Winsoria oil on PASI scores in Imiquimod-induced psoriatic mice (as detailed in Table 1). The two-way ANOVA, followed by Bonferroni's multiple comparison test, showed a significant daily increase in psoriasis symptoms—erythema, edema, and scaling—following topical Imiquimod application (**Imiquimod-induced group: $F = 2918$, **$P < 0.0001$).

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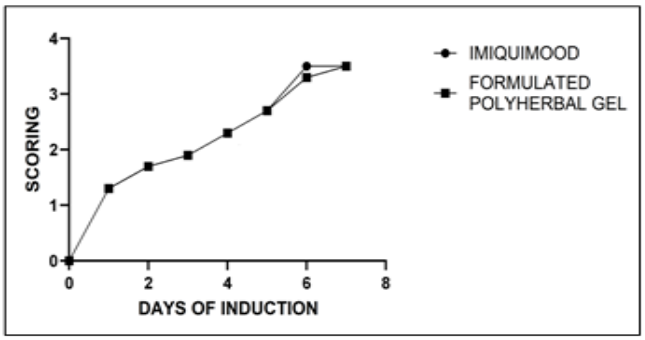
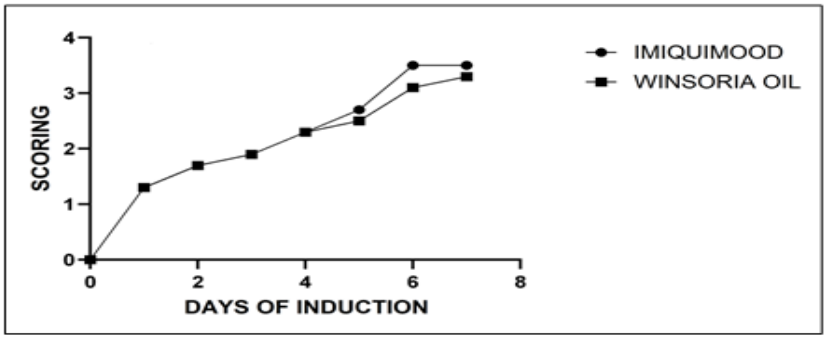
Table 2:

PASI grading of Psoriasis animal before and after treatment with formulated polyherbal gel and Winsoria oil.

Sub. No.	Initial grading after induction of Psoriasis using Imiquimod (PASI)	Final grading after induction of Psoriasis using Imiquimod (PASI)
1. Negative control	NONE	NONE
2. Treatment with standard marketed formulation	Very Marked	NONE
3. Treatment with formulated poly-herbal gel	Very Marked	NONE
4. Treatment with Winsoria oil	Very Marked	NONE



a.



b.

Figure 3:
Psoriasis Induced graph.
a. The effect of formulated polyherbal gel on PASI grading on Imiquimod induced psoriatic rat. b. The effect of Winsoria oil on PASI grading on Imiquimod induced psoriatic rat (Table 1).

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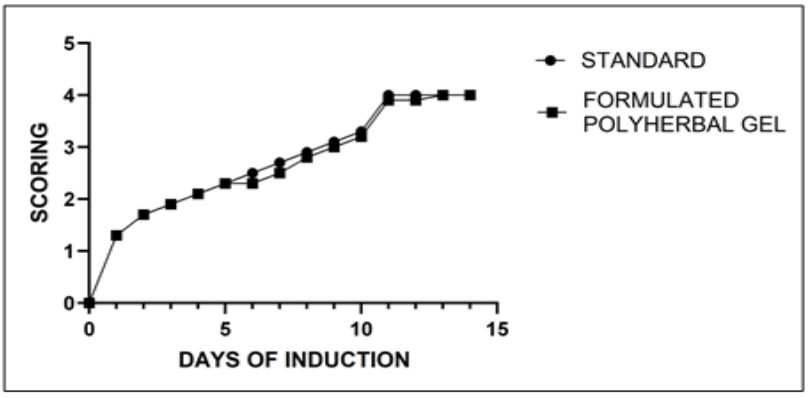
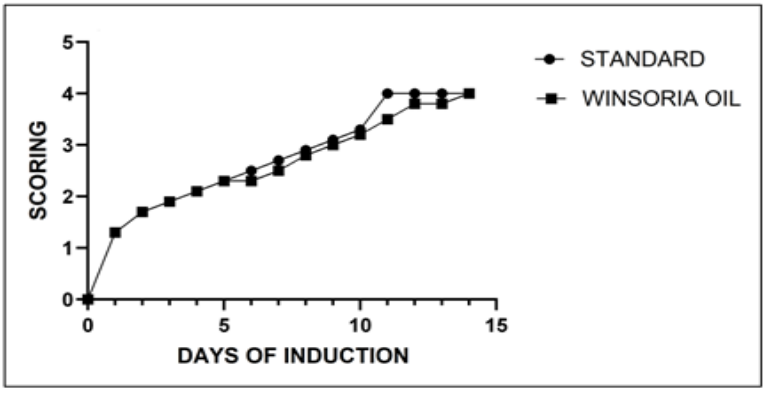
Daily treatment with a formulated poly-herbal gel containing extracts of *Wrightia tinctoria* (Roxb.) R. Br., *Rubia cordifolia* L., and *Hemidesmus indicus* (L.) R. Br., along with Winsoria oil, initiated from day 7 post-imiquimod application, led to a progressive reduction in psoriasis scores in rats. As illustrated in Figure 4, the poly-herbal gel demonstrated a greater therapeutic effect compared to the standard treatment. Two-way ANOVA revealed significant effects for the poly-herbal gel [F(1,14), **P = 0.0070], Winsoria oil [F(1,14), **P = 0.0100], and the standard treatment [F(14,14), ****P < 0.0001], indicating meaningful interactions among the treatment groups.



a.



b.

Figure 4:
Effect of extract on Psoriasis score in rats. Each point indicates psoriasis score vs. standard treated rats.
a. Psoriasis score of formulated polyherbal gel treated animal vs. standard treated animal.

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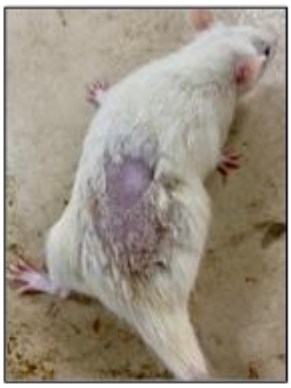
The histopathological examination of ear pinna and dorsal skin treated with formulated herbal gel and Winsoria oil showed remarkable reduction in Parakeratosis, Acanthosis, Epidermal Neutrophilic Infiltration, Supra-papillary thinning of epidermis, Reduction in dilated blood vessels in papillary dermis, Inflammatory cell infiltration, Lymphocytic infiltration and Macrophages in comparison with control group where all above Psoriasis associated parameters were elevated and observed. (Table 2).

a.



b.

c.

d.

Figure 5:
Photographic images of the study.
a. Negative control b. Psoriasis induced c. Test formulation I (Formulate polyherbal gel) d. Test formulation II (Winsoria oil).

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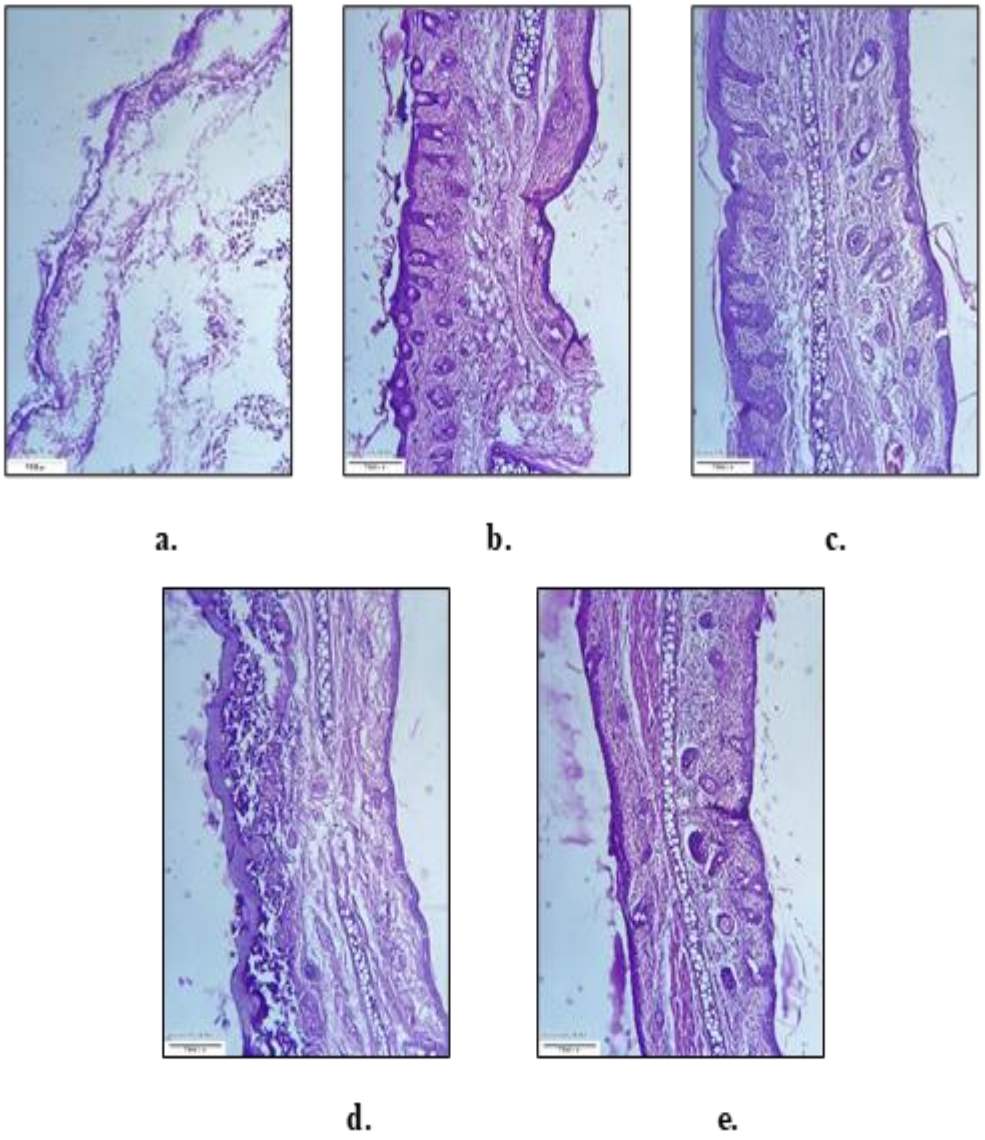

Figure 6:
Histopathology of ear pinna a. Normal b. Disease induced c. Treated with standard d. Treated with formulated polyherbal gel e. Treated with Winsoria oil.

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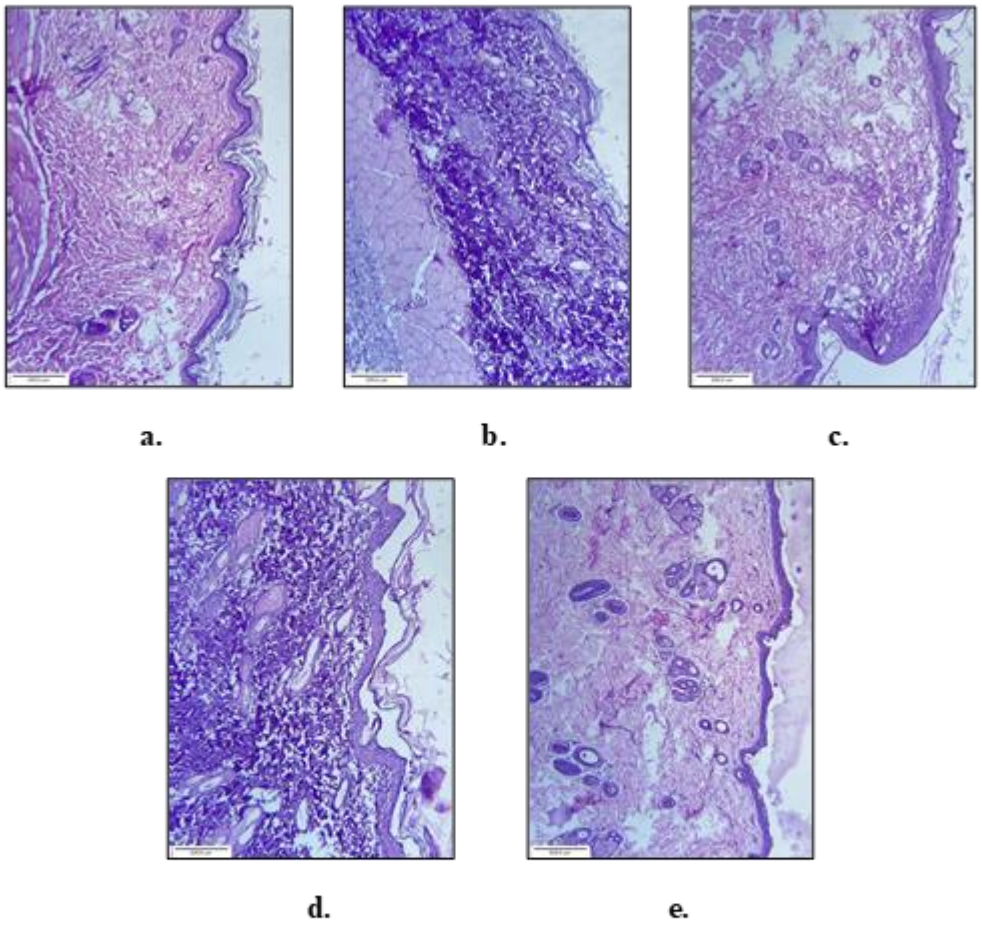

Figure 7:
Histopathology of Dorsal skin
a. Normal b. Disease induced c. Treated with standard d. Treated with formulated polyherbal gel e. Treated with Winsoria oil.

DISCUSSION:

The polyherbal gel formulated with extracts of *Wrightia tinctoria (Roxb.) R. Br., Rubia cordifolia L.,* and *Hemidesmus indicus (L.) R. Br*. was evaluated for parameters such as color, consistency, homogeneity, appearance, greasiness, washability, pH, and spreadability. All results were satisfactory, indicating good quality, uniformity, and reproducibility. Accelerated stability studies showed no significant changes, confirming the formulation's stability.

Psoriasis was induced in rats using the imiquimod model, which produced symptoms like skin thickening, scaling, and erythema after seven days. Treatment with the formulated gel and Winsoria oil resulted in significant improvement, evidenced by reduced skin thickness, decreased scaling, and lower PASI scores.

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Histopathological analysis further confirmed the therapeutic effect. The treated groups showed enhanced collagen formation, angiogenesis, keratinization, and fibroblast activity, indicating tissue repair and regeneration. Additionally, there was a marked reduction in pathological features such as parakeratosis, acanthosis, neutrophilic infiltration, suprapapillary thinning, dilated blood vessels, and inflammatory cell infiltration, including lymphocytes and macrophages.

FUNDING:

No funding was received to conduct the above research work.

CONFLICT OF INTEREST:

All the authors claimed there was no conflict of financial interest in the preparation of the article/manuscript.

ETHICAL APPROVAL:

All the animal procedures were performed with reference to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA, Government of India). The experimental protocol was reviewed and approved by the Institutional Animal Ethics Committee of KLE College of Pharmacy, Belagavi, Karnataka, India, under the approval no. 221/Po/Re/S/2000/CPCSEA. Efforts were made to minimize the number of animals used and their suffering during the experiments.

ABBREVIATIONS:

Not Applicable

SUMMARY AND CONCLUSION:

Literature reveals that allopathic medications have side effects and severe contraindications when treating Psoriasis and associated symptoms. To overcome these side effects and severe contraindications, research has been shifted to the search for herbal medicament's that have fewer side effects and are feasible for the treatment of Psoriasis and associated symptoms.

Traditionally, it is proven that *Wrightia tinctoria (Roxb.) R. Br., Rubia cordiofolia L., Hemidesmus indicus (L.) R. Br.*, has good anti-psoriatic activity and can be used in the treatment of various skin-related diseases. In the present research work, QbD-based formulation, development and evaluation of *in vivo* anti-psoriatic potentials of *Wrightia tinctoria (Roxb.) R. Br., Rubia cordiofolia L., Hemidesmus indicus (L.) R. Br.*, extract loaded poly-herbal gel, and Winsoria oil were performed.

The *Wrightia tinctoria (Roxb.) R. Br., Rubia cordiofolia L., Hemidesmus indicus (L.) R. Br.* Extracts loaded poly-herbal gel and Winsoria oil formulation showed significant results in the reduction of psoriasis-associated symptoms and can help in the treatment and management of psoriasis. The current investigation scientifically validated the anti-psoriatic potential of the formulated poly-herbal gel and Winsoria oil.

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