Research Article

Pharmacological Evaluation Of Wrightia Tinctoria And Rubia Cordifolia In The Treatment Of Psoriasis In Rodents By Imiquimod Inducing Psoriasis In Laboratory Animals

Sunitha Reddy*[1], Dr. M V Naga Bhushanam[2]

[1]Associate Professor, Dept of Pharmacognosy and Phytochemistry, Vijaya institute of pharmaceutical sciences for women, Enikaepadu, Vijayawada. 521108
[2]Professor and Principal, Hindu College of Pharmacy, Amaravathi Road, Guntur -522 002, Andhra Pradesh
Corresponding Author: Sunitha Reddy
sunita.mpharm@gmail.com

Abstract

Psoriasis is a chronic inflammatory skin disorder characterised by erythema, scaling, and epidermal hyperproliferation, often associated with immune dysregulation and increased production of inflammatory cytokines. The present study aimed to evaluate the anti-psoriatic potential of Wrightia tinctoria and Rubia cordifolia in an imiquimod (IMQ)-induced
psoriasis-like rodent model. Ointment formulations containing plant powders were prepared and applied topically to experimental animals following induction of psoriasis using 5% IMQ cream. The severity of psoriasis-like symptoms was assessed using PASI-like scoring
parameters, including erythema, scaling, and skin thickness. Histopathological evaluation and measurement of epidermal thickness were also performed. The results demonstrated that both Wrightia tinctoria and Rubia cordifolia significantly reduced PASI-like scores and epidermal thickness compared to the IMQ control group, indicating notable anti-inflammatory and anti-proliferative effects. Among the treatments, the combination formulation (1:1) exhibited the most pronounced therapeutic effect, showing results comparable to the standard drug
clobetasol. The findings suggest that these medicinal plants possess significant anti-psoriatic activity and their combined formulation may serve as a promising natural alternative for the management of psoriasis.

Keywords: Psoriasis, Herbal formulation; PASI score, Epidermal thickness, Rodent model.

Introduction

Wrightia tinctoria (Roxb.) R.Br., commonly known as the Pala Indigo plant, Sweet Indrajao, or Dyer's Oleander, is a small deciduous tree belonging to the family Apocynaceae. The plant is widely distributed across India, Southeast Asia, and Australia, thriving both in dry and moist ecosystems. In India, it is notably found in the states of Tamil Nadu, Rajasthan, and Madhya Pradesh. The plant has held an important place in traditional medicine,
especially within the systems of Ayurveda, Siddha, and Unani. Different vernacular names reflect its cultural and medicinal significance across regions: it is called 'Indrajau' in Hindi, 'Vetpalai' in Tamil, and 'Mitha Indrajau' in Gujarati (1).
Phytochemical and ethnomedicinal studies have revealed that various parts of the plant, including the bark, leaves, latex, and seeds, possess potent therapeutic values.
Traditionally, the plant has been utilised for the treatment of a wide range of conditions, such as psoriasis, skin diseases, gastrointestinal disorders, fevers, and infectious diseases. In
Siddha medicine, preparations like derived from the leaves of W. tinctoria, are widely
prescribed for their analgesic, anti-inflammatory, and anti-psoriatic properties.(2) The plant is also reported to be useful in jaundice, toothache, diarrhoea, dysentery, and wound healing.
Recent pharmacological studies have validated these claims, attributing activities such as anti-inflammatory, antiulcer, antidiabetic, hepatoprotective, antimicrobial, antifungal, and antiviral effects to its extracts(3).
The therapeutic potential of W. tinctoria is largely due to its diverse phytoconstituents, including steroids, flavonoids, alkaloids, terpenoids, saponins, and phenolic compounds.
Chemical investigations have led to the isolation of bioactive molecules such as lupeol, β-
sitosterol, indirubin, oleanolic acid, ursolic acid, and various flavonoids like kaempferol and quercetin derivatives. (4)These compounds collectively contribute to its multifaceted
pharmacological profile. Given its broad spectrum of biological activities and ethnomedicinal relevance, Wrightia tinctoria has emerged as a promising medicinal resource that continues to draw significant attention from researchers(5).

Figure1: Wrightia tinctoria

In Systema Naturae, published in 1768. Carl von Linnaeus first described this plant, Synonyms cordifolia subsp. pratensis (Maxim.) Kitami., Rubia cordifolia var. coriacea Z.Ying Zhang, Rubia cordifolia var. Manjista (Roxb.) Miq ., Rubia cordifolia var. pratensis Maxim., Rubia cordifolia var. rotundifolia Franch., Rubia Manajith (Roxb). ex-Fleming, Rubia mitis., Rubia manjista Roxb., Rubia pratensis (Maxim.) Nakai, Rubia scandens Zoll.& Moritz Family Juss., 1789 Common Name Qian Cao (Chinese) (6).
Rubia cordifolia is a creeper widely observed in the forests of Pakistan, India, China, Korea, Japan, and Mongolia. Since antiquity, the roots have served as a natural source of vivid red dye for silk and wool, owing to their rich pigment content. The stems are slender and quadrangular. The leaves are simple, opposite, and stipulate; the stipule is interpetiolar. The petiole is 1.5–3 cm long. The blade is lanceolate, 1.5–5 cm × 0.5–2.5 cm, cordate at the base, obtuse-acuminate at the apex, and displays three to five longitudinal nerves. The plant bears small flowers arranged in clusters that appear at the tips of branches or arise from the leaf axils. The calyx is inconspicuous. Tiny floral clusters emerge either at the branch ends or from the junctions where leaves meet the stem. The fruit is a pair of black glossy berries which are globose and 0.5 cm across (Figure 3.5). Figure 3.5. Rubia cordifolia Medicinal Uses In Korea, the plant is used to treat arthritis and gynaecological and other infections(7).

Phytopharmacology of the plant, it produces the naphthol, hydroquinones, furomollugin, mo, ugin, rubilactone and epoxymollugin;257 2-carbamoyl-3-methoxy-1,4-naphthoquinone, 2-carbamoyl-3-hydroxy-1,4 naphthoquinone and dihydro-α-lapachone;258 a series of anthraquinones including 1-acetoxy-3-methoxy-9,10-anthraquinone, soranjidiol, 1-hydroxy- 2-methoxy-9,10 anthraquinone, alizarin 2-methyl ether,257 1,3,6-trihydroxy-2-hydroxymethyl-9,10-anthraquinone-3-O-L-rhamnopyranosyl-D-(6-O-acetyl)-
glucopyranoside,259rubiacordone A,260 cordifoliol and cordifodiol,261 and alizarin;262 the rubiasins A–C263 and the.(8) The medicinal properties of Rubia cordifolia are due to anthraquinones. Proposed Research. Investigation of the pharmacological potential of this compound and its derivatives for developing cancer therapies. Rationale Anthraquinones damage DNA by

locking the II–DNA complex264,265 and have been exploited to develop several, such as (CS 3.238), used for the treatment of acute non-lymphocytic leukaemias, breast cancer (9).

This concept was exemplified in human (SNU-1) cells exposed to 150 µM of danthron, with increased levels of ROS, an increase in cytoplasmic Ca2+ production, activation of CD95and consequent 8 activation, cleaveage of Bid, mitochondrial insult, release of and activation of caspases 9 and 3.269 The methylation of danthron at C3 produces (CS 3.243), obtained from the genus L. (family Polygonaceae Juss.), which abated the survival of human (J5) cells by 50% at a dose of 120 µM, associated with an increase in ROS and C (Rubia cordifolia Rubia cordifolia L. (RCL), widely known as Indian madder or Manjistha, has been deeply rooted in traditional medicinal systems across Asia, with particularly notable use among Uygur, Mongolian, Tibetan, and Yao ethnic communities. Classical Uyghur medical texts record that RCL is employed for the management of dermatological and gynaecological disorders, including mild vitiligo, various fungal infections such as tinea, non-healing wounds, traumatic injuries, and menstrual irregularities like amenorrhea (10).

The Uygur Medicine section of the Chinese Herbal Materia Medica (Zhonghua Bencao) further emphasizes the herb's value in addressing 'cold-dampness' or 'phlegm-dominated diseases,' pathological concepts describing impaired metabolic function, sluggish circulation, and disturbed pigmentary processes in the skin. Interestingly, this compendium notes that RCL can serve as a therapeutic substitute for Plumbago zeylanica L., herb used to treat similar conditions such as vitiligo, arthralgia, chronic itching (pruritus), and eczema, demonstrating the interchangeable nature of certain traditional materia medica. Uygur medical theory associates vitiligo not merely with superficial pigmentary loss but with a systemic imbalance marked by the pathological accumulation of phlegm in dermal tissues. (11)

This leads to microcirculatory blockages, impaired nutrient delivery, and dysregulation of melanogenesis—the process by which skin pigment is synthesised. Modern reviews of historical and contemporary medicinal records reveal that among documented multi-herb formulations used for vitiligo treatment, RCL appears with a frequency exceeding 12%, highlighting its longstanding reputation as a core herbal component in ethnomedicine. This frequency underscores both its therapeutic relevance and the broad confidence placed in its ability to restore pigmentary balance and correct underlying systemic dysfunctions (12).

Figure 2: Rubia cordifolia

The skin is the largest organ of the human body and plays a significant role as the main protective barrier between the internal and external environments. It protects the body against physical injuries, microbes, ultraviolet rays, and toxic chemicals; meanwhile, it also retains water and other vital nutrients in the body to a certain extent. Skin has various kinds of physiological functions and acts as an essential immune organ. Unbalanced immune responses, showing up as abnormal activation of inflammatory pathways, overexpression of inflammatory factors, and autoimmune responses in the skin, are the characteristic features of immune skin diseases (ISDs). Atopic dermatitis (AD), psoriasis, vitiligo, pemphigus, and systemic lupus erythematosus (SLE) are currently several ISDs whose pathogenesis has been proven to be influenced by immune balance. (13)

The underlying cause of ISDs is associated with a breakdown of immune homeostasis, and improper metabolism, microbiomes, environment, genetics, and epigenetics can all lead to immune imbalance and accelerate the disease. Research on the physiology of ISDs has also contributed significantly to the development of multi-omics technology, as more targets have been discovered and used in the development of clinical treatments (14).

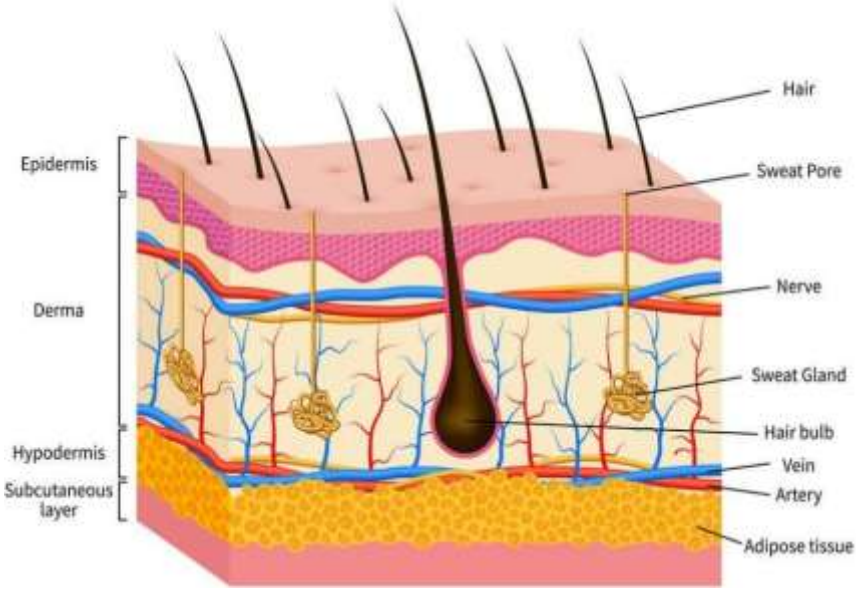
The demand for natural cosmetics is expanding as well. Because of their eco-friendliness and dermatological suitability, inexpensive products obtained from agroforestry and industry are considered to be safer and healthier. subsequently cellulose and chitin are the most common polysaccharides. It has excellent biocompatibility and nontoxic characteristics, on top of being broadly found and inexpensive to purchase. Chitosan, a by-product of deacetylation, is soluble in water and is utilised in many kinds of companies, but chitin's insolubility limits its use. Chitosan oligosaccharides (COS; formerly known as chitooligosaccharides) together with other chitosan derivatives are often discussed for their possible medicinal advantages. These applications pertain to drug delivery systems, wound healing, anti-oxidant activity, anti-inflammation, anti-microbial effects, immune stimulation, and anti-cancer effects (15).

The body's ability to respond to a variety of internal and external stressors, such as fasting and hypoglycaemia, illness, and surgery, is heavily impacted by endogenous glucocorticoids such as cortisol. Exogenous corticosteroids are a vital tool in a physician's arsenal to combat inflammation, and glucocorticoids have the capability to pharmacologically inhibit inflammatory processes. Endogenous glucocorticoids have been demonstrated to be critical to maintaining skin homeostasis, decreasing inflammation, and regulating immune system responses. Long-term use of exogenous (pharmacological) glucocorticoids, however, has been shown to have adverse effects on this organ, such as increased susceptibility to infection, delayed wound healing, and induction of skin atrophy (16).

A mechanistic understanding of how NSA interacts with the skin's own protective and reparative systems is required to address this situation, which not only allows researchers to develop management protocols that optimise therapeutic outcomes and reduce complication rates.

The dual aspects of skin health are its biological function and appearance. Smoothness, elasticity, an absence of obvious problems, and a healthy water-oil balance are characteristics of healthy skin. The support of internal skin core functions are accountable of these beautiful external skin manifestations. In this review, we define the three major functional triads of skin: self-repair (regenerative capacity through cell proliferation and extracellular matrix (ECM) remodelling), and equilibrium resistance (physical and biochemical barriers against pathogens and environmental stressors) (17).

Figure-3: Longitudinal section of skin



Materials and Methods

Materials: Plant Materials

- Fresh leaf powder of Wrightia tinctoria (Family: Apocynaceae)
- Fresh roots of Rubia cordifolia (Family: Rubiaceae)

Chemicals and Reagents

- Distilled water
- Carboxymethyl cellulose (CMC)
- Coconut oil (20g, approx.22 ml)
- Lanolin (8g)
- Beeswax (12g)

Equipment and Apparatus: Analytical balance / digital weighing balance, tray for drying plant materials, grinder/mixer, Soxhlet apparatus/maceration flask

Animals: Albino Wistar Rats

Method for anti-Psoriatic Activity

Drying and Powdering

The clean plant material was evenly spread on trays and shade-dried at room temperature (25 to 30 °C) for seven to ten days. Direct sunlight was avoided to prevent degradation of phytoconstituents. After complete drying, the material was ground into a powder using a grinder. The powder was then sieved through a 40-mesh screen to achieve a uniform particle size. The powder was stored in an airtight container until extraction.[10]

Preparation of Ointment Base

The necessary raw materials for the ointment base preparation were weighed with a precision of a calibrated laboratory balance. The oil phase consisted of coconut oil (20 g, approximately 22–23 mL), while beeswax (12 g) served as the main stiffening agent. Additionally, lanolin (8 g) was chosen as an emulsifier and a means for evenly mixing the ingredients. The coconut oil and beeswax were weighed and properly placed in a clean, heat-resistant beaker to prevent direct heating during melting. The heating of the mixture was done gradually and held at 70–80 °C until the beeswax was completely dissolved and mixed with the coconut oil. Slowly, the very warm lanolin was added to the mixture, which was continuously stirred using a glass rod or a magnetic stirrer to ensure proper emulsification.

Stirring was uninterrupted until all the ingredients were completely mixed together and a single phase was reached, with no wax particles left unmelted. The beaker was then taken off the heat, and the mixture was allowed to cool slowly to room temperature while gentle stirring was continued during the cooling to prevent phase separation and to get a uniform semi-solid texture. After cooling, the ointment base was smooth and soft with uniform consistency; thus, it was good for small-scale laboratory use and incorporation of active pharmaceutical ingredients.[11]

Preparation of Wrightia tinctoria Ointments

Preparation of 1% Wrightia tinctoria Ointment

A digital balance was utilised to weigh 9.9 g of the ointment base, which had been prepared earlier, with high precision, and it was then moved to a clean ointment slab. In a different case, 0.1 g of the Wrightia tinctoria powder was measured out on butter paper. The filtered powder from the plant was added to the ointment base little by little. A stainless-steel spatula was used for mixing in accordance with the principle of geometric dilution to ensure that the drug powder was evenly distributed throughout the base. The mixture was kept on the ointment slab until a smooth, homogeneous, and lump-free ointment was produced. The final formulation was then collected with the spatula and placed in a clean, airtight container. The container was marked with the formulation name, strength, date of preparation, and batch number for identification and record-keeping purposes.[11]

Preparation of 2% Wrightia tinctoria Ointment

The ointment base was methodically weighed to get 9.8 g and then set on a neat ointment slab. On the other hand, 0.2 g of Wrightia tinctoria powder was also weighed and put aside. The weighed plant powder was then gradually added to the ointment base using the

geometric dilution method. With every addition, the mixture was thoroughly spread and blended with a stainless-steel spatula to make sure the powder was evenly distributed throughout the base. Continue mixing until a smooth, homogenous ointment without any visible particles is obtained. The prepared ointment was then meticulously transferred into a new, airtight jar while trapping no air. Finally, the jar was tightly sealed and correctly tagged for identification.[12]

Preparation of Rubia cordifolia Ointments

Preparation of 1% Rubia cordifolia Ointment

Initially, the ointment base was weighed out precisely to get 9.9 g using a digital scale and then placed on a clean mixing slab. In another container, 0.1 g of Rubia cordifolia
powder was accurately measured. Gradually, the weighed powder of the plant was added to the ointment base, little by little. A spatula was used to mix according to the method of geometric dilution to make sure that the powder and the base were mixed evenly. The
paste was worked continuously till a smooth, uniform, and properly textured ointment was accomplished. The 1% ointment prepared was then put into a clean, air-tight container,
labelled correctly, and stored in the right place.[9]

Preparation of 2% Rubia cordifolia Ointment

The previously prepared 9.8 g ointment base was put on a neat mixing surface. In another bowl, 0.2 g of Rubia cordifolia powder was measured accurately. The measured plant powder was then gradually mixed with the ointment base using the geometric dilution technique. The powder was mixed very well with the base to get the powder evenly distributed, and at the same time, a spatula was used to continuously smooth and remove any lumps to get a consistent, homogeneous ointment. The final formulation was poured off and moved to a clean, airtight container, which was later tagged correctly.[13]

Anti- Psoriatic Activity

Before starting the research, the Institutional Animal Ethics Committee (IAEC) gave its approval and allocated a reference number IAEC/VPCV/25-26/1 , IAEC/VPCV/25-26/2 following the CPCSEA guidelines. The whole process of selecting and arranging the experimental animals was carried out randomly and in an organised manner. Cages that housed the animals were labelled accordingly, and wherever it was possible the investigator who did clinical scoring remained blind to the group assignments. On Day -1, the hair from the dorsal area, around 2 by 3 centimetres, was gently taken off using electric clippers and the animals were allowed to recover for 24 hours to make sure that any irritation caused by shaving was gone. Imiquimod (IMQ) was the agent used to make the skin condition resemble psoriasis. [14]

The first application of IMQ consisted of 62.5 mg of 5% IMQ cream being applied daily once to the shaved dorsal skin from Day 0 to Day 6 by a gloved finger or sterile spatula, with care taken to avoid mechanical injury; about 10 mg of IMQ cream was used for each ear in the ear model. Starting from Day 0, topical test formulations, vehicle controls, or clobetasol, which is the reference standard, were administered 30-60 minutes after the IMQ application to ensure that the same exposure and absorption conditions were maintained throughout the study. Although the application of IMQ was limited to the first six days of the study (Day 0–6), the topical treatments were continued once daily for a total duration of 21 days (Day 0–20) to evaluate both therapeutic efficacy and sustained effects. A scoring system similar to PASI was utilised throughout the study at a fixed time point every day to carry out the clinical assessment of skin inflammation. The scoring was based on three parameters: erythema, scaling, and thickening, and they were graded on a scale of 0-4, resulting in a cumulative score ranging from 0 to 12. At set spots, the thickness of the skin was determined in millimetres with the help of digital calipers and the weight of the body was taken on all days to check the health of the system and the toxicity in case. The images of the treated skin were taken by a standardised digital camera with the same distance and lighting conditions so that the possibility of bias in observing was reduced. Humane endpoints were observed strictly, and animals showing more than 15% body weight loss, severe ulceration, or distress were euthanised in a humane way with documentation and justification for all such cases.[15]

On the 21st day, the animals were put under anaesthesia using approved methods of the institution, and blood samples were taken through cardiac puncture for serum biochemical and cytokine analyses. After the euthanasia was done, skin, spleen, and lymph nodes were removed, and the weights of the spleen were recorded as both absolute and relative values. The skin samples were split into two parts; one part was kept in 10% neutral buffered formalin for histopathological evaluation, and the other part was snap-frozen in liquid nitrogen for biochemical and molecular analyses. The tissues were fixed in formalin, processed, embedded in paraffin, sectioned at 4-5 µm, and stained with hematoxylin and eosin.

Quantification of epidermal thickness and inflammatory cell infiltration was done using image analysis software by measuring 3-5 randomly selected fields per section and all histological evaluations were done by an unblinded method. Analyses of serum and skin homogenates were done to check for the presence of inflammatory cytokines like IL-17A, IL-22, IL-23, and TNF-α through the use of ELISA kits following the manufacturer's instructions and, where appropriate, RNA was extracted, and quantitative PCR was performed for gene expression analysis using suitable housekeeping genes for normalisation. Additional

optional evaluations consisted of myeloperoxidase activity measurement as a means of assessing neutrophil infiltration and oxidative stress markers like malondialdehyde and superoxide dismutase. [16]

Data were presented as mean ± SEM and analysed with one-way ANOVA followed by Tukey's post hoc test for multiple group comparisons, while two-way repeated-measures ANOVA was applied for PASI scores over time; non-parametric data were analysed with the Kruskal-Wallis test followed by Dunn's post hoc test. Exact p-values and the statistical software used (e.g., GraphPad Prism) were stated. The therapeutic efficacy of the treatment was evaluated by comparing clinical scores, skin thickness, histopathological features, and cytokine levels with the IMQ-treated control group, which showed sustained decreases in epidermal hyperplasia, inflammatory infiltration, and IL-17/IL-23 axis cytokines over the 21-day period as signs of effectiveness, along with the absence of systemic toxicity as shown by stable body weight and normal biochemical parameters. Among the precautionary measures were prevention of any skin abrasion during shaving, IMQ application was withheld for 24 hours post-shaving, an IMQ plus vehicle control group was included to cater for any formulation-related irritation, pilot studies were conducted when necessary to optimise IMQ dosage and duration, blinded scoring and photographic documentation were maintained to reduce bias and strict adherence to humane endpoints, along with detailed record-keeping, were the standard practices.[14]

Results For Psoriasis

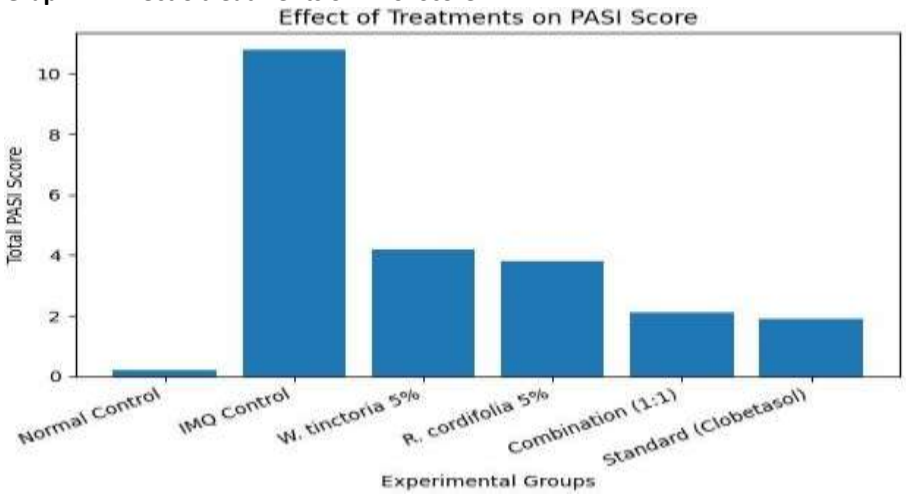
Table 1: Effect on PASI-like Score

Group	Erythema	Scaling	Thickness	Total Score (Mean ± SEM)
Normal Control	0	0	0	0.2 ± 0.1
IMQ Control	3.6	3.7	3.5	10.8 ± 0.4
W. tinctoria 5%	1.4	1.5	1.3	4.2 ± 0.3
R. cordifolia 5%	1.3	1.4	1.1	3.8 ± 0.4
Combination (1:1)	0.6	0.7	0.8	2.1 ± 0.2
Standard (Clobetasol)	0.5	0.6	0.7	1.9 ± 0.1

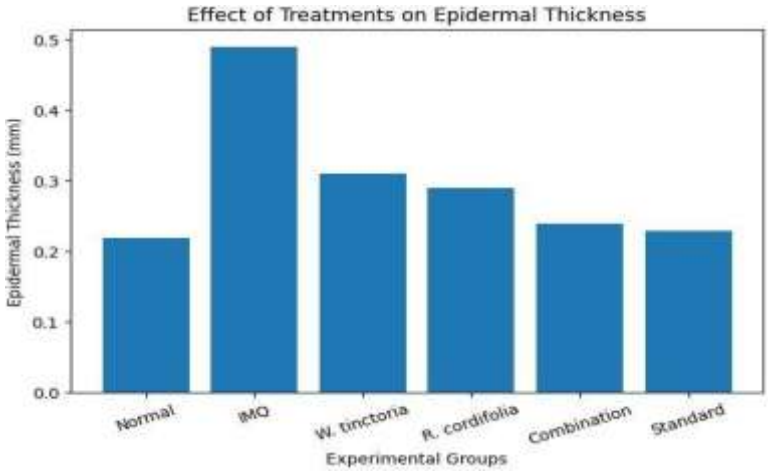
Table 2: Effect on Skin Thickness

Group	Epidermal Thickness (mm)	% Reduction
Normal	0.22 ± 0.02	—
IMQ	0.49 ± 0.05	—
W. tinctoria	0.31 ± 0.04	48%
R. cordifolia	0.29 ± 0.03	55%
Combination	0.24 ± 0.03	65%
Standard	0.23 ± 0.02	68%

Graph-1 : Effect o treatments on PASI Score



Graph-2: Effect of Treatments on Epidermal Thickness



UP S	Day-1	Day-2	Day-3	Day-4	Day-5
Group-1					
Group-2					
Group-3					
Group-4					

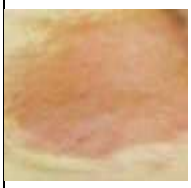
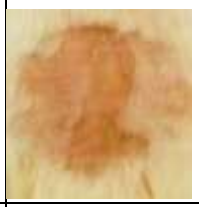
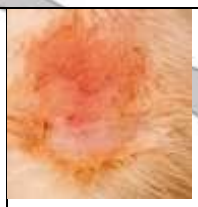
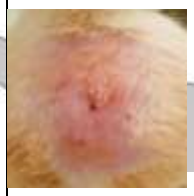
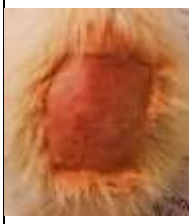
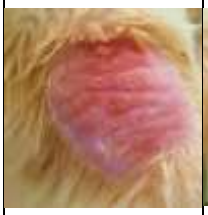
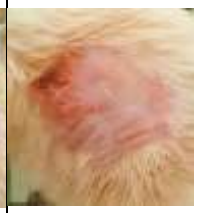
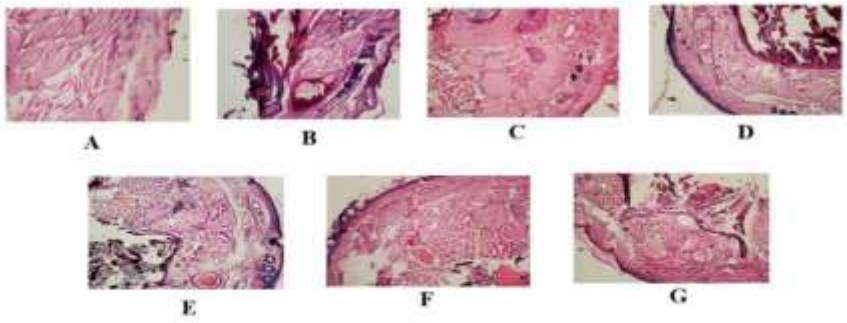
Group-5					
Group-6					

Figure 4: Histology of Skin



A. Control group
B. Disease control
C. Standard salicylic acid
D. 1% *Wrightia Tinctoria*
E. 2% *Wrightia Tinctoria*
F. 1% *Rubia Cardifolia*
G. 2% *Rubia Cardifolia*

Discussion

The present analysis confirms very efficient effects of Wrightia tinctoria and Rubia cordifolia, both individually and in combination, against IMQ-induced psoriasis-like skin inflammation. The IMQ control group displayed a significant rise in PASI-like scores and epidermal thickness, which was a confirmation that psoriatic pathology, characterized by erythema, scaling, and epidermal hyperplasia, was successfully induced. The use of W. tinctoria and R. cordifolia separately brought about a notable паси score and epidermal thickness reduction as compared to IMQ control, which was indicative of their anti-inflammatory and anti-proliferative properties [14,15,16].The most impressive result was achieved with the combination treatment (1:1), which showed a marked clinical improvement with large drops in clinical severity scores and epidermal thickness that came very close to the effects obtained with the standard drug clobetasol. The greater percentage reduction in epidermal thickness seen with combination therapy points to a possible synergistic interaction between the phytoconstituents of both plants, hence, enhancing their efficacy [18,19]

Conclusion

The study unequivocally points out the capability of Wrightia tinctoria and Rubia cordifolia to be used in the treatment of IMQ-induced psoriasis-like skin inflammation. Individual treatment with the plant extracts led to significant diminishing of erythema, scaling, skin thickness, and overall disease severity, which are signs of their anti-inflammatory and anti-proliferative activities. This higher effectiveness could be due to the combined action of bioactive phytoconstituents in both the plants which have the power to intercept inflammatory mediators and restore normal epidermal turnover. To sum up, the findings of this research are in favour of the use of the combined herbal formulation as a promising, effective, and less harmful alternative to psoriasis therapy which will eventually lead to more research on its molecular mechanisms and clinical applicability.

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