

The effect of 10% pegagan (centella asiatica (L.) urban) extract cream on melanin amount in the guinea pig skin vitiligo model

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Abstract

Background Pegagan is one of the plants used by the community for medicinal purposes; it contains a variety of compounds, including the following: Pegagan contains antioxidant and anti-inflammatory effects. The in vivo effects of repigmenting chemicals generated from centella asiatica extract have not been explored prior to this study.

Objective To demonstrate that 10% pegagan extract cream affects melanin levels in guinea pig models of vitiligo.

Methods This is a strictly experimental study with a post-test-only control group design involving the administration of 10% centella asiatica extract cream. In this study, 16 male guinea pigs were divided into two groups. Both groups of guinea pigs had cream containing hydroquinone administered to their backs daily for eight days. The control group then did not get any treatment. The treatment group received a 10% Pegagan extract lotion for fourteen days. Histopathological examination of tissue stained with Masson-Fontaine. The amount of melanin is based on the ratio of pixels in the melanin area to the total number of pixels in the epidermal tissue.

Results The group that got 10% Pegagan extract cream had the highest concentration of melanin, and the difference was statistically significant (p0.05).

Conclusion The conclusion of this study is that 10% centella asiatica extract cream improves the level of melanin in the skin of guinea pigs with a vitiligo model.

Key words

Centella extract; Melanin; Hydroquinone.

Introduction

Vitiligo is an acquired skin pigmentation disorder characterized by macules or patches of depigmentation that are well-defined and result from the loss of melanocytes. The complex pathophysiology of vitiligo is largely unknown.¹ According to commonly accepted hypotheses of the pathogenesis of vitiligo, hereditary and non-

genetic variables combine to alter the function and survival of melanocytes, which consequently lead to autoimmune melanocyte annihilation.^{2,3} Several explanations, including genetic, immunological, neurological, and biochemical hypotheses associated to oxidative stress and reactive oxygen species (ROS), support the theory of the pathogenesis of vitiligo.^{1,4,5}

Cosmetic issues and psychological burdens have a substantial impact on the quality of life of vitiligo patients, particularly those with dark

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skin. The impact of vitiligo on a patient's quality of life includes diminished social function, decreased productivity, and a loss of self-confidence; hence, they require the most effective therapy.^{6,7}

Suman et al. (India, 2021) discovered that 92.5% of vitiligo patients have higher Dermatology Life Quality Index (DLQI) and depression scores, which are substantially related with reduced life quality.⁸ Asri E et al. (Indonesia, 2019) found a correlation between the severity of vitiligo and the quality of life among 34 vitiligo patients who received treatment at the Dermatology and Venereology Polyclinic at Dr. M. Djamil Padang Hospital.

The modalities of vitiligo treatment include medicinal, surgical techniques, phototherapy with Narrow Band Ultraviolet B (NB-UVB), topical and systemic corticosteroids, topical calcineurin inhibitors, immunomodulators, and antioxidant therapy.^{10,11} There is currently no one therapeutic technique that is both successful and satisfactory in the treatment of vitiligo and has minimum side effects, making vitiligo therapy a problem in the field of dermatology.¹² The lack of animal models for vitiligo hinders understanding of the disease pathophysiology and the development of treatments.¹³

Utilizing hydroquinone, monobenzone, and hydrogen peroxid (H₂O₂), researchers are attempting to create an animal model of vitiligo. However, test animals do not perfectly resemble human vitiligo, making it more challenging to design novel medications.¹⁴⁻¹⁷

Hydroquinone concentrations of 2% to 5% can promote depigmentation and inflammation at the site of exposure within three to four weeks. Within 8-10 days of exposure to 2%-5% hydroquinone, depigmentation can occur.¹⁸ By promoting melanin formation and preventing

melanin from dissolving and destroying itself, natural substances have therapeutic potential as vitiligo treatments. Gamier, green tea, turmeric, and centella asiatica are some of the natural components that are used. Centella asiatica is a tropical annual plant that blooms continuously throughout the year.¹⁹⁻²¹

Centella asiatica is growing in popularity in Indonesia due to its numerous health advantages. Centella asiatica has grown in popularity as a natural skincare component with numerous advantages for the skin. Capsules, lotions, powders, and other forms of market-available medications have been encountered.²²

Methods

This is an experimental study with a control group design consisting only of post-test measures.

In the form of a local guinea pig strain, the sample represents a portion of the population (*Cavia porcellus*). Inclusion criteria: Healthy, weighing between 250 and 350 grams, male gender, about 2-3 months old. Exclusion criteria: they passed away while the study was still being conducted. Based on these estimates, the minimum number of samples necessary for each experimental group is 16 guinea pigs. This study sample consisted of two groups, therefore a minimum of 32 guinea pigs were necessary for the experiment.

Hydroquinone 5% Cream Depigmentation

All groups of guinea pigs had their back hair cut, and subsequently lotion containing 5% hydroquinone was applied to both groups for eight days. The treatment group was then given a cream containing 10% Centella asiatica extract, while the control group received no therapy. Following this, euthanasia was

administered, and skin tissue was removed from his back. The tissue was subsequently submerged in a formalin solution containing 10% formaldehyde. To analyze cells containing melanin, histological preparations were produced from guinea pig skin.

Cream Application

The treatment group guinea pigs received 10% Centella asiatica extract cream, which was administered once daily to a maximum of 0.2 mg/cm² of their skin surface for 14 days.

Histopathological analysis

A biopsy located on the back of samples was conducted in order to obtain microscopic preparations. Microscopic preparations were created using the paraffin method with Masson Fontana staining.

Results

Masson-Fontana stain contains black melanin. **Figure 1** depicts the description of melanin in the epidermal tissue of guinea pig skin under 400x magnification and image J analysis. There are apparent black granules, a red nucleus, and pale pink cytoplasm.

In the control group, melanin pigment levels were lower than in the treatment group, which received a 10% centella asiatica extract cream. The proportion of epidermal melanin was then estimated using the average percentage in specific area. Using image J software, the area of melanin pigment in the basal layer of the epidermis was quantified in both groups, with the control group measuring 0.191% and the treatment group measuring 1.717% (**Figure 2**). This indicates that 10% centella asiatica extract cream affects the amount of melanin in guinea

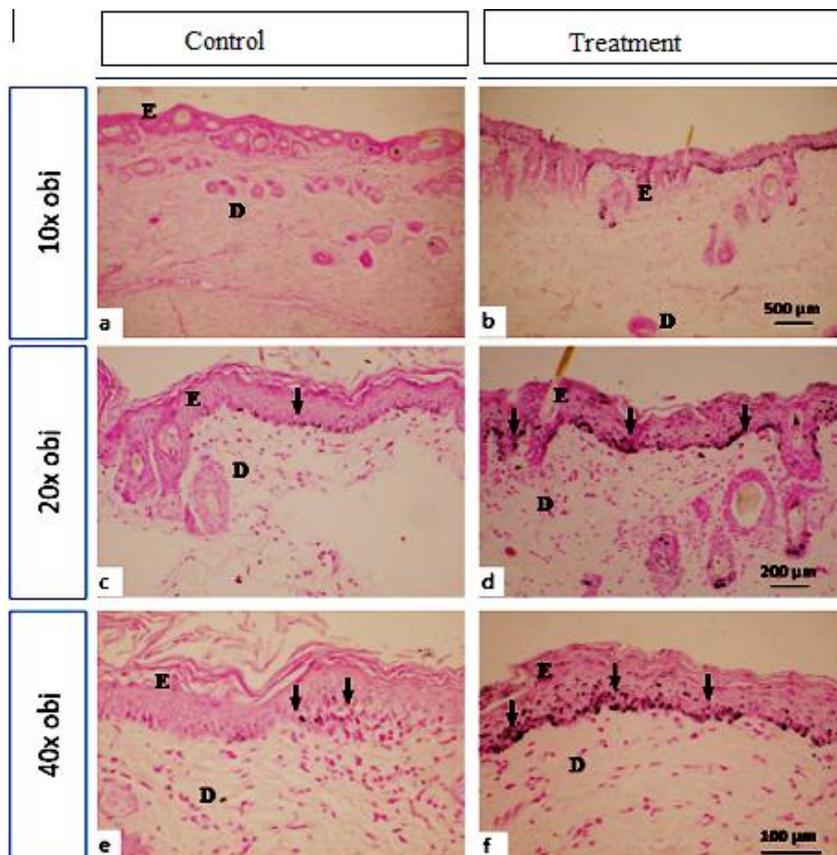


Figure 1 The epidermis (E) and dermis (D) are visible in the photomicrograph of guinea pig skin tissue (D). The Fontana Masson stain () discolors melanin, notably in the basal epidermis and hair follicles, to a dark brown color. The control group (a, c, and e), as well as the treatment group (b,d,f). In the control group, the epidermis was visible and the basal layer had little melanin. In certain areas, there was no noticeable pigment. Compared to the control group, the treatment group with 10% Centella asiatica extract cream showed a higher density of melanin in the basal layer. The upper panel consists of a 20x objective Fontana Masson and bottom panels are 40x.

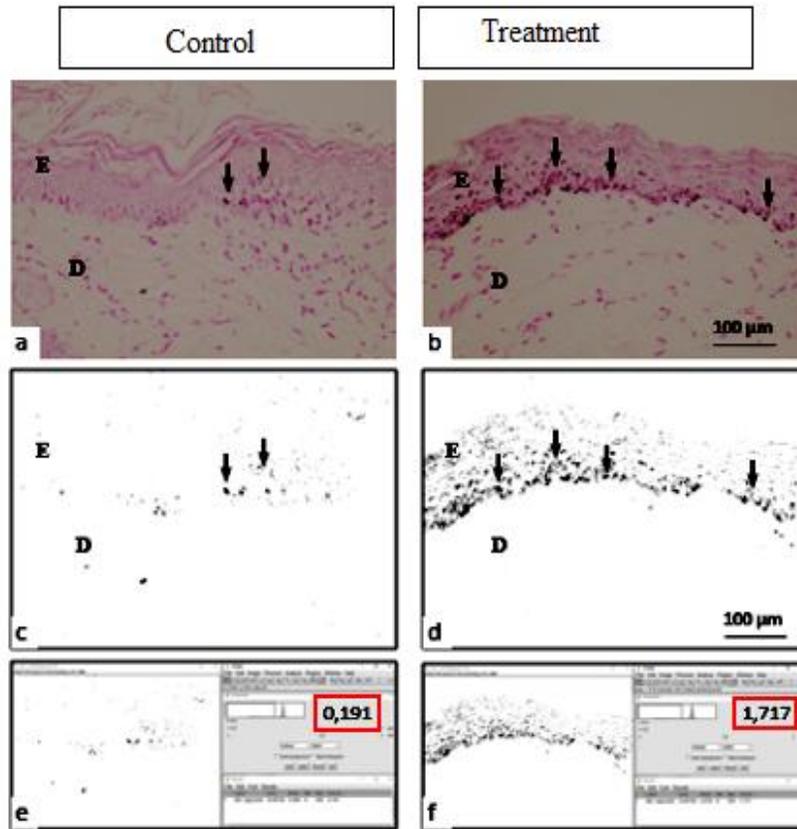


Figure 2 Evaluation of the proportion of melanin in guinea pig epidermis. Pig skin including both the epidermis and dermis (D). Due to melanin, the epidermis is stained black by the Fontana Masson stain. The proportion of regions containing melanin pigment was determined at 400x magnification. ImageJ was used to identify and quantify the proportion of black-stained spots on the Fontana Masson preparation for the evaluation (ImageJ 1.49v software, National Institute of Health, Bethesda, MD, USA). Hydroquinone control group (a,c,e), and Centella asiatica treatment (b,d,f). ImageJ examination of the percentage of melanin area revealed that the Centella asiatica group had a higher percentage of melanin area than the control group. From top to bottom; Fontana Masson, melanin area isolation, proportions of melanin area evaluation.

pig skin, suggesting that centella asiatica extract is useful for increasing melanin in guinea pigs with vitiligo. The statistical analysis found a statistically significant increase in melanin levels in the treatment group, which supports this conclusion.

Discussion

Extensive study has been undertaken on antioxidant effect of Centella asiatica, with studies revealing that it contains significant antioxidant activity and neuroprotective benefits.²³ The research conducted by Pitelli et al. (Brazil, 2009) demonstrated that the antioxidant activity of centella asiatica extract was linked to its phenolic and flavonoid content, as well as its anticancer potential against cancer cell lines by reducing lead-induced oxidative stress in male rats. Extracts of centella asiatica that are rich in flavonoid components have the

most potent antioxidant properties. To allow its employment as a component in the development of innovative drugs and as a source of antioxidant and antitumor medicinal raw materials.

As a model that reflects the functioning of human body systems, the guinea pig was chosen as an investigational sample. Guinea pigs are ideal as research test animals because they are readily available, affordable and biologically similar to humans in that they contain both pheomelanin and eumelanin. Compared to mice and rabbits, guinea pigs are more timid, do not bite, and cannot climb or jump; hence, they can be housed in groups relatively easily.²⁴

Using Fontana Masson staining, histochemical analysis of hydroquinone-treated guinea pig skin tissue revealed differences in melanin deposition between the control group and the centella

asiatica extract cream treatment. In the control group, guinea pig skin displayed minimal black-stained melanin deposition in the basal layer, an uneven distribution of melanin along the epidermis and the absence of melanin pigment in certain areas of the epidermis.

In the treatment group with centella asiatica extract cream, the proportion of melanin-containing areas was greater with a higher melanin density and a wider distribution of melanin than in the control group, however the majority of melanin was deposited at the basal and upper spinous layer. An increase in the proportion of pigmented areas revealed an improvement in melanogenesis after exposure to hydroquinone. This protection can occur either directly by increasing melanin production by melanocytes or indirectly by repairing damaged skin tissue with antioxidant effects, consequently restoring the function of skin cells, including melanocytes and keratinocytes in melanin uptake.

This mechanism cannot be verified by the current study and further research is required. An increase in melanin deposition indicates the potential of centella asiatica extract for hypomelanosis, especially chemically-induced hypomelanosis. Centella asiatica is a natural antioxidant that suppresses oxidation reactions by binding with free radicals and highly reactive substances, thus reducing cell damage.²⁵ The antioxidant activity of phenolic substances results from the oxidation-reduction cycle, which is essential for absorbing and neutralizing free radicals and preventing peroxide degradation.²⁶ This is because plant-based antioxidants can protect the skin against reactive oxygen species and free radicals.

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