WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
Coden USA: WJPRAP Impact Factor 8.453
Volume 14, Issue 20, 688-697. Research Article ISSN 2277–7105

ANTI-INFLAMMATORY AND ANTI-APOPTOTIC EFFECTS OF BUTTERFLY PEA GEL ON PHOTODAMAGED SKIN

***Dorr Hassan Abdalla Hassan, Dr. K. Jesindha Beyatricks**

Department of Pharmaceutics, Hillside College of Pharmacy, Bangalore-560062, Karnataka, India.

Article Received on 18 Sept., 2025,
Article Revised on 08 October 2025,
Article Published on 16 October 2025,
https://doi.org/10.5281/zenodo.17365884

***Corresponding Author**
Dorr Hassan Abdalla Hassan
Department of Pharmaceutics, Hillside College of Pharmacy, Bangalore-560062, Karnataka, India.

How to cite this Article: Dorr Hassan Abdalla Hassan (2025) Anti-Inflammatory And Anti-Apoptotic Effects Of Butterfly Pea Gel On Photodamaged Skin. World Journal of Pharmaceutical Research, 14(20), XXX–XXX.

ABSTRACT

Ultraviolet B (UVB) radiation is a major environmental factor contributing to skin damage through excessive generation of reactive oxygen species (ROS). This oxidative stress activates inflammatory pathways, increases pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α), and triggers apoptosis via caspase-3, ultimately leading to photodamage and premature skin aging. Plant-derived antioxidants have been extensively investigated for their potential to counteract UVB-induced cellular damage. Clitoria ternatea (butterfly pea) flower extract is particularly rich in flavonoids and anthocyanins, compounds known for strong free radical scavenging activity. Recent animal studies have shown that topical gel formulations of butterfly pea flower extract significantly downregulate TNF-α and caspase-3 expression in UVB-exposed rats, thereby reducing inflammation and apoptosis. These findings suggest that butterfly pea gel may serve as a promising natural approach for protecting the skin against UVB-induced photodamage. **Methods:** Tests were conducted on both the experimental and control groups. There was no UVB exposure in the healthy group. After five days of daily exposure to a minimal erythema dose of 160 mJ/cm2, the negative controls and treatments 1 and 2 received treatment with a gel-based extract that contained 5% and 10% of the extract, respectively. The extraction process's maceration step involved the use of a 96% ethanol solution. On day 14, the levels of gene expression in the skin tissue were assessed using Real-Time Quantitative Reverse Transcription PCR. **Results:** Higher doses of the extract had a bigger effect, and the treatment group's TNF-α and caspase-

3 expression levels dropped. **Conclusion:** The gel extract greatly decreased the UVB-induced TNF-α and caspase-3 production in rats.

KEYWORDS: Butterfly pea, topical gel, anti-oxidant, anti-inflammatory, Ultraviolet B (UVB) Radiation.

INTRODUCTION

UVB radiation is the type of UV light that reaches the upper dermis after passing through the epidermis. There, it increases the concentration of reactive oxygen species (ROS), which damages DNA in skin cells.[1] The release of several proinflammatory molecules, including tumour necrosis factor-alpha (TNF-α), is one indicator of increased inflammation brought on by excessive ROS production. UV irradiation changes dermal collagen through the collagen breakdown pathway (matrix metalloproteinase proteins [MMPs]), and by blocking the procollagen synthesis pathway resulting in the loss of collagen content.3 UV-induced ROS damage DNA, and trigger lipid peroxidation and protein degradation in skin cells. ROS also lessen the activity of antioxidant enzymes in the skin, such as glutathione peroxidase and superoxide dismutase.[2]

The butterfly pea plant (Clitoria ternatea L.) has been traditionally valued for its medicinal properties, with the roots, seeds, and leaves all demonstrating therapeutic potential, particularly in reducing inflammation. The petals of the butterfly pea flower are especially rich in bioactive compounds, containing up to twelve anthocyanins along with various flavonoids, which contribute strong antioxidant activity.

The flower's potential for skin health and rejuvenation is particularly relevant to photodamage, which is caused by exposure to ultraviolet (UV) radiation. Photodamage involves a complex cascade of events, including inflammation and increased cell death (apoptosis). The butterfly pea flower is rich in bioactive compounds, primarily flavonoids and anthocyanins, which are responsible for its therapeutic properties.

Recent research have indicated that butterfly pea extract has significant antioxidant activities that inhibit the production of ROS and reduce inflammation, which inhibits the increase in MMP, avoids fibroblast cell apoptosis, and inhibits the decrease in collagen.[1]

Another study showed that anthocyanins are potent antioxidants that, when taken orally or topically, lower ROS.[3]

The impact of butterfly pea extract on TNF-α and caspase-3 expression levels in skin with low collagen levels brought on by UVB exposure has not, however, been investigated in any research.[3]

Thus, this work investigated how the expression levels of TNF-α and caspase-3 in Wistar rats exposed to UVB were affected by topically applied butterfly pea flower extract gel.

METHODS

MATERIALS AND INSTRUMENTS

Butterfly pea extract, water-based gel, rat caspase-3 primer set (F: 5′-GTGGAACTGACGATGATATGGC-3′; R: 5′-CGCAAAGTGACTGGATGAACC-3′ and TNF-α F: 5′-AAATGGGCTCCCTCTCATCAGTTC-3′; R: 5′ -TCTGCTTGGTGGTTTGCTACGAC-3′), RNA later solution, and neutral-buffered formalin were the materials used in this investigation. A microscope (Olympus, Tokyo, Japan), a 25-watt UVB tool, micropipettes, glassware and tools, a refrigerator (4 °C), a freezer (-20 °C and -80 °C), a probe sonicator, a vortex, and Falcon tubes were among the equipment utilized.

Preparation of butterfly pea flower extract

The butterfly pea flowers were extracted at Diponegoro University's Integrated Laboratory after being bought from Balai Besar Penelitian dan Pengembangan Tanaman Obat dan Obat Tradisional Tawangmangu, Central Java, Indonesia. The maceration procedure with methanol was used to finish the extraction. The floral extract was obtained by drying out the filtrate using evaporation.

Qualitative phytochemical analysis

To find the secondary metabolites, the crude extract was tested. The Wagner method was used to identify alkaloids, the Willstatter method for flavonoids, the Lieberman–Burchard method for triterpenoids, and 1% FeCl3 for tannins.

Rats exposed to UVB

The Bioethics Commission for Medical/Health Research, Faculty of Medicine, Sultan Agung Islamic University, Semarang, approved all animal studies under Komisi Bioetik No. 304/VIII/2022. We purchased twenty-four male Wistar rats (Rattus novergicus), weighing 200 g and three months old, from the Islamic University of Sultan Agung's Faculty of Medicine. Prior to the start of the trial, the rats were assessed and placed in quarantine for

three days. Every rat was given a regular food and unlimited water, and they were split up into four groups of six. Rats with UVB-exposed collagen were included in the first, second, and third groups. There was no UV exposure for the fourth group. To optimize the effects of UVB radiation on the skin, the rats' left dorsum hair was shaved.

A total of 800 mJ/cm2 of UVB was administered five times at an intensity of 160 mJ/cm2/day.

Preparation of the topical gel therapy

The epidermis and dermis were altered by topically treating the skin's surface. A water-based gel with topical therapeutic dosages of 5% and 10% was created. The rats were given the topical gel extract once daily for two weeks following day six of UVB exposure. A 50 mg sample of skin tissue was used to extract total RNA using the FAVORGEN RNA isolation kit. 25 μg of total RNA from the skin samples was converted to cDNA using the ReverTra AceTM qPCR RT Master Mix. Rat-specific primers and SYBR Green DNA polymerase were used in a polymerase chain reaction analysis to measure the expression levels of TNF-α, caspase-3, and the housekeeping gene glyceraldehyde-3-phosphate dehydrogenase. The annealing step involved 40 cycles of the reaction at 60 °C.

Statistical analysis

SPSS software (version 20.0; SPSS Inc., Chicago, IL, USA) was used to analyze the data. To find differences between the groups, one-way analysis of variance and a post-hoc test were used in the comparative analyses. A p-value of less than 0.05 was deemed significant.

RESULTS

The extract of butterfly pea flowers contains bioactive substances. The qualitative test used colorimetry as a phytochemical screening method. By observing the hues that each component created, the secondary metabolites (phytochemical substances) were evaluated. assays including flavonoid, alkaloid, saponin, tannin, steroid, and triterpenoid assays were included of the phytochemical screening. The presence of the significant classes of phytochemicals in an extract can be determined with the help of this kind of phytochemical screening study. The findings suggested that flavonoids in butterfly pea flower extract might be a possible source of antioxidant action. The total flavonoid content of the ethanol and ethyl acetate extracts of butterfly pea flowers was examined using a quantitative flavonoid test.

Table 1 displays the findings of three measurements of the total flavonoid levels. A sample of butterfly pea flower extract had an average total flavonoid concentration of 682,0238.

Table 1: Total flavonoids in the butterfly pea flower.

Extract Concentration	Total Flavonoid Compound
1000 ppm	690,2143
1000 ppm	671,6429
1000 ppm	684,2143

Collagen density in the skin of UVB-exposed rats is increased by butterfly pea flower extract gel.

In this study, rats were exposed to UVB light at an intensity of 160 mJ every 15 minutes for five days in a row, and then euthanized under anesthesia. Skin tissue samples were examined using Masson's trichrome staining to measure collagen density, and the results showed that the collagen expression in UVB-exposed rats was significantly lower than in healthy controls. As shown in Figure 1, healthy rat skin had a higher collagen density (shown by the blue staining) than the UVB-irradiated rat skin.

TNF-α and caspase-3 expression levels in dermal fibroblasts Real-Time Quantitative Reverse Transcription (PCR) was used to examine the impact of the butterfly pea flower extract topical gel on the relative level of TNF-α mRNA expression. The treatment group's dorsal skin showed a substantial decrease in TNF-α and caspase-3 mRNA expression levels compared to the control group. Nonetheless, the UVB group exhibited greater levels of TNF-α and caspase-3 mRNA expression compared to the therapy group.

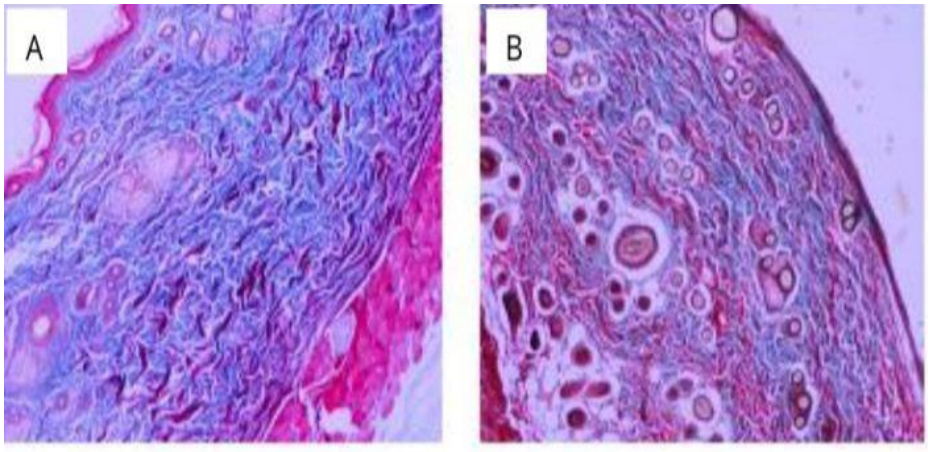
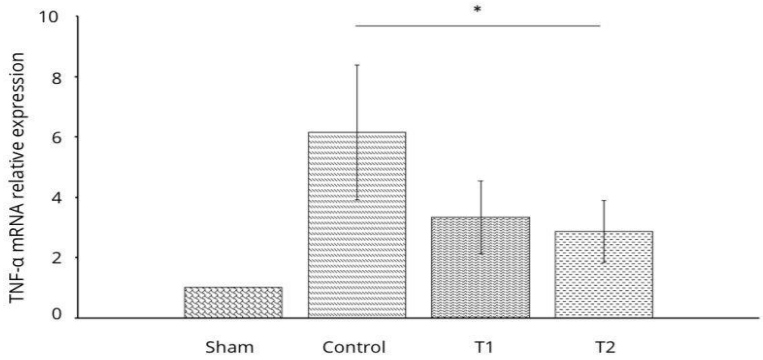
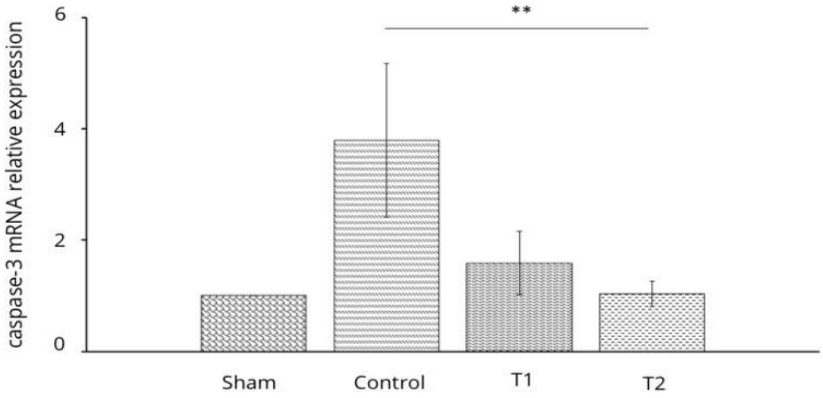


Figure 1: (A) Healthy rat collagen (B) Reduced collagen density caused by UVB exposure of rat skin tissue.



*significant difference in the control group ($p < 0.05$); indicates a significant difference in the UVB group (N = 6 per group).

Figure 2 shows the relative amounts of TNF-α expression in the dorsal skin as determined by real-time polymerase chain reaction: (Control) group with UV-exposed collagen loss, (Sham) group not exposed to UV radiation, (T1) group with collagen exposed to UVB-treated topical gel from 5% of butterfly pea extract, (T2) group with collagen exposed to UVB-treated topical gel from 10% extract of butterfly pea, and (Control) group with collagen exposed to UVB-treated topical gel.



*significant difference in the control group ($p < 0.01$); indicates a significant difference in the UVB group (N = 6 per group).

Figure 3 shows the relative amounts of caspase-3 expression in the dorsal skin as determined by a real-time polymerase chain reaction.

(Control) group with UV-exposed collagen loss, (Sham) group not exposed to UV radiation, (T1) group with collagen exposed to UVB-treated topical gel from 5% of butterfly pea extract, (T2), and group with collagen loss exposed to UVB-treated topical gel from 10% of butterfly pea extract.

Figure 2 displays the TNF-α gene expression level analysis results on day 14 following UVB exposure. The results demonstrate that the expression of TNF-α in rat skin exposed to UVB was reduced by the gel topical therapy based on butterfly pea flower extract. Although it was not significant when compared to T1 ($p > 0.05$), the TNF-α gene expression level in T2 was significantly different from that of the control and healthy groups ($p < 0.05$). T1 was considerably different from the healthy rats ($p < 0.05$), but not statistically different from the control ($p > 0.05$), according to the findings.

Figure 3 displays the findings of the investigation of the caspase-3 gene expression level on day 14 following UVB exposure. The findings demonstrate that topical gel therapy based on butterfly pea flower extract reduced caspase-3 expression in rat skin exposed to UVB rays. The expression of the caspase-3 gene was not significant when compared to T1 ($p > 0.05$; Mann-Whitney), but it was significantly different in T2 from that of the control and healthy groups ($p < 0.05$).

Additionally, the results indicates that T1 differed considerably from the healthy rats ($p < 0.05$) but not significantly from the control ($p > 0.05$).

DISSCUSION

UV rays cause tissue damage by activating signal transduction pathways and increasing the generation of ROS. Furthermore, ROS generated by UVB irradiation raises the concentrations of inflammatory factors such TNF-α, which modulate cell apoptosis by activating caspase-3, causing a decrease in collagen and other skin problems.[4,5]

It has been suggested that the butterfly pea flower possesses strong antioxidant properties. A coffee extract's high flavonoid content, including anthocyanins, quercetin alkaloids, saponins, and tannins, determines its antioxidant activity. Because they absorb UV rays 31.99 and function as antioxidants and anti-inflammatory substances, anthocyanins, a family of flavonoids, have the potential to be photoprotective agents.[6,13]

In the current investigation, photodamage was prevented by topically administering 10% butterfly pea flower extract gel, which significantly decreased TNF-α levels ($p < 0.05$) and downregulated the expression levels of the TNF-α and caspase-3 genes. The TNF-α level may have dropped as a result of the flavonoids in the butterfly pea flower extract.

According to a prior study, anthocyanins play a part in Nrf-2 activation, which directly deactivates NF-kB.[8]

According to recent research, NF-B is essential for the emergence of skin inflammation brought on by UVB radiation. Skin inflammation in response to UVB rays may be reduced by suppressing NF-kB expression. According to earlier research, NF-kB activation causes the release of inflammatory cytokines such TNF-α, interleukin (IL)-6, and interferon-γ. The cytokine TNF-α, which is released by local macrophages during infection and cellular stress brought on by outside causes like UV light exposure, is one of the triggers that activates the NF-kB signaling pathway. When activating stimuli are not present, NF-kB dimers are kept in the cytoplasm in conjunction with inhibitory members of the NF-kB inhibitory (IkB) protein family.[9,10]

UVB exposure releases the NF-KB bonds and inhibiting factors so that NF-kB is activated. Previous studies have reported the role of a butterfly pea flower extract in inhibiting the expression of NF-kB. Suppressing the transcription factor NF-kB is associated with the cleavage of the inflammatory cytokine pathway, including TNF-α. This aligns with the results of this study, which found a decrease in TNF-α expression after the administration of butterfly pea flower extract gel.[11,12]

Cell death is regulated by TNF-α, which also signals the control of immunological homeostasis. Caspases are a cascade of apoptotic cysteine proteases that initiate and carry out apoptosis, and they are strongly linked to the TNF-α-associated apoptotic process. Utilizing the following Fas Associated Via Death Domain adapter protein, the TNFRSF1A Associated Via Death Domain adapter protein receives the death signal from the TNF-α receptor and controls The signaling cascade that causes death activates caspase-8 to caspase-3, which results in cell death. The inhibited production signal of apoptotic enzymes, such as caspase-3 is associated with lower TNF-α expression.[17]

Additionally, the T2 group's level of caspase-3 expression was found to be much lower than that of the control group. This outcome might stem from group 2's lower level of TNF-α expression. This finding is consistent with earlier research showing that giving butterfly pea flower extract gel to UVB-exposed mice results in decreased levels of caspase-3 and TNF-α expression in their skin tissue, which may have significance for preventing skin cell death.

Mason's trichrome staining revealed that the test group of rats' collagen density was lowered by UVB exposure, as seen by a less blue tint on the stained slides. This result shows that after 5 days of treatment at a level of 160 Mj/cm2 for 15 minutes daily, UVB was able to successfully generate an inflammatory response of the skin.

UVB irradiation led the epidermis to produce ROS, which in turn released IL-6, and an excess of MMPs that were activated by the protein transcription factor AP-1 MMPs reduce collagen density and break down collagen such ,as the collagen level was not verified in the skin.

CONCLUSION

The butterfly pea flower extract gel proved useful as a topical treatment for photodamage induced by excessive decreases in caspase 3 and TNF-α gene expression levels on the UVB-exposed skin of rats. This study will serve as the basis for additional practical research, leading to the production of photoaging products, such as hyperpigmentation therapy.

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