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Inhibitory Effects of Petai Peel Extract Gel on Tyrosinase and TRP1 Gene Expression in UVB-Exposed Mouse Skin

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ABSTRACT

Background: UVB irradiation can induce the formation of Reactive Oxygen Species (ROS), which causes the activation of melanin synthesis through the activation of tyrosinase and tyrosinase-related protein-1 (TRP1). Secondary metabolites in stink bean peel extract inhibit ROS production due to exposure to UVB rays. This study aims to determine the effect of administering stink bean peel extract gel on the expression of the tyrosinase and TRp1 genes in mouse skin tissue exposed to UVB.

Method: The research design was a posttest-only control group with a completely randomized design method. The samples studied were 24 mice exposed to UVB light with a wavelength of 302 nm and an energy of 390mJ/cm2/day 3 times a week for 2 weeks. This research was carried out in four groups: the healthy group, the negative control group, treatment 1 (T1) with 10% stink bean peel extract gel, and treatment 2 (T2) with 20% stink bean peel extract gel. Tyrosinase and TRP1 gene expression were analyzed using qRT-PCR.

Results: qRT-PCR analysis showed that there was a significant decrease in tyrosinase and TRP1 gene expression between groups T1 (tyrosinase 3,19±2,12 and TRP1 4,96±3,42) and T2 (tyrosinase 0,65±0,44 and TRP1 2,22±1,18) compared to negative control (tyrosinase 17,92±3,77 and TRP1 35,91±4,52).

Conclusion: The administration of stink bean peel extract gel has shown promising results in reducing the expression of tyrosinase and TRP1 genes in hyperpigmentation mice exposed to UVB light. This suggests that stink bean peel extract could be a safe and effective therapeutic approach for preventing UVB-induced hyperpigmentation.

Keywords : UVB exposure, stink bean peel extract, tyrosinase, TRP1

INTRODUCTION

Hyperpigmentation is the formation of black pigment in the skin due to increased melanin caused by exposure to ultraviolet B (UVB) radiation [1]. UVB radiation can stimulate melanin production in skin cells, especially melanocytes, responsible for creating a black color in the skin [2]. Tyrosinase and tyrosinase-related protein 1 (TRP-1) are enzymes involved in melanin biosynthesis [3]. Tyrosinase plays a role in the early stages of melanin synthesis, while TRP1 is involved in the maturation and distribution of melanin [4]. Overexpression of these enzymes can lead to excessive melanin production, contributing to hyperpigmentation [5]. Some standard hyperpigmentation therapies, such as retinol, hydroquinone, and tranexamic acid, can cause cancer with long-term use [6]. Therefore, safe and effective therapeutic approaches are needed to prevent UVB-induced hyperpigmentation, one of which is using natural antioxidant compounds.

The use of chemical agents such as arbutin, azelaic acid, kojic acid, and hydroquinone in preventing hyperpigmentation has been reported to have adverse side effects, including genotoxicity, skin irritation, contact dermatitis, and increased risk of skin cancer [7]. In 2015, about 4.2% of 142 subjects exposed to three times the minimal erythema dose (MED) of UVB 390mj/cm2 experienced hyperpigmentation [8]. In Indonesia, hyperpigmentation cases account for about 0.25-4% of all skin disease cases [9,10]. Standard treatment for skin hyperpigmentation focuses on physical and chemical protection against UVB exposure without affecting melanin production pathways such as tyrosinase and TRP1 [7]. Natural antioxidants have been proven to inhibit melanin synthesis and prevent hyperpigmentation [11]. One natural antioxidant is the extract of Parkia speciosa, known as stink bean or bitter bean. Parkia speciosa skin contains bioactive compounds such as flavonoids and tannins, which have been proven to have antioxidant and anti-inflammatory activities [12,13]. Polyphenol compounds in stink bean skin, such as cinnamic acid, ferulic acid, caffeic acid, and ferulic acid, have antioxidant activity by inhibiting melanocyte degradation and preventing hypermelanogenesis [13,14]. Previous research has reported that antioxidant compounds can inhibit the expression of tyrosinase and TRP1 in melanocyte cells, preventing excessive melanin production [15]. However, no studies have examined the effect of stink bean skin extract on tyrosinase and TRP1 gene expression in UVB-induced hyperpigmentation models.

Previous research has shown that the administration of extracts containing flavonoids and polyphenols, either through topical application or oral administration, can reduce free radical levels, which has the potential to prove the effect of stink bean skin extract on tyrosinase and TRP1 gene expression in rat skin experiencing UVB-induced hyperpigmentation [16]. Based on this information, this study aims to evaluate the effect of stink bean skin extract administration on tyrosinase and TRP1 gene expression in Wistar rats experiencing UVB-induced hyperpigmentation.

MATERIAL AND METHODS

Animal ethics

This investigation utilized a post-test-only control group design with complete randomization, incorporating six replicates per treatment. Male Wistar rats (Rattus norvegicus) aged 2-3 months, weighing 200-250 grams, served as the research subjects. The study received approval from the ethical committee at the Faculty of Medicine, Universitas Islam Sultan Agung Semarang (No. 448/XI/2023/Komisi Bioetik).

Study design

This study comprised four groups: the control group is healthy rats without UVB exposure; the negative control is UVB-exposed rats treated with base gel; treatment 1 (T1) is UVB-exposed rats treated with 10% stink bean peel extract gel; and treatment 2 (T2) is UVB-exposed rats treated with 20% stink bean peel extract gel. The sample size was six rats per group.

Stink Bean Extract Preparation

Approximately 600g of stink bean peel was processed, dehydrated, and ground into powder. The powder underwent maceration with 70% ethanol, followed by filtration and re-maceration. A rotary evaporator removed the ethanol, resulting in a thick extract stored at 2-8°C [17–19].

Gel Formulation

The gel was formulated by combining 200mg of gel base with either 10% w/w (20mg) or 20% w/w (40mg) of stink bean peel extract. Lemon essential oil was added to mask the odor, and the mixture was homogenized.

UVB Exposure and Treatment Protocol

After a 5-day adaptation period, rats were anesthetized, and their back hair was shaved. UVB exposure (390 mJ/cm2 MED) was administered six times over two weeks. Treatment groups received daily topical application of the extract gel for 14 days. On day 15, the rats were sacrificed, and skin tissue samples were collected using a biopsy punch, followed by isogenic isolation for qRT-PCR analysis [20–23].

Melanin Visualization

Fontana-Masson staining was employed to visualize melanin in skin tissue samples, with melanin granules appearing black against a pink background.

qRT-PCR analysis of tyrosinase and TRP1 gene

RNA extraction from skin tissue samples was performed using TRIzol® reagent (Invitrogen Life Technologies). At the same time, cDNA synthesis was carried out using the iScript cDNA Synthesis Kit (Bio-Rad iScript gDNA Clear cDNA Synthesis Kit) with a C1000 Thermal Cycler (Bio-Rad) for Reverse Transcriptase PCR (RT-PCR). To determine the expression of tyrosinase and TRP1 genes, the PCR-RFLP technique was employed using 2x PCR Master mix solution (iNtRON®, catalog number 25027) in 0.2 mL vials with a total volume of 50 µL per sample and the PCR was conducted using an Applied Biosystems Veriti thermal cycler. The PCR mix components included specific forward and reverse primers for tyrosinase and TRP1 genes, TRIzol reagent, High-Capacity cDNA Reverse Transcription kit, and SYBR Green for amplification. The expression levels of tyrosinase and TRP1 genes were calculated as a ratio compared to the expression of the housekeeping gene GAPDH, with the final measurement expressed as the mRNA level ratio of the target genes to the housekeeping gene [24–28].

Statistical Analysis

Data analysis was performed using SPSS 22.0. Normality and homogeneity were assessed using Shapiro-Wilk and Levene's tests. One-way ANOVA with Post Hoc Turkey's and Shapiro Wilk and Levene tests were used for normally distributed data with homogeneous variance, respectively. Non-normal or non-homogeneous data were analyzed using Kruskal-Wallis and Mann-Whitney tests. Statistical significance was set at $p<0.05$.

RESULTS

Stink Bean Peel Extraction

The stink bean peel extract in this study was obtained through maceration using ethanol as a solvent, yielding an extract yield of 8.00%. Phytochemical screening results of the stink bean peel extract showed that it positively contained compounds from the phenol, phenolic, tannin, flavonoid, terpenoid, and saponin groups (Appendix 2). In this study, the determination of total flavonoids and phenolics in the stink bean peel extract was also conducted using spectrophotometric methods. One

gram of stink bean peel extract contained 65.27mg ± 1.20 flavonoids and 44.70mg ± 1.22 phenolics. These results prove that most compounds in the stink bean peel extract are from the flavonoid group.

Validation of Hyperpigmentation Model

This study we used a hyperpigmentation model. The animal models were induced with hyperpigmentation through UVB irradiation at 302 nm with an energy intensity of 390mJ/cm2 three times a week for two weeks. Hyperpigmentation validation was observed on day 14 in one healthy rat and one negative control rat. Fontana-Masson staining showed a significant increase in melanin production, marked by brown pigment in the epidermis (melanocyte cells). In the group given UVB irradiation (negative control), the amount of melanin increased by up to 46.5% (Figure 1).

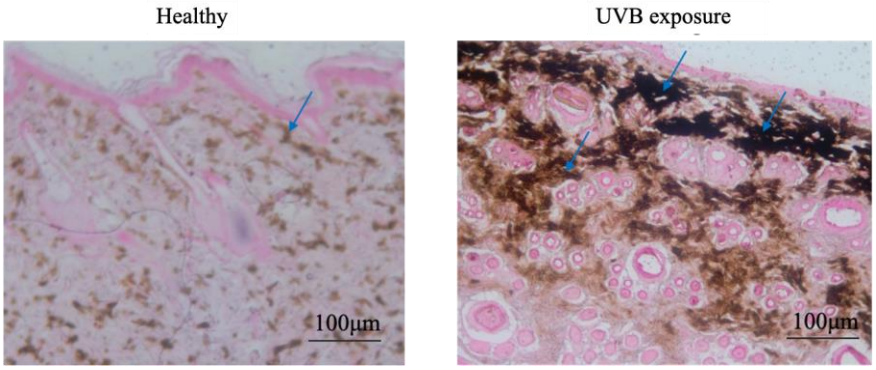

Figure 1. Validation of photoaging using Fontana-Masson staining (left) Healthy rats and (right) Rats exposed to UVB irradiation. Blue arrows indicate positive melanin, 100x magnification.

Effect of 10% and 20% Stink Bean Peel Extract Gel on Tyrosinase Gene Expression

In this study, we found that stink bean peel extract gel was able to significantly decrease the expression of tyrosinase and TRP1 genes in hyperpigmentation model rats in a dose-dependent manner. Based on the research results in Table 5.1, the mean tyrosinase gene expression in group T2 was the lowest (0.65±0.44), followed by the mean tyrosinase gene expression in group T1 (3.19±2.12). The negative control treatment group had the highest ratio at 17.92±3.77 (Figure 2). The mean expression value of the healthy group was used as the baseline ratio value, so all treatment groups were compared to the healthy group with a ratio value of one. Stink bean peel extract gel administration reduced tyrosinase gene expression in male Wistar rats with a hyperpigmentation model.

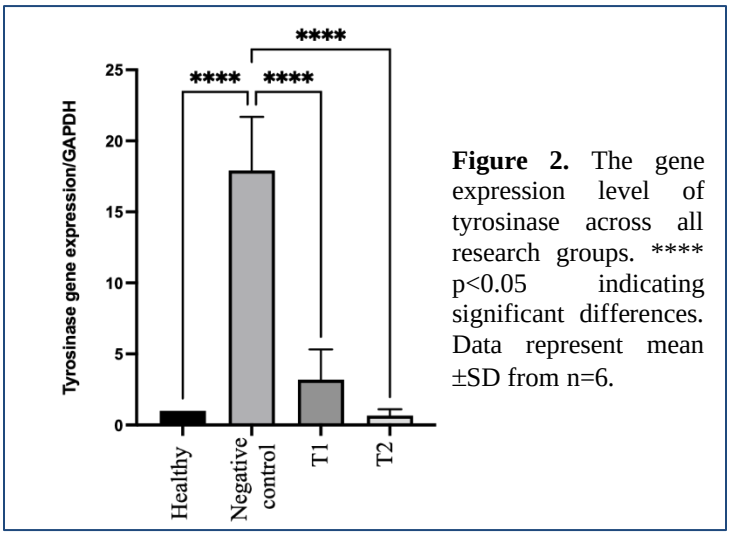

Figure 2. The gene expression level of tyrosinase across all research groups. **** $p<0.05$ indicating significant differences. Data represent mean ±SD from n=6.

Effect of 10% and 20% Stink Bean Peel Extract Gel on TRP1 Gene Expression

In this study, we found that stink bean peel extract gel could significantly decrease the expression of the TRP1 gene in hyperpigmentation model rats in a dose-dependent manner (Figure 3). The mean TRP1 gene expression in group T2 (2.22±1.18) was the lowest, followed by the mean TRP1 gene expression in group T1 (4.96±3.42). The negative control group had the highest TRP1 gene expression data at 35.91±4.52 (Figure 3).

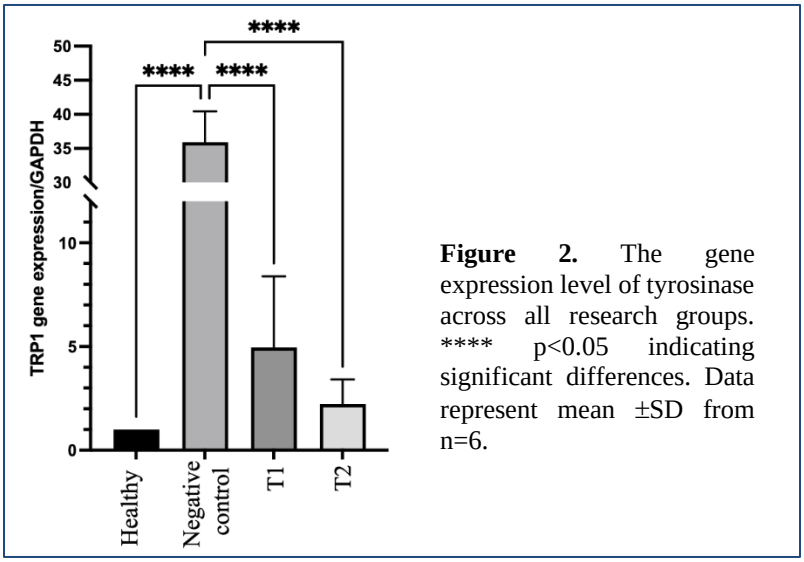

Figure 2. The gene expression level of tyrosinase across all research groups. **** $p<0.05$ indicating significant differences. Data represent mean ±SD from n=6.

DISCUSSION

UVB radiation exposure is a significant risk factor for skin hyperpigmentation, characterized by increased expression of melanin-forming enzymes such as tyrosinase, TRP1, and TRP2 [29,30]. UVB radiation has increased oxidative stress due to DNA damage, activating melanin formation pathways such as the nuclear factor kappa beta (NF-kB) pathway and the melanocyte-inducing transcription factor (MITF) pathway [31,32]. In the melanogenesis process, tyrosinase plays a role in converting L-tyrosine to L-3,4-dihydroxyphenylalanine (L-DOPA) [33]. L-DOPA is then oxidized to L-DOPAquinone, forming eumelanin and pheomelanin, causing skin darkening. Recent research confirms that stink bean peel extract containing various secondary metabolites such as flavonoids, tannins, and saponins can suppress ROS formation due to its antioxidant activity [34–36]. The extract's ability to suppress ROS could prevent melanin production [37,38].

This study aimed to determine the effect of stink bean peel extract gel on tyrosinase and TRP1 gene expression in a rat hyperpigmentation model. Test animals were exposed to UVB radiation at 302 nm with an energy intensity of 390mJ/cm2 thrice a week for two weeks. The results showed that in the negative control group, there was an increase in melanin content in rats up to 46.5%. The application of stink bean peel extract gel was proven to significantly decrease tyrosinase and TRP1 gene expression in group T1 (10% stink bean peel extract gel) and T2 (20% stink bean peel extract gel) compared to the hyperpigmentation control group (negative control). This indicates that stink bean peel extract gel can prevent hyperpigmentation by decreasing tyrosinase and TRP1 gene expression.

Secondary metabolite compounds from stink bean peel extract, such as flavonoids, phenolics, saponins, tannins, and terpenoids, are thought to inhibit MITF activity by inhibiting the PI3K/Akt pathway [4,32,39]. Previous research has also reported that flavonoid compounds can inhibit TGF-β through the cAMP/protein kinase A pathway and induce GLI2, then suppressing MITF, the central transcription factor in melanogenesis [40]. Inhibited MITF will prevent tyrosinase enzyme synthesis, so L-tyrosine cannot be converted to L-DOPA. Decreased L-DOPA levels have also been reported to decrease TRP1

and TRP2 expression [41]. Secondary metabolite compounds in the extract, such as flavonoids and phenolic compounds, are reported to inhibit TGF-β, thereby suppressing melanogenesis by signaling through specific ligand heteromeric receptors. These serine/threonine kinase receptors phosphorylate and activate (R)-Smad receptors. This leads to the formation of complexes with (Co)-Smad, Smad4, and regulation of target gene transcription, which ultimately suppresses the expression of melanogenesis-regulating enzymes such as tyrosinase, TRP1, and TRP2 [4,30].

Stink bean peel extract inhibits tyrosinase, inhibiting TRP1 and TRP2 activities, which correlate with eumelanin and pheomelanin formation pathways. Suppression of excessive TRP1 expression can inhibit melanin synthesis. Previous research has reported that increased TRP1 expression correlates with increased melanin due to UVB irradiation [30,42]. However, increased TRP2 expression is associated with melanoma cell proliferation. In this study, the application of stink bean peel extract gel significantly and dose-dependently prevented hyperpigmentation. This indicates that applying stink bean peel extract gel decreases tyrosinase and TRP1 gene expression, thus preventing melanin formation in melanocyte cells.

CONCLUSION

In conclusion, this study provides strong evidence for using stink bean peel extract gel as a natural and effective treatment for UVB-induced hyperpigmentation. These findings open new avenues for developing novel, plant-based skincare products for managing hyperpigmentation disorders.

Authors' contributions

HP, TS, CC and AP contributed to the conception of the work. NDA, SAH, SP, and MF contributed to the acquisition of the work. HP and NDA contributed to the analysis and interpretation of data. AP, CC and NDA contributed to drafting the work. HP, MF and SAH contributed to revising the work critically. NDA contributed to the revising of the manuscript. TS and AP is responsible for giving the final approval of the manuscript.

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Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Competing interests

The authors declare that they have no competing interests.

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