Induction of Epidermal Pigmentation in ICR Mice through Weekly Sunlight Exposure: A Pilot Experimental Study

Inducción de la pigmentación epidérmica en ratones ICR mediante exposición semanal a la luz solar: un estudio experimental piloto

Asiyah Nanda Che Azit[1], Afiqah Nuwaira Ahmad Yasir[1], Humaira Fatini Mohd Faisal[1], Siti Syairah Mohd Mutalip[1*], Yuslina Zakaria[1], Delima Engga Maretha[2].

Highlights

- Epidermal hyperpigmentation in mice can be effectively induced through controlled sunlight exposure.
- Prolonged sunlight exposure is essential to achieve a visible induction of epidermal hyperpigmentation.
- Increasing the frequency of sunlight exposure sessions enhances the overall pigmentation response in mice.

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SHORT COMMUNICATION

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ABSTRACT

Introduction. One reason for hyperpigmentation is elevated melanin deposition in the epidermis. Studies on hyperpigmentation are being increasingly reported, and one of the challenges of studying hyperpigmentation is to induce a large number of pigments on the epidermis of laboratory mice to mimic the condition in humans. **Objective.** Hence, this study aimed to conduct a pilot study to determine the number of pigments produced on the epidermis following weekly sunlight exposure. **Materials and Methods.** This study involved 40 *Mus musculus* (mice) of the ICR strain that were exposed to noon (11 am to 1 pm) sunlight once a week with different durations (ranging from 15 minutes to 120 minutes) for 3 weeks. The number of pigments produced was counted at the end of the experiment, and the obtained data were statistically analysed. **Results and Discussion.** Pigmentations were observed in almost all studied groups, with the highest in the group exposed for three weeks with the longest exposure time (105 – 120 minutes). Two-way ANOVA analysis showed that both sunlight exposure duration ($p < 0.0001$) and weekly exposure over time ($p = 0.031$) had a significant influence on pigmentation. Post-hoc Tukey HSD analysis showed that 105–120 minutes of exposure significantly increased pigmentation than other groups ($p < 0.05$). **Conclusion.** The present findings showed the appearance of pigmentations on the epidermis, but most of these conditions are insufficient to induce the large number of pigments needed to conduct studies on hyperpigmentation. Further studies are required to obtain the optimal effective frequency and durations of sunlight exposure to induce the right amount of pigmentation for scientific research.

RESUMEN

Introducción. Una de las causas de la hiperpigmentación es el aumento en la deposición de melanina en la epidermis. Los estudios sobre hiperpigmentación se han incrementado en los últimos años, y uno de los principales desafíos consiste en inducir una cantidad considerable de pigmentos en la epidermis de ratones de laboratorio para reproducir las condiciones observadas en humanos. **Objetivo.** El presente estudio tuvo como objetivo realizar un estudio piloto para determinar la cantidad de pigmentos producidos en la epidermis tras la exposición semanal a la luz solar. **Materiales y métodos.** Se utilizaron 40 ratones *Mus musculus* de la cepa ICR, expuestos a la luz solar del mediodía (de 11 a. m. a 1 p. m.) una vez por semana, con diferentes duraciones (entre 15 y 120 minutos) durante 3 semanas. Al finalizar el experimento se cuantificó el número de pigmentos producidos, y los datos obtenidos fueron analizados estadísticamente. **Resultados y discusión.** Se observaron pigmentaciones en casi todos los grupos estudiados, siendo más notorias en el grupo expuesto durante tres semanas con el mayor tiempo de exposición (105–120 minutos). El análisis de varianza de dos vías (ANOVA) mostró que tanto la duración de la exposición solar ($p < 0{,}0001$) como la exposición semanal a lo largo del tiempo ($p = 0{,}031$) tuvieron una influencia significativa en la pigmentación. El análisis post hoc de Tukey HSD evidenció que la exposición de 105–120 minutos incrementó significativamente la pigmentación en comparación con los demás grupos ($p < 0{,}05$). **Conclusión.** Los resultados obtenidos demostraron la aparición de pigmentaciones en la epidermis; sin embargo, la mayoría de estas condiciones no fueron suficientes para inducir la cantidad de pigmentos necesaria para desarrollar estudios sobre hiperpigmentación. Se requieren investigaciones adicionales para determinar la frecuencia y duración óptimas de la exposición solar que permitan inducir el nivel adecuado de pigmentación para fines científicos.

[1]Faculty of Pharmacy, Universiti Teknologi MARA, Cawangan Selangor, Kampus Puncak Alam, 42300, Selangor, Malaysia.
[2]Faculty of Science & Technology, Universitas Islam Negeri Raden Fatah, Palembang, South Sumatra, Indonesia.

INTRODUCTION

Hyperpigmentation is one of the common skin disorders that happens due to excessive sunlight exposure [1-2]. Pigmentation is caused by elevated melanin deposition or a heightened number of melanocytes in the epidermis [3]. Melanin is an essential component in producing skin pigmentation [4-5] and acts as a photoprotection by absorbing ultraviolet radiation (UV) from the sunlight [6]. The functionality of melanin varies from skin pigmentation to UV radiation protection, radical scavenging, and even thermal regulation. Skin, hair, and eye colours are influenced by melanin pigments, particularly by the balance between the two types of melanin pigments which are eumelanin (dark/brown pigment) and pheomelanin (red/yellow pigment) [7].

Hyperpigmentation affects a significant proportion of the global population, especially in regions with high sun exposure. It has been one of the treatment focuses by dermatologists and scientists worldwide [8] due to the associated psychosocial distress, cosmetic concerns, and increased demand for dermatological interventions. Studies for an effective alternative approach are being widely conducted, including research on bioactive compounds from natural products to formulate cosmetic products such as oils, serums, creams, lotions, and many more [9-11]. Most studies are being conducted in scientific laboratories involving experimental mice and rats. However, one of the challenges in conducting studies on hyperpigmentation is to induce a sufficient amount of pigment on the skin to mimic the occurrence of hyperpigmentation in humans. This is important because an insufficient amount of pigments may result in poor or invalid data.

Previous studies have utilized UV lamps or chemical inducers to simulate hyperpigmentation in murine models [12-13]. However, such methods may require specialized equipment or may not fully replicate natural sunlight exposure. Few studies have evaluated the effectiveness of direct sunlight exposure as a reliable method to induce pigmentation in mice under controlled conditions [14]. Hence, the present pilot study was conducted to determine if weekly sunlight exposure could cause a sufficient amount of pigmentation in the epidermis of mice, which can be used for further research on hyperpigmentation. It is hypothesized that longer and more frequent exposure increases pigmentation.

MATERIALS AND METHODS

This study involved 40 female mice (*Mus musculus*) of the ICR strain (obtained from the Laboratory Animal and Facility Management (LAFAM), Universiti Teknologi MARA (UiTM), Selangor, Malaysia), aged between 6–8 weeks old and weighing 20–25 g. The mice were divided into four groups with five mice each, in two different settings (Set 1 and Set 2). The groups were Group A (negative control) and Groups B, C, and D as the experimental groups for Set 1; and Group E (negative control) and Groups F, G, and H for Set 2. The difference between these two settings was the duration of sunlight exposure **(Table 1)**. This research was conducted between March and July 2024 at the Laboratory Animal Facilities and Management (LAFAM), Faculty of Pharmacy, Universiti Teknologi MARA (UiTM), Selangor, Malaysia. All mice were exposed to sunlight once a week for 3 weeks.

Table 1: The durations of sunlight exposure (between 11 am to 1 pm)

Set 1		
Group	**Treatment**	**Duration (minutes)**
Group A (n=5)	No treatment (negative control)	
Group B (n=5)	Exposed to sunlight	15 – 20
Group C (n=5)	Exposed to sunlight	30 – 45
Group D (n=5)	Exposed to sunlight	45 – 60
Set 2		
Group E (n=5)	No treatment (negative control)	
Group F (n=5)	Exposed to sunlight	45 – 60
Group G (n=5)	Exposed to sunlight	75 – 90
Group H (n=5)	Exposed to sunlight	105 – 120

Maintenance of mice

All 40 mice were housed in the open system cages in a 12-hour light/dark cycle at room temperature (20-25˚C). Corn cob was used as bedding and tissue rolls were used as the enrichment tool. Mice were provided with food pellets and water ad libitum. Mice were allowed 3 days for acclimatisation.

Shaving

After acclimatisation, an area of 4x4 cm on the back of each mouse was shaved to expose the skin for the experiment. This shaving procedure was repeated regularly before and after each treatment session for the three week duration of the experiment to optimise the observation.

Observation and Analysis

Upon completion of the treatments, the presence of pigmentations on the epidermis of the mice was observed. Results were captured and the amount of pigments was recorded. Pigments were categorized into 3 groups: Small (<40 pigments), Medium (<40-80 pigments), and Many (>80 pigments). Statistical analysis was performed using R version 4.2.3. A two-way analysis of variance (ANOVA) was conducted to assess the effects of sunlight exposure group (8 levels: Groups A–H) and exposure week (3 levels: Week 1–3) on the number of pigments observed on the mice's epidermis.

The model used was:

$$Y_{ij} = \mu + \alpha_i + \beta_j + \varepsilon_{ij}$$

Where:

- Y_{ij}: number of pigments in the i^{th} group and j^{th} week
- μ: overall mean
- α_i: effect of the i^{th} group (exposure duration)
- β_j: effect of the j^{th} week (exposure time point)
- ε_{ij}: random error (assumed normally distributed)

The assumption of homogeneity of variance was assessed using Levene's test prior to ANOVA. Post-hoc comparisons were conducted using Tukey's HSD to identify pairwise differences between groups and across weeks, with statistical significance set at $p < 0.05$.

RESULTS

The obtained results showed the presence of pigments in nearly all mice in the experimental groups **(Figure 1)**. All mice in Set 1, including the control group, exhibited pigments in small quantities. Meanwhile, the appearance of pigments in Set 2 varied across all categories of 'Small' (<40 pigments), 'Medium' (<40-80 pigments) (Group G), and 'Many' (>80 pigments) (Group H) **(Figures 2 and 3)**.

Statistical comparisons between the control and experimental groups in Set 1 revealed a significant increase ($p<0.05$) in the number of pigments in Groups B, C, and D compared to the control group (Group A). Similarly, results from Set 2 indicated a significant increase ($p<0.05$) in pigmentation in Groups F, G, and H compared to the control group (Group E). Two-way ANOVA revealed significant effects of sunlight exposure group ($F(7, 14) = 23.26, p < 0.0001$) and exposure week ($F(2, 14) = 4.49, p = 0.031$) on the number of epidermal pigments. Post-hoc Tukey HSD analysis showed that Group H (105–120 minutes) exhibited significantly higher pigmentation than all other groups ($p < 0.05$), and a significant increase in pigment count was observed between Week 1 and Week 3 ($p = 0.0244$). These findings suggest that frequent and prolonged sunlight exposure can induce a sufficient level of pigmentation for experimental purpose.

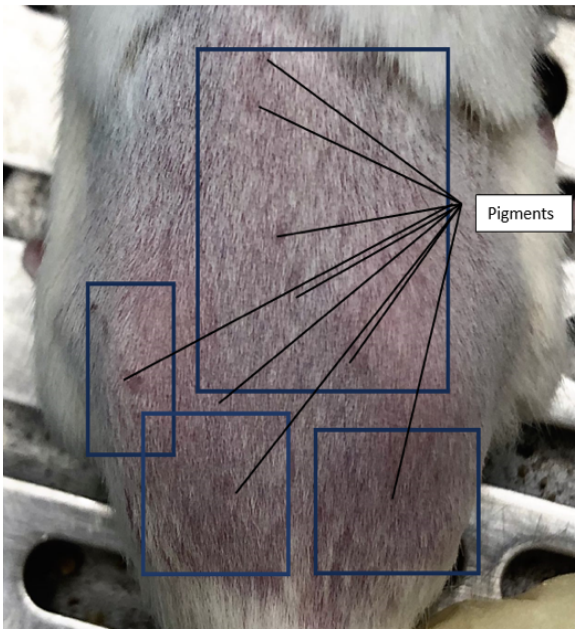

Figure 1. Example of the pigmentation observed on the epidermis of mice following weekly sunlight exposure, with labelled areas indicating the presence of pigments

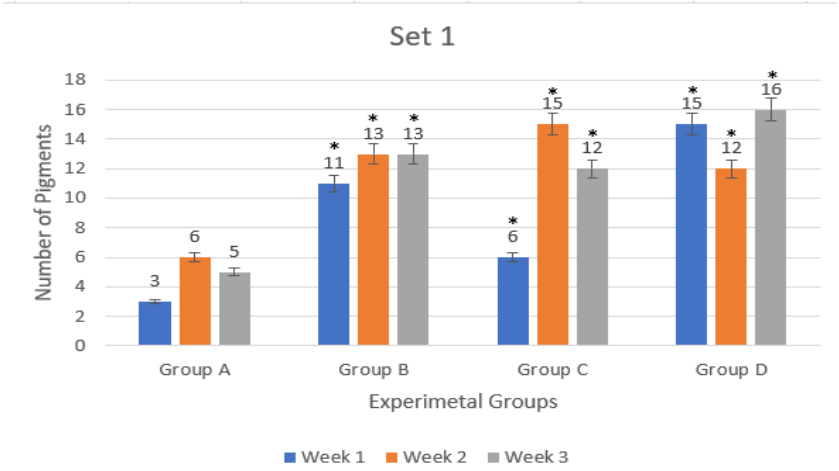

Figure 2. Average number of pigments observed on the mice epidermis in Set 1 following weekly sunlight exposure for 3 weeks. A p-value less than 0.05 ($p < 0.05$) was considered statistically significant. The asterisks (*) indicate significant increases in pigmentation compared to the control group (Group A). Error bars represent the standard error of the mean (S.E.M.).

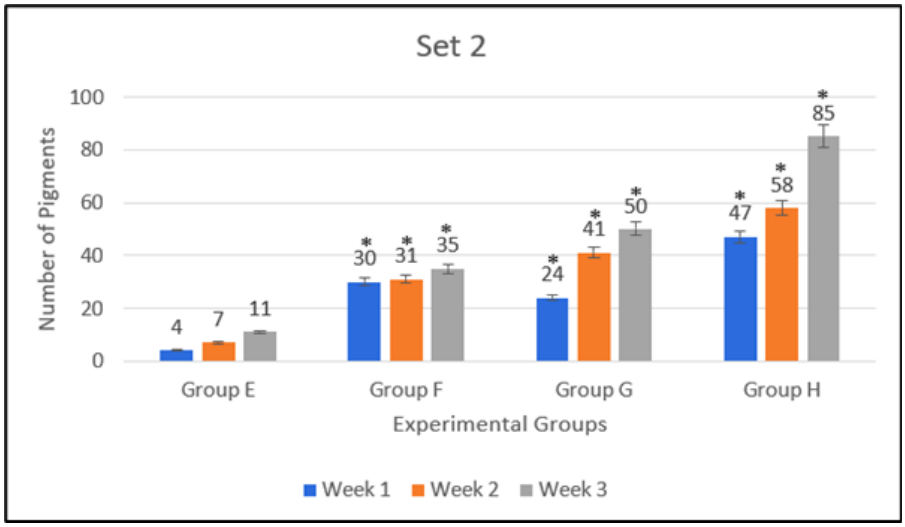

Figure 3: Average number of pigments observed on the mice epidermis in Set 2 following weekly sunlight exposure for 3 weeks. A p-value less than 0.05 ($p < 0.05$) was considered statistically significant. The asterisks (*) indicate significant increases in pigmentation compared to the control group (Group E). Error bars represent the standard error of the mean (S.E.M.).

DISCUSSION

Skin pigmentation, particularly hyperpigmentation, is strongly associated with sunlight and UV radiation. In the context of human health, there are several external factors affecting sunlight-induced hyperpigmentation, including migration, frequent long-distance traveling, and lifestyle changes, and these factors have been long reported to exert some health consequences [15-18]. Due to these conditions, hyperpigmentation has been one of the focuses of clinical skin disorders among dermatologists and scientists. Rapid research is being conducted to find the optimal skin care solution for hyperpigmentation, and these studies mostly involve laboratory animals. However, one of the major problems of conducting animal studies is to induce a sufficient amount of pigmentation that mimics the hyperpigmentation in humans. Hence, this pilot study was conducted to determine the effect of weekly sunlight exposure on the pigment production in mice epidermis.

The obtained results indicated the presence of pigments in all groups of Set 1, but these were limited to small numbers (<40 pigments). In Set 2, the appearance of pigments varied across all categories: Small (<40 pigments), Medium (40-80 pigments), and Many (>80 pigments). Notably, the control groups (Group A and Group E) also exhibited pigments, but the amount is too small and their presence can be considered as spontaneous. In addition, each group in Set 1 and Set 2 recorded an increase in the number of pigments in comparison between Week 1 to Week 3, indicating that the longer the duration of sunlight exposure, the higher the number of pigments being produced. These data aligned with our hypothesis that different durations of sunlight exposure induce different amounts of pigmentation.

The scientific reason behind the varying amount of pigment production could not be fully comprehended in this pilot study, however, there is a possibility that it could correlate with the duration of sunlight exposure given to each of the groups. The prolonged exposure may allow UV radiation from the sunlight to penetrate the epidermis and cause an increase in melanin synthesis [19]. The penetration of UV radiation on the skin can cause damage to skin cells and DNA, leading to the formation of pigments, wrinkles, and many other conditions [1]. This is possible due to a report on UV irradiation that regulates the oxidative stress mechanism in the cell, causing the accumulation of reactive oxygen species (ROS) and causing DNA mutation in the mitochondria [20]. UV radiation can cause DNA damage directly or indirectly by activating photo-reactivity, leading to the absorption of UV photons onto pyrimidine bases and causing dimerization of two adjacent pyrimidines [21-22], as well as increasing melanin production [23-24].

Limitation of the Study

The present findings reported are based on the observations made on the surface of the mice epidermis following a limited frequency of sunlight exposure over the three weeks. The duration was limited to 3 weeks to observe the initial results before proceeding with increased frequency. Future studies should incorporate longer exposure periods and larger sample sizes, along with detailed skin histological analysis to better understand the underlying mechanisms of pigmentation.

CONCLUSION

The current pilot study has demonstrated the presence of pigments in all experimental groups. However, the quantity of pigments produced was quite low and insufficient for further studies on hyperpigmentation. Future experiments should explore different frequencies and durations of sunlight exposure to increase pigment production.

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ETHICAL CONSIDERATIONS

This study was approved by the Committee on Animal Research and Ethics of Universiti Teknologi MARA (UiTM CARE) under approval number UiTM CARE: 443/2024, dated 8 March 2024, and valid from March to August 2024. The research was conducted in accordance with the ethical standards established by the institution and complied with international guidelines for the care and use of laboratory animals.

DECLARATION OF COMPETING INTEREST

The authors have declared no conflict of interest.

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