Research Article

Development and evaluation of *Bougainvillea glabra* leaf hydroalcoholic extract gel against ultraviolet-induced skin inflammation in Wistar rats

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ABSTRACT

Background: Ultraviolet (UV) radiation induces oxidative stress, inflammation, and structural skin damage. *Bougainvillea glabra* (BG) leaves are rich in flavonoids and phenolic compounds with reported antioxidant and anti-inflammatory properties. **Aim:** The aim of this study was to formulate and evaluate hydroalcoholic extract gel of BG leaves and assess its protective effect against UV-induced skin inflammation. **Materials and Methods:** Hydroalcoholic extract (ethanol: water, 70:30) was prepared by maceration and subjected to preliminary phytochemical screening. Carbopol 940-based gels containing 1% and 5% w/w extract were formulated and evaluated for physicochemical properties, drug content, and stability. Anti-inflammatory activity was assessed in UV-irradiated Wistar rats by evaluating erythema, edema, body weight, catalase (CAT), lipid peroxidation (LPO), tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), and histopathological changes. **Results:** The extract showed an 8% yield and contained flavonoids, alkaloids, tannins, phenolic compounds, carbohydrates, and saponins. The optimized F2 formulation exhibited acceptable pH, viscosity, spreadability, drug content, and stability. UV irradiation significantly increased oxidative stress and inflammatory markers, whereas BG gel reduced LPO, TNF-α, and IL-6 while restoring CAT activity. The 5% gel showed superior efficacy, comparable to hydrocortisone cream, and markedly improved skin histopathology. **Conclusion:** The 5% BG hydroalcoholic gel demonstrated significant antioxidant and anti-inflammatory effects, indicating its potential as a natural topical therapy for UV-induced inflammatory skin disorders.

Keywords: *Bougainvillea glabra*, hydroalcoholic extract, skin inflammation, topical gel, ultraviolet irradiation

Introduction

Skin serves as the body's primary protective barrier against physical, chemical, and biological insults.[1] Continuous exposure to ultraviolet (UV) radiation is one of the major environmental factors responsible for cutaneous injury, leading to oxidative stress, inflammation, premature skin aging, immunosuppression, and increased risk of skin disorders. UV irradiation promotes excessive generation of reactive oxygen species (ROS), which damage cellular lipids, proteins, and DNA while activating inflammatory signaling pathways that trigger the release of cytokines such as tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6). Persistent oxidative stress and inflammation ultimately disrupt skin integrity, resulting in erythema, edema, collagen degradation, and delayed tissue repair.[2]

Topical corticosteroids are commonly used for inflammatory skin conditions due to their strong anti-inflammatory activity. However, long-term use is associated with adverse effects, such as skin atrophy, irritation, delayed wound healing, and increased susceptibility to infections. These limitations have encouraged the exploration of safer plant-based alternatives with antioxidant and anti-inflammatory potential.[3]

Medicinal plants are rich sources of bioactive phytoconstituents including flavonoids, phenolic compounds, tannins, alkaloids, and saponins, many of which exhibit strong free radical scavenging

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and anti-inflammatory properties. Herbal topical formulations are increasingly preferred due to localized drug delivery, reduced systemic side effects, improved safety, and better patient compliance.[4]

Bougainvillea glabra (BG) Choisy (family Nyctaginaceae) is an ornamental medicinal plant widely distributed in tropical and subtropical regions. Conventionally, it has been used in the management of inflammatory disorders, cough, gastrointestinal disturbances, diabetes, and microbial infections. Phytochemical studies have reported that its leaves contain flavonoids, phenolic compounds, alkaloids, tannins, and other secondary metabolites responsible for antioxidant, antimicrobial, wound-healing, and anti-inflammatory activities, suggesting potential protection against UV-induced oxidative and inflammatory damage.[5]

Hydroalcoholic extraction is an efficient method for extracting both polar and moderately non-polar phytoconstituents from plant materials. Incorporation of such extracts into Carbopol-based gels provides a stable semisolid dosage form with good spreadability, enhanced skin retention, and improved patient acceptability for topical application.[6]

Therefore, the present study was undertaken to prepare a hydroalcoholic extract of BG leaves, formulate Carbopol-based topical gels at different concentrations, evaluate their physicochemical properties, and assess their protective effect against UV-induced skin inflammation in Wistar rats using biochemical, gross, and histopathological parameters.

Materials and Methods

Plant material and authentication

Fresh leaves of BG were collected from Ashti, Maharashtra, India, in September 2025. The plant was taxonomically authenticated by Dr. Prashant A. Gawande, Professor and In-charge, Herbarium, Department of Botany, Sant Gadge Baba Amravati University, Amravati, Maharashtra, India. A voucher specimen (No. NY/BO/GL/510/1/28-10/2025) was deposited in the Herbarium, Department of Pharmacognosy, Dr. Rajendra Gode Institute of Pharmacy, Amravati, India [Figure 1].

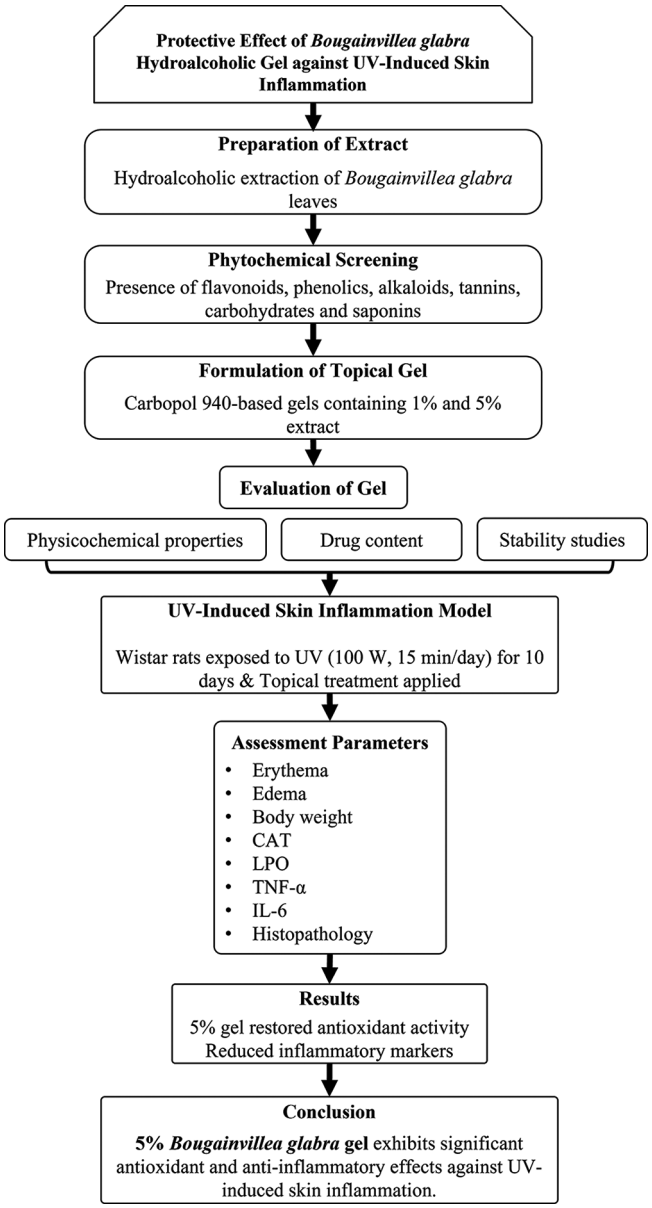

Figure 1: Schematic representation of the experimental workflow for the formulation and evaluation of *Bougainvillea glabra* hydroalcoholic gel against ultraviolet-induced skin inflammation

Preparation of hydroalcoholic extract

The collected leaves were shade-dried, coarsely powdered, and passed through sieve No. 40. Approximately 100 g of the powder was defatted with petroleum ether (500 mL) for 24 h and subsequently extracted by maceration using a hydroalcoholic solvent (ethanol: water, 70:30 v/v) for 4–5 days at room temperature. The extract was filtered through muslin cloth followed by Whatman No. 1 filter paper, concentrated on a water bath to obtain a semisolid mass, and stored in airtight containers after determining the percentage yield.

Preliminary phytochemical screening

The hydroalcoholic extract was qualitatively screened for alkaloids, carbohydrates, flavonoids, tannins, phenolic compounds, steroids, terpenoids, glycosides, saponins, proteins, and amino acids using standard phytochemical methods.

Formulation of BG gel

Topical gels containing 1% and 5% (w/w) hydroalcoholic extract of BG were prepared using Carbopol 940 as the gelling agent. Carbopol was dispersed in purified water, while methylparaben and propylparaben dissolved in propylene glycol were incorporated into the hydrated base.[7] The extract, dissolved in ethanol, was added under continuous stirring, followed by dropwise addition of triethanolamine to adjust the pH (5.5–6.5) and induce gelation. The final volume was adjusted with purified water, and the formulations were stored in airtight containers at room temperature. Three formulations containing 0.15%, 0.30%, and 0.45% Carbopol

940 were prepared for each extract concentration to optimize physicochemical properties.

Physicochemical evaluation of gel formulations[8]

The formulated gels were evaluated for appearance, homogeneity, pH, viscosity, spreadability, drug content, and stability. pH was measured using a digital pH meter, viscosity with a Brookfield viscometer, spreadability by the parallel plate method, and drug content by UV–visible spectrophotometry at 275 nm. The optimized formulations were subjected to short-term stability studies for 30 days under refrigerated (4 ± 2°C) and room-temperature (30 ± 2°C) conditions.

Experimental animals

Healthy Wistar albino rats (150–200 g; 7–8 weeks old) of either sex were housed under standard laboratory conditions (25 ± 2°C, 60–65% relative humidity, 12 h light/dark cycle) with free access to standard diet and water and acclimatized for 1 week before the study. The experimental protocol was approved by the Institutional Animal Ethics Committee, Dr. Rajendra Gode Institute of Pharmacy, Amravati (Approval No. 2240/IAEC/25-26/11), and conducted in accordance with the CCSEA guidelines, Government of India.

Experimental design

Animals were randomly divided into six groups ($n = 5$):

- Group I: Normal control (no UV exposure)
- Group II: Disease control (UV irradiated)
- Group III: Standard (1% hydrocortisone cream)
- Group IV: UV + 1% BG gel
- Group V: UV + 5% BG gel
- Group VI: Placebo (gel base without extract).

The dorsal skin was shaved before the experiment. Groups II–VI were exposed to UV radiation (100 W) for 15 min daily for 10 consecutive days. Treatments were applied topically 2 h after UV exposure once daily, and gross skin changes were recorded throughout the study period.

Gross skin evaluation

Skin inflammation was assessed visually for erythema, edema, dryness, and lesion formation. Erythema and edema were scored from 0–4 and edema from 0–4 according to the modified Draize scale, and the Primary Irritation Index (PII) was calculated.[9]

Biochemical estimation[10]

At the end of the study, animals were sacrificed and dorsal skin tissues were collected. Tissue homogenates (10% w/v) were prepared in phosphate buffer (pH 7.4) and centrifuged at 3000 rpm for 15 min at 4°C, and the supernatant was used for biochemical analysis. Catalase (CAT) activity was determined by monitoring hydrogen peroxide decomposition at 240 nm, while lipid peroxidation (LPO) was estimated using the TBARS method by measuring malondialdehyde formation at 532 nm.

Estimation of pro-inflammatory cytokines

TNF-α and IL-6 levels in skin tissue were quantified using rat ELISA kits according to the manufacturer's instructions. Absorbance was measured at 450 nm using a microplate reader, and concentrations were calculated from standard curves.

Histopathological examination[11]

Skin tissues were fixed in 10% neutral buffered formalin, processed through graded alcohols, embedded in paraffin, sectioned at 4–5 µm thickness, and stained with hematoxylin and eosin (H&E). Sections were examined under a light microscope for epidermal integrity, dermal edema, inflammatory infiltration, collagen arrangement, and follicular architecture.

Statistical analysis

Physicochemical evaluation data are expressed as Mean ± SD ($n = 3$), whereas animal experimental data are expressed as Mean ± SEM ($n = 5$). Statistical analysis was performed using one-way analysis of variance followed by Dunnett's *post hoc* test using GraphPad Prism version 10. $P < 0.05$ was considered statistically significant.

$P < 0.05$ = *
$P < 0.01$ = **
$P < 0.001$ = ***
$P < 0.0001$ = ***.

Results

Extraction yield and phytochemical analysis

Hydroalcoholic extraction of BG leaves yielded 8% (w/w) of a dark green semisolid extract using an ethanol–water (70:30 v/v) solvent system. Preliminary phytochemical screening revealed the presence of carbohydrates, alkaloids, flavonoids, tannins, phenolic compounds, and saponins, while steroids, terpenoids, proteins, amino acids, and glycosides were absent. The presence of flavonoids and phenolics suggests strong antioxidant potential contributing to the observed pharmacological activity.

Evaluation of gel formulations

Organoleptic characteristics

All gel formulations exhibited acceptable organoleptic properties. The 1% BG gel was clear and translucent amber, whereas the 5% BG gel appeared deep reddish-brown and glossy due to higher extract concentration. All formulations were homogeneous, smooth, and free from grittiness or phase separation, indicating good physical acceptability for topical use.

pH

The pH of all formulations ranged from 6.10 to 6.32, within the physiological skin pH range, suggesting good compatibility and minimal risk of irritation [Table 1].

Viscosity

Viscosity increased with increasing Carbopol 940 concentration, indicating stronger gel network formation. The optimized F2 formulation showed balanced viscosity, providing suitable consistency for topical application [Table 1].

Spread ability

Spreadability decreased with increasing viscosity, showing an inverse relationship. However, all formulations demonstrated satisfactory spreadability, with F2 exhibiting an optimal balance between ease of application and consistency [Table 1].

Drug content

Uniform drug distribution was observed across all formulations. The F2 batch showed maximum drug content (≈99%), indicating efficient incorporation of extract and formulation uniformity [Table 1].

Stability study

Both optimized formulations remained stable over 30 days under refrigerated and room-temperature conditions, with only minor, non-significant variations in physicochemical parameters and no visible instability [Table 1].

Drug content was estimated spectrophotometrically at 275 nm using the hydroalcoholic extract as an in-house reference standard. The absorbance measured at 275 nm represents the cumulative UV absorption of phenolic and flavonoid constituents present in the extract and does not correspond to the quantification of any single phytochemical marker.

Gross evaluation of UV-induced skin inflammation

UV exposure induced marked erythema, edema, dryness, and lesion formation in the disease control group. In contrast, treatment groups showed progressive improvement. The 5% BG gel demonstrated superior protective effects, with results comparable to hydrocortisone. The PII confirmed severe irritation in the disease group and slight irritation in the 5% BG gel-treated group [Figure 2].

Effect on body weight

UV irradiation caused a reduction in body weight in the disease group, indicating systemic stress. Treatment with hydrocortisone and BG gels prevented this decline, with the 5% formulation showing greater efficacy and values closer to the normal control group [Table 2].

Effect on oxidative stress and pro-inflammatory cytokines

UV irradiation significantly induced oxidative stress and inflammation, as evidenced by a marked reduction in CAT activity and a significant increase in LPO, IL-6, and TNF-α levels compared with the normal control group. Treatment with both BG gel formulations significantly restored CAT activity while reducing LPO and the pro-inflammatory cytokines IL-6 and TNF-α in a concentration-dependent manner. The 5% BG gel demonstrated greater antioxidant and anti-inflammatory effects than the 1% BG gel, with biomarker levels approaching those observed in the standard hydrocortisone-treated group, indicating superior protection against UV-induced skin inflammation [Table 3 and Figure 3].

Histopathological findings

Disease control animals exhibited severe epidermal and dermal damage with inflammatory infiltration and collagen disruption [Figure

Table 1: Physicochemical evaluation of *Bougainvillea glabra* gel formulations

Parameter	1% BG Gel			5% BG Gel		
Batch	F1	F2	F3	F1	F2	F3
Carbopol 940 (%)	0.15	0.30	0.45	0.15	0.30	0.45
pH	6.32±0.04	6.21±0.05	6.10±0.03	6.32±0.04	6.21±0.05	6.10±0.03
Viscosity (cps)	21,500±100	34,800±110	41,200±120	24,300±110	36,500±120	43,700±130
Spreadability (g•cm/s)	7.9±0.11	6.4±0.09	5.3±0.07	7.3±0.10	5.9±0.08	4.8±0.06
Absorbance (275 nm)	0.356±0.02	0.421±0.03	0.388±0.02	0.782±0.04	0.865±0.05	0.814±0.04
Drug content (%)	90.42±0.38	99.68±0.42	94.14±0.40	93.25±0.45	98.36±0.39	94.87±0.41

Values are expressed as mean±SD (n=3). Statistical analysis was performed using one-way ANOVA followed by Tukey's multiple comparison test. Statistical significance was defined as *P<0.05, **P<0.01, *P<0.001, and **P<0.0001. BG: *Bougainvillea glabra*, cps: Centipoise, SD: Standard deviation

Table 2: Effect of *B. glabra* gel on body weight

Day	Normal group	Disease group	Standard	1% BG Gel	5% BG Gel	Placebo
1	235±2.14	243±2.36	220±1.95	242±2.08	240±2.02	230±2.11
7	248±2.42	237±2.17	232±2.06	247±2.21	244±2.14	227±1.96
14	262±2.65	230±2.03	243±2.28	249±2.30	249±2.24	234±2.04

Values are expressed as Mean±SEM (n=5). Statistical analysis was performed using one-way ANOVA followed by Dunnett's multiple comparison test. Statistical significance was considered at *P<0.05

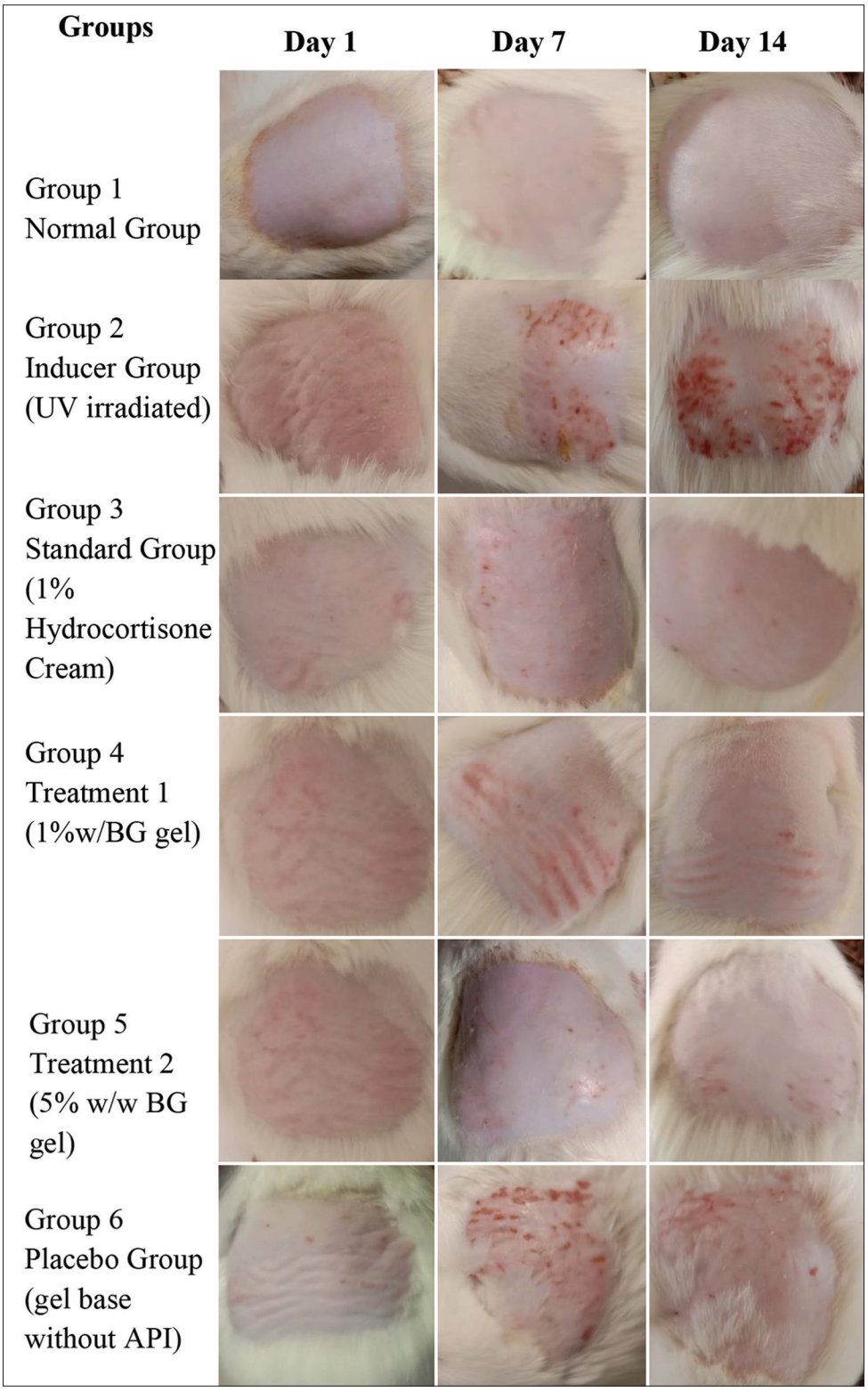

Figure 2: Gross observation of skin changes in experimental groups

4]. Treatment with BG gels improved tissue architecture in a dose-dependent manner. The 5% gel showed near-normal histological recovery comparable to standard treatment, while placebo showed no significant protection [Figure 2].

Discussion

The present study demonstrated that the hydroalcoholic extract of BG can be successfully formulated into a Carbopol-based topical gel

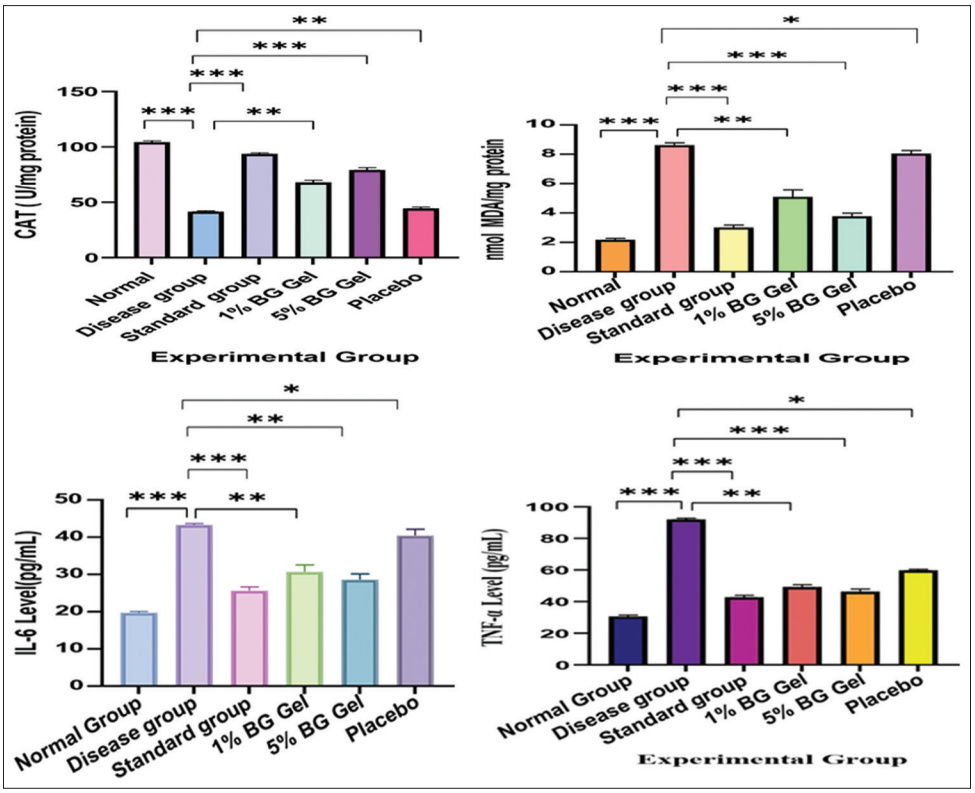

Figure 3: Graph representing the effect of *Bougainvillea glabra* gel on oxidative stress and inflammatory biomarkers

Table 3: Effect of *Bougainvillea glabra* Gel on oxidative stress and inflammatory biomarkers

Group	Catalase (U/mg protein)	Lipid peroxidation (nmol MDA/mg protein)	IL-6 (pg/mL)	TNF-α (pg/mL)
Normal control	104.46±0.48	2.18±0.04	19.73±0.14	30.66±0.35
Disease control	41.58±0.32	8.63±0.07	43.32±0.15	92.03±0.33
Standard	93.84±0.40	3.02±0.07	25.65±0.43	42.83±0.50
1% BG Gel	68.02±0.87	5.00±0.08	30.69±0.83	49.40±0.59
5% BG Gel	79.18±0.94	3.84±0.24	28.61±0.56	46.35±0.72
Placebo	44.38±0.57	8.04±0.09	40.45±0.79	59.82±0.28

Values are expressed as mean±SEM (n=5). Statistical analysis was performed using one-way ANOVA followed by Tukey's multiple comparison test. P<0.05, *P<0.01, **P<0.001, and *P<0.0001 versus the Disease Control group. MDA: Malondialdehyde

with acceptable physicochemical properties and significant protective effects against UV-induced skin inflammation. The formulation effectively reduced oxidative stress, inflammatory cytokines, and histopathological damage, with the 5% gel showing consistently exhibiting greater efficacy compared to the 1% formulation.

The hydroalcoholic extraction yielded 8% (w/w), and preliminary phytochemical screening confirmed flavonoids, phenolics, alkaloids, tannins, carbohydrates, and saponins. These findings are consistent with study reported that BG is rich in flavonoids and phenolic compounds which contribute to its for antioxidant and anti-inflammatory activities and highlighted hydroalcoholic extraction as an efficient method for bioactive recovery.[12]

The formulated gels showed exhibited desirable organoleptic properties, skin-compatible pH, satisfactory viscosity, spreadability, drug content, and stability. The optimized F2 formulation exhibited the best overall characteristics. Similar observations have been reported for Carbopol-based herbal gels with good stability and efficient topical drug delivery.[13]

Exposure to UV radiation generates excessive ROS, leading to LPO, DNA damage, and activation of nuclear factor-kappa B (NF-κB) and mitogen-activated protein kinase (MAPK) pathways, which increase TNF-α and IL-6 production. In this study, CAT activity was reduced after UV exposure but significantly restored by BG gel, particularly the 5% formulation, likely due to flavonoid-mediated activation of antioxidant pathways such as Nrf2.[14]

Similarly, LPO was markedly increased in the disease control group a reduced in a concentration-dependent manner by BG gel, indicating membrane-protective antioxidant effects. The

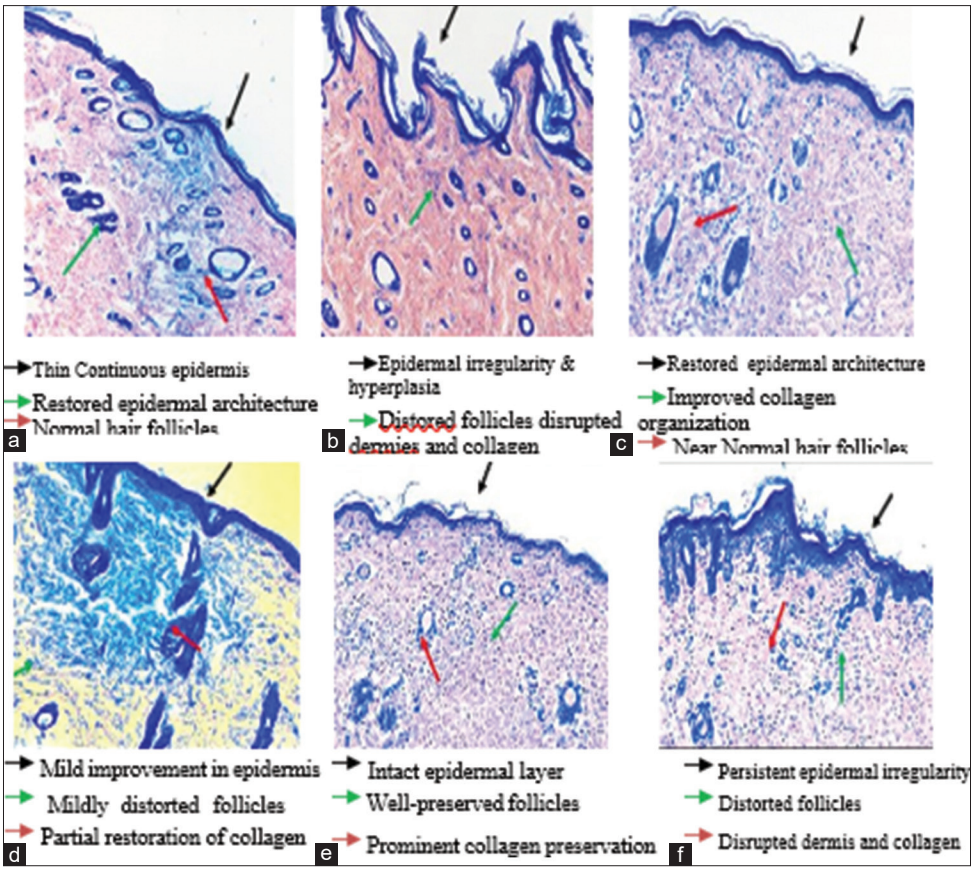

Figure 4: Histopathological findings (a) Normal group, (b) inducer group, (c) standard group, (d) 1%w/w *Bougainvillea glabra* (BG) gel, (e) 5% w/w BG gel, and (f) placebo group

inflammatory cytokines TNF-α and IL-6 were also significantly suppressed, supporting evidence that flavonoids inhibit NF-κB signaling and downstream inflammatory mediators.[15,16] Gross and histopathological findings confirmed improved skin architecture, reduced erythema, edema, and inflammatory infiltration, especially with the 5% gel.

The protective effects of BG gel against UV-induced skin injury are likely mediated through multiple antioxidant and anti-inflammatory mechanisms. The flavonoids and phenolic compounds present in the hydroalcoholic extract are known to directly scavenge ROS, thereby reducing oxidative stress and LPO. Previous studies have suggested that these phytoconstituents may activate the nuclear factor erythroid 2-related factor 2 (Nrf2)/antioxidant response element pathway, resulting in enhanced expression of endogenous antioxidant enzymes, including CAT. Attenuation of oxidative stress may subsequently suppress activation of pro-inflammatory signaling pathways such as NF-κB and MAPK, leading to reduced production of inflammatory mediators including TNF-α, IL-6, cyclooxygenase-2, and inducible nitric oxide synthase. Reduced oxidative stress may also limit activation of matrix metalloproteinases, thereby preserve collagen fibers and maintain dermal structural integrity. Although these molecular pathways were not directly investigated in the present study, the observed restoration of CAT activity, reduction in LPO and inflammatory cytokines, together with improved epidermal architecture, reduced inflammatory cell infiltration, and better collagen organization in histopathological analysis, are consistent with these previously reported mechanisms. The superior efficacy observed with the 5% gel further supports a concentration-dependent pharmacological response and highlights the potential of BG gel as a promising natural topical therapy for UV-induced inflammatory skin disorders.

Overall, the protective effect of BG is attributed to the synergistic action of flavonoids and phenolics, reducing oxidative stress and inflammation. The 5% formulation demonstrated the highest efficacy, supporting its potential as a safer herbal alternative for UV-induced skin inflammation.

Conclusion

The present study demonstrated that the hydroalcoholic extract of BG leaves can be successfully formulated into a stable Carbopol-based topical gel with acceptable physicochemical properties. The developed formulation significantly attenuated UV-induced oxidative stress and inflammatory responses in Wistar rats. The 5% gel showed superior antioxidant and anti-inflammatory activity compared to the 1% formulation and was comparable to standard hydrocortisone treatment. Overall, BG gel may serve as a promising natural therapeutic alternative for UV-induced skin inflammation.

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