Antipsoriatic and Inhibitory effects of an oral dosage form containing Bioflavonoids on inflammatory cytokines IL-1α, IL-1β, IL-6, IL-8, IL-17 and TNF-α

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ABSTRACT

Objective: The present study aimed to develop and evaluate orally administrable dosage form containing bioactive flavonoids viz., Luteolin-7-O-β-D-Glucuronide (II), Kaempferol 3-O-[2-O-(6-O-feruloyl)-β-D-glucopyranosyl]-β-D-galactopyranoside (III) from the bark of Givotia rottleriformis and Formononetin-7-O-β-D-glucoside (VI) from the leaves of Cassia tora. **Methods:** The formulation was developed and evaluated as per official compendia. The developed formulation was evaluated for antipsoriatic activity using rat UV ray photo dermatitis model and cytokine inhibition assay. **Results:** Evaluation parameters observed optimum range of formulations and the tablets were suitable for oral administration. The formulation exhibited antipsoriatic activity by good reduction in the thickness of epidermis, significant retention of the stratum granulosum and the absence of movement of neutrophils in UV-B induced psoriasis. In cytokine inhibition assay, the formulation showed remarkable inhibition of IL-17 and TNF- α, key cytokines involved in the pathogenesis of psoriasis at higher concentration. **Conclusion:** The study can be concluded that the developed formulation can be effective and safety as dietary supplements with health benefits to psoriatic patients.

Key words: *Givotia rottleriformis*, *Cassia tora*, Psoriasis, Formulation, Cytokines.

INTRODUCTION

Psoriasis is a chronic inflammatory skin disorder characterized by hyperproliferation and aberrant differentiation of keratinocytes, inflammation in dermis and epidermis and leukocyte infiltration.[1] In normal condition, epithelial turn over takes place in about 311 hrs (12.5 days) and divisions of a single keratinocyte occur in about once in 60 days. In psoriatic state, the epithelialization occurs in about 36 hrs and so the division of keratinocytes which reduces to 10 days (240 hrs). This phenomenon collectively leads to hyperkeratinized state.[2] Course of treatment available for treating psoriasis includes use of the combinations of conventional methods like using coal tar preparations, dithranol, calsipotriol, topical corticosteroids and controlled UV radiations. However, serious side effects are associated with them. Systemic treatment is considered if extensive psoriasis fails to respond to local measures.[3]

Recent literature data continue to support the fact that polyphenolic compounds, found in most plants, can have a positive effect on many chronic diseases.[4] Natural polyphenols, recognized as potent antioxidants, are multifunctional molecules that can act as anti-inflammatory and antiproliferative agents through the modulation of multiple signaling pathways. This characteristic could be advantageous for the treatment of multi-causal diseases, such as psoriasis. Polyphenols are ubiquitous constituents of plants and possess a broad spectrum of biological activities such as immune system

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activities, oxygen radical scavenging, antimicrobial, anti-inflammatory and antitumor activities.[5]

We therefore tried to investigate the possible positive effects of flavonoids from the antipsoriatic plants viz., *Givotia rottleriformis* bark and *Cassia tora* leaves. *Givotia rottleriformis* Griff. Ex Wight moderately sized tree, is commercially valuable in building Catamarans of the family *Euphorbiaceae*. The bark and seeds of the tree are used in indigenous medicine in the treatment of inflammatory diseases such as rheumatism, psoriasis and dandruff.[6] The leaves and seeds of the plant *Cassia tora* L., Fabaceae, traditionally, is claimed to be useful in the treatment of psoriasis and other skin diseases.[7]

MATERIALS AND METHODS

Plant material

The plant specimen *Givotia rottleriformis* bark was collected in the forest of Attur, Salem district, Tamilnadu and the plant *Cassia tora* leaves was collected in Chennai, Tamilnadu.. It was identified and authenticated by Dr. P. Jayaraman, Director of the Plant Anatomy Research Centre, Tambaram, Chennai. A voucher specimen was deposited for further reference under No. PARC/2011/2140 and PARC/2011/2141 respectively.

Extraction and isolation

About 500 g of the powdered *Givotia rottleriformis* bark was extracted using a Soxhlet apparatus with ethanol (70% v/v) (18 h). The extracted solution was filtered and concentrated in a rotary evaporator under reduced pressure (rotary vacuum flash evaporator). The crude ethanol extract (25 g) thus obtained was subjected to chromatography (Silica gel 120 mesh, 500 g) with gradient elution using solvents of increasing polarity, hexane, chloroform, ethyl acetate and methanol. Three flavonoidal glycosides were isolated viz., Rutin (**I**), Luteolin-7-O-β-D-Glucuronide **(II)** and Kaempferol 3-*O*-[2-*O*-(6-*O*-feruloyl)-*β*-D-glucopyranosyl]-*β*-D-galactopyranoside **(III)**.

About 500 g of the leaves of *Cassia tora* powder were extracted using a Soxhlet apparatus with ethanol (70% v/v) (18 h). The extracted solution was filtered and concentrated in a rotary evaporator under reduced pressure (rotary vacuum flash evaporator). The crude ethanol extract (34 g) thus obtained was subjected to chromatography (Silica gel 120 mesh, 500 g) with gradient elution using solvents of increasing polarity, hexane, chloroform, ethyl acetate and methanol. Three flavonoidal glycosides were isolated viz., Quercetin-3-O-β-d-glucuronide (**IV**), Luteolin-7-O-β-glucopyranoside **(V)** and Formononetin-7-O-β-D-Glucoside **(VI)**.[8]

In-vitro anti-psoriatic activity

Keratinocyte is the primary cell found in the epidermis, the outermost layer of the skin constituting 90% of the cells. The function of the keratinocyte is the formation of the keratin layer that protects the skin and the underlying tissues from the environmental damages such as the heat, UV. Anti-psoriatic activities are reflected by inhibition of keratinocyte proliferation. Hence the potency of the ethanol extract of the *Givotia rotleriformis* bark, *Cassia tora* leaves and isolated flavonoids were screened using HaCaT human keratinocyte cell line.

HaCaT Cell Inhibition assay

In vitro antipsoriatic activity was carried out in HaCaT human keratinocyte cell line. Human HaCaT keratinocytes were obtained from NCCS, Pune, India. The cells were seeded at a concentration of 1.0×10^5 cells/ml in a 96 well microtitre plate and grown in Dulbecco's modified Eagle's medium (DMEM, Gibco) supplemented with 10% fetal bovine serum (BioWest). After 24 h, the supernatant was decanted and the monolayer was washed once. Then 100 µl of test drug dilution, ethanol extract of *Givotia rottleriformis* bark, *Cassia tora* leaves and isolated flavonoids **I-VI** prepared with above media was added per well in microtitre plates. The plates were then incubated at 37°C for 3 days in 5% CO_2 atmosphere. Antiproliferant activity was assessed by performing the Sulphorhodamine B (SRB) assay.[9]

Cells were fixed by adding 25 µl of ice-cold 50% trichloroacetic acid on top of the growth medium and the plates were incubated at 4°C for 1 h, after which plates were washed to remove traces of medium, drug and serum. SRB stain (50 µl; 0.4% in 1% acetic acid) (Sigma) was added to each well and left in contact with the cells for 30 min after which they were washed with 1% acetic acid, rinsing 4 times until only dye adhering to the cells was left. The plates were then dried and 100 µl of 10 mM Tris buffer (Sigma) added to each well to solubilise the dye. The plates were shaken gently for 5 min and absorbance read at 550 nm using a micro plate reader (Biorad, USA). Data obtained at different concentrations were used for IC_{50} calculations.

Formulation of tablets[10]

The beneficial medicinal effects of phytomedicine typically result from synergistic actions acting at single or multiple target sites associated with a physiological process by eliminating the problematic side effects associated with the predominance of a single xenobiotic compound in the body. Hence the bioactive flavonoids (compound II, III, VI) has been converted into dosage form for acceptance, palatability and evaluated for product performance.

Preparation of granules

The tablets were prepared by classic wet granulation method. The compound **II, III** and **VI** (1:1:1) were mixed uniformly in mortar and pestle for 15 minutes. Then microcrystalline cellulose was added, mixed well and granulated by using starch solution as granulating agent. The granules were sifted through sieve no 20, and then it was dried.[10]

Characterization of granules

The powder blend was evaluated for its physical characteristics-bulk density, tapped density, angle of repose, compressibility index, and Hausner's ratio[11]

Preparation of tablets

The dried granules were lubricated with magnesium stearate. Finally the lubricated granules were compressed into tablets by using Rimek punching machine.

Evaluation of tablets

The formulated herbal tablets were evaluated for quality control tests such as appearance, thickness**,** hardness**,** friability**,** weight variation test**,** disintegration, drug content and *In vitro* drug release as per British Pharmacopoeia (BP), 2009.

Stability studies

The formulated tablets were subjected to stability studies as per ICH guidelines. Samples were withdrawn at predetermined time intervals and then evaluated.

Animals

Healthy Male *Wistar* rats (120-170 g) and *Swiss* albino mice (25-30 g) obtained from the institutional animal house were used for the study. Animals were housed in polypropylene cages and were left 7 days for acclimatization to animal room maintained under controlled condition (a 12 h light–dark cycle at 22 ± 2°C) on standard pellet diet and water *ad libitum*. All animals were taken care of under ethical consideration as per the guidelines of CPCSEA with due approval from the Institutional Animal Ethics Committee. The Institutional Animal Ethics Committee (IAEC) approved the protocol (Registration no. 52 CPCSEA dated 12.8.2012).

Acute toxicity

Acute toxicity studies were carried out using mice as per Economic Co-operation and Development OECD 425 guidelines.[12] Mice (6/group) were divided into four groups. A limit test at a dose of 2000 mg/kg body weight was carried out of ethanol extract of *Givotia rottleriformis* and *Cassia tora* and 300 mg/kg of combined flavonoids **II, III** and **VI** (1:1:1). The animals were observed for clinical signs and mortality for a period of 15 days and body weight changes were recorded every week.

Bio activity evaluation of the developed formulation

In-vivo antipsoriatic activity using Rat UV ray photo dermatitis model

The exposure of the rat skin to UV radiation using UV-B bulb (wavelength<290 nm) has induced pro-inflammatory reaction in the skin that resembles the one observed in psoriasis, evident by the altered skin parameters such as increase in epidermal thickness to almost double the normal size, absence of stratum granulosum and the movement of neutrophils towards epidermis which are typical of psoriasis. The close resemblance of inflammatory process produced by ultraviolet radiation to the one exhibited in psoriasis.[13]

Drug tested

The ethanolic extract of bark of *Givotia rottleriformis* (400 mg/kg BW, p.o.) and the *Cassia tora* leaves (400 mg/kg BW, p.o.), formulation (30 mg/kg BW, p.o.) and reference standard retinoic acid (0.5 mg/Kg, p.o.).

Procedure

The hairs of the rat skin, on one side of the flank, were depilated by clipping with a scissors followed by careful shaving taking precaution to avoid injury to the skin. The animals were then placed on a curved wooden block and their legs tied around it, to avoid contact with the floor. This arrangement prevented the movement of the animal during its subsequent exposure UV radiation. Except for an area of 1.5×2.5 cm on the depilated skin, the entire animal was covered with a UV resistant film. The uncovered area of 1.5×2.5 cm was then irradiated for 20 min with a UV-B lamp kept at a vertical distance of 20 cm from the skin. Irradiation of the depilated rat skin with ultraviolet radiation is known to produce a biphasic erythema. Immediately after irradiation, initial faint erythema appears, and disappears within 30 min. The second phase of erythema starts 6 h after the irradiation and gradually increases, peaking between 24 and 48 h. This reaction is confined to the exposed area and has a sharp boundary. It develops a brownish-red colour. By 48 to 72 h, silvery white scale appears on the erythematous lesion. These scales are relatively thick and begin to fall beyond 72 h. Animals were treated orally with the test drug once daily, 5 times a week, 12 h after irradiation for 2 weeks. Two hours after the last treatment animals were sacrificed; longitudinal sections of the tail skin were made and prepared for histological examination with hematoxylin- eosin staining.[14]

Histopathological Examination

Sections were examined for presence of Munro's microabscesss, elongation of rete ridges, and capillary loop dilation by direct microscopy.

The vertical epidermal thickness between the dermo-epidermal junction and the lowest part of the stratum

corneum (n = 3 measurements per scale, n = 3 scales per animal, n = 6) were examined. The percentage relative epidermal thickness of all the groups was calculated in comparison to the positive control group (100%; n = 54 measurements per treatment).

It was also examined for Mean thickness of stratum corneum and stratum granulosum.

All measurements were made at a magnification of 400X using OLYMPUS microscope having a digital camera attachment and software to take measurements.

Cytokine inhibition assay

Keratinocytes secrete a number of cytokines and chemokines that either activate or suppress immune responses. Any local or systemic stimulus may stimulate keratinocyte cytokines production [15] Cytokine or growth factor secretion by epidermal keratinocytes can be sufficient to recruit immune cells into the skin and induce a hyperplastic epidermis with hyperkeratosis and reproduce features of psoriatic disease.

Psoriatic keratinocytes are able to produce and release IL-1α, IL-1β, IL-6, IL-8, IL-17 and IL-20, all of them involved in the development of different alterations which compose the complex and intricate net of psoriasis pathogenesis. The cellular composition of the inflammatory infiltrate within the psoriatic plaques as well as hyperproliferation of keratinocytes and so the whole pathogenetic process of psoriasis appears to be mediated by these cytokines.[16] The serum TNF-*a*, IL-1, IL-6, IL-8, IL-12, and IL-17 levels were significantly higher in active psoriatic patients than in controls. Regulation of the inflammatory events initiated or perpetuated by keratinocytes could so represent an important strategy for the treatment of psoriasis and other chronic inflammatory skin diseases.[17] Thus inhibition of TNF-*a*, IL-1, IL-6, IL-8, IL-12, and IL-17 could be employed as criteria for the evaluation of anti-psoriatic activity.

Assay for the inhibitory effects on IL-lα, IL-1β, IL-6, IL-8, IL-17 and TNF-α biosynthesis

Endotoxin (LPS) from *Escherichia coli* 055:B5 was obtained from Difco (Detroit, MI). Heparin was purchased from Takeda (Osaka, Japan) and ELISA kits from RayBio® (RayBiotech, Inc.).

Blood collection

About 20 mL of blood collected from healthy human volunteers after an overnight fast of 10–12 h. containing 20U heparin/ml by venapuncture and 30% solution is prepared by suspending in supplemented RPMI-1640 medium containing 100 U/ml penicillin and 100 mg/ml streptomycin.

Procedure

Lipopolysaccharide stimulated human peripheral mononuclear cells (LPS) (1 μg/ml) was dissolved in the supplemented RPMI-1640 media at a concentration of 3 μg/ml. The test sample was dissolved in DMSO at concentrations of 1, 3, 10, and 30 μg/ml and each of these concentrations was diluted with the supplemented RPMI-1640 media (1:100). Only DMSO was contained in control suspension. Equal volumes from each of three solutions (whole blood, LPS and test sample) were mixed and the mixture was incubated at 37°C in a humidified atmosphere of 5% CO_2–95% air for 18—24 h. The supernatant of culture prepared by centrifugation was stored at -20°C until the assay of cytokine. The concentrations of the human cytokines (IL-lα, IL-1β, IL-6, IL-8, IL-17, TNF-α) were assayed using an ELISA kits.[18] The ratio (%) of inhibition of the cytokine release was calculated by the following equation:

Inhibition (%) = 100×(1-T/C)

where T represents the concentration of the cytokine in the culture supernatant with the test compound, and C represents the concentration of the cytokine in the culture supernatant with the solvent (control).

Statistical analysis

Values were represented as mean ± SEM. Data were analysed using one-way analysis of variance (ANOVA) and group means were compared using the Tukey-Kramer Multiple Comparison test using Instat-V3 software. P values < 0.05 were considered significant.

RESULTS AND DISCUSSION

HaCaT Cell Inhibition assay

The cytotoxic effect of ethanol extract and isolated flavonoids **I-III** from *Givotia rottleriformis* bark and compounds **IV-VI** from *Cassia tora* leaves were evaluated using HaCaT cells, a rapidly multiplying human keratinocyte cell line, as a model of epidermal hyperproliferation in psoriasis. Among the isolated compounds,

Table 1: Inhibitory concentration against HaCaT cell line by Sulphorhodamine B assay

Sample	IC_{50} value (μg/ml)
Ethanol extract of *Givotia rottleriformis*	310.30
Ethanol extract of *Cassia tora*	290.48
Compound I	180.70
Compound II	56.50
Compound III	76.50
Compound IV	58.65
Compound V	146.20
Compound VI	87.74
Asiaticoside	31.50

compounds **II, III** and **VI** showed significant antiproliferant activity in HaCaT cells. The results were validated using asiaticoside as positive control and tabulated in Table 1.

Formulation of Tablets

These bioactive flavonoids were promoted to formulation of combinational tablet dosage form and evaluation for their product performance using various evaluation parameters such as weight variation, hardness, friability, thickness, disintegration & dissolution study.

Preparation of granules

The type and concentration of additives used were optimized and the final formula was achieved. The composition of herbal tablets was given in Table 2.

Characterization of granules

The powder blend was evaluated for its physical characteristics bulk density, tapped density, angle of repose, compressibility index, and Hausner's ratio. The results were given in Table 3.

Evaluation of tablets

About 100 tablets were formulated and will be extending it to pilot plant scale in future. The formulated herbal tablets were evaluated for quality control tests such as appearance, thickness**,** hardness**,** friability**,** weight variation test**,** disintegration, drug content and *In vitro* drug release. The results were given in Table 4. The result of the stability study of the formulation was given in Table 5.

The results showed that granules containing bioactive flavonoids exhibited good flowability and the values obtained fell within the acceptable range for good powder flow. Values of angle of repose were significantly below 35°C, which showed that the granules had low interparticle cohesion and hence good flowability. Hausner's ratio less than or equal to 1.25 indicates good flow, while Hausner's ratio greater than 1.25 indicates poor flow. Therefore, the granules were within the specified limits for good flow, Also, Carr's index of 5 to 16 indicates good flow, while 18 to 23 shows fair flow.[19] The results of compressibility index indicate that the prepared granules had good flowability and consolidation properties. When the Carr's Index (CI) and Hausner's Ration (HR) are adequate, the powder flows at minimum bulk density. A high bulk density, that is, a low porosity, will result in a low deformation potential, a lack of space for deformation during compression will cause less intimate contact between the particles within the tablets, resulting in weak tablets.[20] The results showed that the granules had low bulk and tapped densities and hence, exhibited good properties required for the production of good quality tablets.

The tablets weight had percentage deviation below 5%. Tablets friability results showed that the tablets passed the friability test as the friability values were significantly below 1%. Therefore, the tablets can comfortably withstand handling, packaging and transportation without compromising the properties of the tablets. The results of disintegration time test showed that formulated tablets complied with the official requirements of uniformity of weight. From the results it can be concluded that the newly developed tablet met Pharmacopoeial specifications limit, and the tablets were suitable for oral administration.

Table 2: Formulation of herbal tablets

Ingredients	1 tablet (mg)	100 tablets (gm)
Compound II	10	1
Compound III	10	1
Compound VI	10	1
Starch solution	QS	QS
Microcrystalline cellulose	60	6
Magnesium stearate	4	0.4

Table 3: Pre compression parameters of granules

Parameters	Recorded values
Angle of repose (^{0})	24.44± 0.12
Bulk density (g/ml)	0.66 ± 0.04
Tapped density (g/ml)	0.72 ± 0.04
Compressibility index (%)	11.90±0.04
Hausner's Ratio	1.14±0.03

Table 4: Results of Post compression parameters of Herbal Tablets		
Tablet parameter	**Recorded values**	
Appearance	Tablet surface was smooth and elegant nature	
Thickness (mm)	2.52 ± 0.24	
Hardness (kg/cm²)	5.2 ± 0.1	
Friability (%)	0.90 ± 0.12	
Avg. wt. (mg)	98.42± 1.49	
% Weight Variation	1.52± 0.02	
Disintegration	2 min 33 sec	
Drug content (%)	Compound II	99.55± 0.96
	Compound III	98.62± 0.96
	Compound VI	98.54± 0.86
% *In vitro* drug release	Compound II	98.27± 0.19
	Compound III	99.37± 0.19
	Compound VI	98.33± 0.19
SD values (n=6)		

Table 5: Stability studies as per ICH guidelines				
Tablet	**Recorded values**			
parameter	**1st month**		**3rd month**	**6th month**
Appearance	smooth and elegant nature		smooth and elegant nature	smooth and elegant nature
Thickness (mm)	2.52 ± 0.26		2.50 ± 0.28	2.50 ± 0.32
Hardness (kg/cm²)	5.2 ± 0.1		5.1 ± 0.1	5.1 ± 0.1
Friability (%)	0.92 ± 0.16		0.94 ± 0.18	0.98 ± 0.20
Avg. wt. (mg)	98.22± 1.69		98.02± 1.80	97.02± 1.82
% Weight Variation	1.58± 0.02		1.62± 0.06	1.68± 0.08
Disintegration	2 min 33 sec		2 min 32 sec	2 min 29 sec
Drug content (%) (Compounds)	II	9.53± 0.98	99.32± 0.84	98.32± 0.99
	III	98.52± 0.96	98.12± 0.98	97.18± 0.66
	VI	98.52± 0.82	98.22± 0.86	97.22± 0.88
% *In vitro* release (Compounds)	II	98.17± 0.29	97.87± 0.39	97.22± 0.86
	III	99.27± 0.19	98.96± 0.20	98.06± 0.24
	VI	98.24± 0.29	97.84± 0.39	97.24± 0.39
SD values (n=6). Herbal tablets at Storage Temp 40°C ± 2 oC /RH75% ± 5%				

Acute toxicity

The LD_{50} determination was done in mice by OECD guideline 423. The tested samples were found to be safe and did not produce any mortality after 15 days. The selection of dose was made based upon the minimum concentration of drug required for therapeutic action which will be economically fruitful for further research and formulation.

In-vivo antipsoriatic activity

Histopathologically, numbers of features are observed in fully developed lesions in psoriasis such as Munro's microabscess, regular elongation of rete ridges, and capillary loop dilation which are shown in Table 6 and in Figure 1. In comparison to positive control group, all other groups led to significantly decreased relative epidermal thickness. Mean thickness of the epidermis, stratum corneum and stratum granulosum in the positive control group, and the groups that were treated with the ethanol extract of *G. rottleriformis* and *C. tora*, and formulation were tabulated in Table 7 and Figure 2.

In case of positive control group, section showed regular elongation of rete ridges, capillary loop dilation with minimal grade lesion of diagnostic Munro's microabscess and marked increase in relative epidermal thickness as compared to other groups. In case of etha-

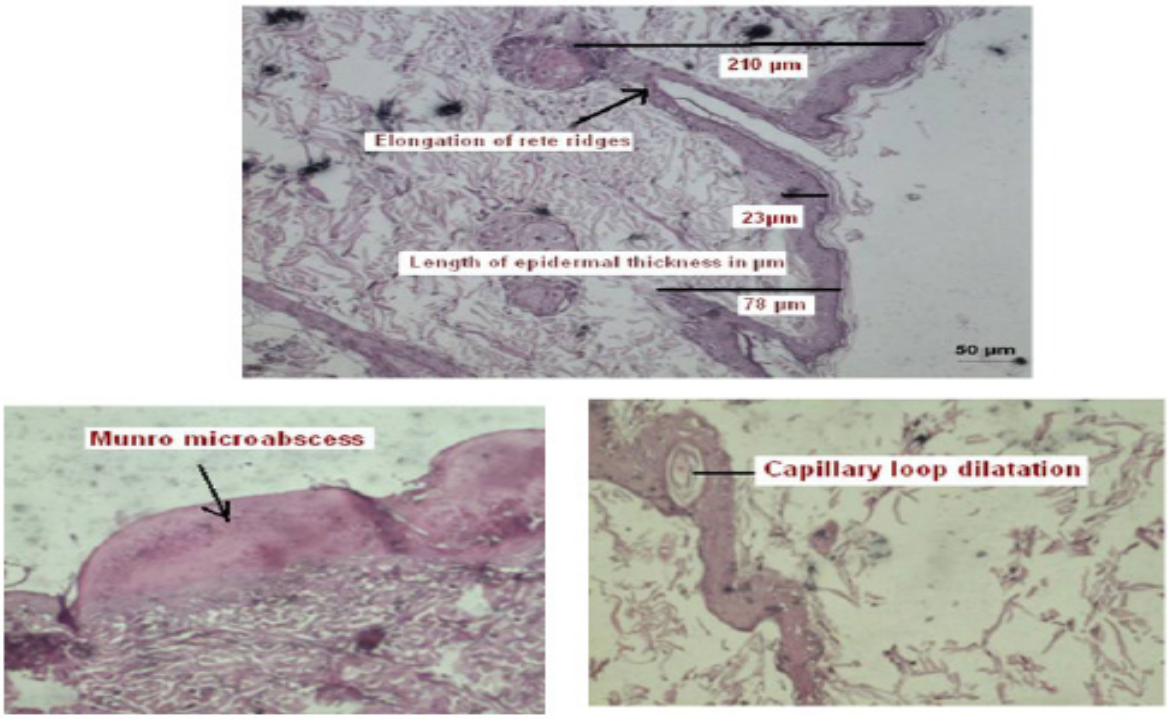

Figure 1: Photomicrographs of positive control rat skin on UV-B-induced psoriasis

nol extract of *G. rottleriformis* 400 mg/Kg, there was a minimal grade lesion of elongation of rete ridges along with capillary loop dilation in the section and absence of Munro's microabscess. In ethanol extract of *C. tora* 400 mg/Kg, there was no lesion of Munro's microabscess, capillary loop dilation along with elongation of rete ridges in the section of skin of rats. The irradiated rat skin treated with formulation containing flavonoids has shown a significant reduction in the total epidermal thickness and also significant retention of the stratum granulosum, the absence of movement of neutrophils, further substantiates that the formulation containing flavonoids has been very useful in containing the changes that occur in the skin due to irradiation. The significant increase in the thickness of stratum corneum has been observed in the formulation containing flavonoids treated group.

The formulation significantly decreases total thickness of the epidermis indicating that its presence in the formulation has an influence to retard the hyper proliferation of the keratinocytes that occurs when the skin is exposed to UV radiation. The significant increase in the thickness of the stratum corneum is probably due to its ability to enhance the keratinisation process which is a protective strategy adopted by the skin when exposed to penetrating radiation. The presence of the granulosum layer in the skin treated with formulation and is completely absent in the positive control, indicates that the formulation containing flavonoids is probably able to suppress the altered process of differentiation of the keratinocytes. Further, formulation containing flavonoids has brought about useful changes in the epidermis of the irradiated skin, which shows that the drug may be useful in psoriasis. Hence, the developed formulation has shown antipsoriatic activity by good reduc-

Table 6: Effect of formulations on Histopathological features on UV-B-induced psoriasis in rats

Treatment	Munro's microabscess	Elongation of rete ridges	Capillary loop dilation
Positive control	++	+++	++
Standard	-	-	-
Ethanol extract of *Givotia rottleriformis*	-	+	+
(400 mg/Kg)	-	+	+
Ethanol extract of *Cassia tora*			
(400 mg/Kg)	-	-	-
Formulation (30 mg/Kg)	-	-	-

Note: + mild or slight grade lesion; ++ moderate grade lesion; +++ severe grade lesion; – no lesion

Table 7: Relative Epidermal thickness and different layers of epidermis on UV-B-induced psoriasis in rats			
Treatment	% Relative Epidermal thickness (µm)	Thickness of stratum corneum (µm)	Thickness of stratum granulosum (µm)
Positive control	94.86±6.82	2.95±0.83	Absent
Standard	22.37±2.59***	15.62±1.20	12.83±0.38
EE of *G. rottleriformis* (400 mg/Kg)	40.60±3.58**	6.20±1.40	3.86±0.24
EE of *C. tora* (400 mg/Kg)	38.57±2.80**	8.43±0.69	4.94±0.80
Formulation 30 mg/Kg	28.53±5.25***	13.84±0.74	8.12±0.44
* EE-ethanol extract; n = 6; values are expressed as mean ± SEM. Data were analyzed by one-way ANOVA followed by Tukey multiple comparison test. The values are *P< 0.05; **P< 0.01; ***P< 0.001 when compared against control.			

tion in the thickness of epidermis, significant retention of the stratum granulosum and the absence of movement of neutrophils in UV-B induced psoriasis.

Cytokine Inhibition assay

Inhibitory effects of the test samples on IL-lα, IL-1β, IL-6, IL-8, IL-17, TNF-α biosynthesis are given in Table 8 as the inhibitory percentages. For the interpretation of the results, percentage values are classified under four groups; an inhibition between 70 and 100% is accepted as high, values between 40 and 69% as moderate, 20 and 39% as low and an inhibition less than 20% is considered to be insignificant.

Psoriasis can be described as a T-cell-mediated disease, with a complex role for a variety of cytokines and other factors. Interaction between T lymphocytes and keratinocytes, via cytokines, is likely to play a pivotal role in the pathogenic process in psoriasis. The Th1 cytokines (TNF-*a*, IFN-*γ*, and IL-12) and some proinflammatory cytokines (such as IL-6, IL-8, and IL-18) are influenced in the serum of psoriatic patients.[21]

Th17 cells are stimulated by IL-23 (which shares the p40 subunit with IL-12) to produce IL-17 and also IL-22, which has recently been shown to be a major driver of acanthosis in psoriasis, and so is a novel target for treatment.[17] The exact role of TNF-*a* in the pathomechanism of psoriasis is still unclear, but anti-TNF-*a* therapy is highly effective in psoriasis indicating that this cytokine has, together with IFN-*γ*, a central role in the pathogenesis. IFN-*γ* and TNF-*a* induce IL-6, IL-8, IL-12, and IL-18 and constitute an important link in the cytokine network in the pathogenesis of psoriasis.[22] Moreover, the intradermal administration of IFN-*γ* into nonlesional skin of psoriatic patients causes the appearance

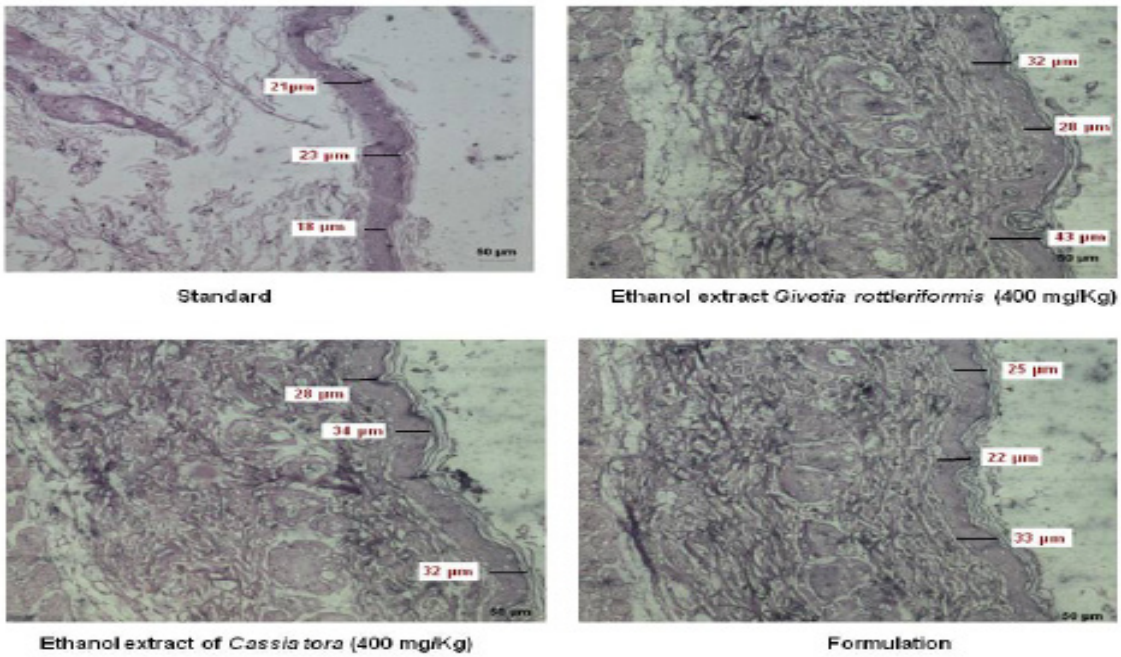

Figure 2: Histopathological features on UV-B-induced psoriasis in drug treated rats

Table 8: Inhibitory effects of the ethanol extracts from Givotia rottleriformis, Cassia tora and developed formulation on IL-lα, IL-1β, IL-6, IL-8, IL-17, TNF-α Biosynthesis

Treatment	Final concentration (µg/ml)	Inhibitory ratio (%)					
Formulation		IL-lα	IL-1β	IL-6	IL-8	IL-17	TNF-α
	1	0	-1	2	-10	-6	12
	3	6	5	8	3	10	14
	10	11	8	13	12	19	27
	30	22	30	31	23	44	70
EE of G. rottleriformis	1	-2	-22	0	-31	-16	-13
	3	3	-9	-8	-16	-13	16
	10	8	11	15	3	2	26
	30	19	24	27	25	13	40
EE of C. tora	1	-3	-21	-12	-16	-7	-9
	3	12	-3	-1	5	13	16
	10	10	10	17	11	23	22
	30	30	24	19	27	31	45

of lesions at the inoculation site. IL-6 mediates T-cell activation, stimulates proliferation of keratinocytes[21] and, at the beginning of acute inflammation, mediates the acute phase responses.[23] In fact, data currently available suggest that this cytokine exerts a critical role as a potent chemoattractant for neutrophils and T lymphocytes, as well as a factor prompting keratinocyte proliferation.

Antje R. Weseler et al.[24] reported that the flavones fisetin, morin, or tricetin attenuated LPS-induced increases in concentrations of TNF-α in blood from COPD patients (chronic obstructive pulmonary disease) and IL-6 in blood from T2D patients (Type 2 diabetes), indicating a potential application as nutraceutical agents for these patient groups. In an earlier work, Vijayalakshmi et al.,[25] reported that the flavonoid quercetin showed significant reduction in epidermal thickness with respect to control in Perry's mouse tail model. Jadranka Skuric et al.[26] reported that the flavonoids from propolis offer some protection against psoriatic complications through their roles as inhibitors of inflammation and as free radical scavengers on animal model psoriasis, induced by the Di-n-Propyl Disulfide iritant (PPD).

CONCLUSION

In the present study, the formulation developed containing flavonoidal glycosides viz., Kaempferol, Luteolin and Formononetin showed remarkable inhibition of IL-17 and TNF- α, key cytokines involved in the pathogenesis of psoriasis at higher concentration. At higher concentration, both the ethanol extract of *G. rottleriformis* and *C. tora* exhibited remarkable inhibition against TNF-α. This result demonstrates that the flavonoidal glycosides Kaempferol, Luteolin and Formononetin exert strong anti-TNFα. and anti-IL-17 effects in ex vivo LPS-stimulated blood. So the study can be concluded that the developed formulation can be effective and safety as dietary supplements with health benefits to psoriatic patients. Therefore, additional clinical investigation of these compounds is indicated to evaluate the efficacy and safety of their application as dietary supplements with health benefits to psoriatic patients.

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