Journal of Social Research

P-ISSN:2827-9832
E-ISSN: 2828-335x
Vol.5, No.5, April 2026
http://ijsr.internationaljournallabs.com/index.php/ijsr

Effect of Papaya Leaf Extract (*Carica papaya L.*) Oral on Malondialdehyde (MDA) Levels in Vitiligo Model Mice

Nurul Hidayati*, Nurrachmat Mulianto, Arie Kusumawardani, Muhammad Eko Irawanto, Suci Widhiati
RSUD Dr. Moewardi Surakarta, Indonesia
Email: dr.nurulhidayati01@gmail.com*

Keywords:
Vitiligo; antioxidant; papaya leaves; C. papaya; Malondialdehyde

ABSTRACT

Vitiligo is a multifactorial skin disorder associated with oxidative stress and increased malondialdehyde (MDA) levels. Papaya leaf extract (*Carica papaya L.*) has antioxidant and anti-inflammatory properties that may inhibit its pathogenesis. This research aims to analyze the effect of papaya leaf extract on MDA levels in vitiligo model mice. This was an *in vivo*experimental study using C57BL/6 mice divided into control and treatment groups, including papaya leaf extract (1500 mg) and/or 0.05% clobetasol propionate. Vitiligo was induced with 40% monobenzone for 16 days, followed by 28 days of treatment. Serum and tissue MDA levels were measured using ELISA and analyzed with the Kruskal–Wallis and Dunn tests ($p < 0.05$). Administration of 40% monobenzone cream significantly increased serum MDA levels in vitiligo model mice ($p \leq 0.001$) compared to mice without vitiligo. The administration of papaya leaf extract significantly reduced serum MDA levels ($p \leq 0.001$) in vitiligo mice compared to untreated vitiligo mice. Tissue MDA levels in vitiligo mice given combination therapy of papaya leaf extract and 0.05% clobetasol propionate cream were significantly different ($p \leq 0.001$) compared to those receiving papaya leaf extract alone or standard topical therapy. Papaya leaf extract lowers serum MDA levels in vitiligo model mice. Tissue MDA levels in vitiligo model mice were lower with combination therapy of papaya leaf extract and 0.05% clobetasol propionate cream compared to papaya leaf extract alone or standard topical therapy.

INTRODUCTION

Vitiligo is a multifactorial disease characterized by the loss of functional melanocytes (Shiu et al., 2022; Touni et al., 2023; Xuan et al., 2022). The mechanisms underlying melanocyte destruction are multifactorial and include genetic factors, autoimmune responses, oxidative stress, the formation of inflammatory mediators, and melanocyte detachment mechanisms. Both the innate and adaptive immune systems play roles in the pathogenesis of vitiligo (Faraj et al., 2022; He et al., 2022; Inoue, 2026; Speeckaert et al., 2024). Vitiligo can affect the skin, mucous membranes, and hair, presenting with characteristic lesions in the form of well-demarcated, milky-white macules (depigmentation) that are amelanotic (Bergqvist and Ezzedine, 2020; Liao and Chen, 2023). Recent global epidemiological studies indicate that vitiligo affects approximately 0.4% of the world's population and 0.12% in East Asia (Haulrig et al., 2024). The incidence is reported more frequently in women, possibly due to greater attention to aesthetic concerns compared to men. Epidemiological data on vitiligo in Indonesia remain limited, and there are no specific data describing its incidence in each province (Saiboo et al., 2023).

Reactive oxygen species (ROS) produced during melanogenesis make melanocytes highly susceptible to oxidative stress, thereby affecting their survival and melanin synthesis (Speeckaert et al., 2023). Melanocytes contain high levels of polyunsaturated fatty acids (PUFAs) and relatively low levels of antioxidants, leading to increased susceptibility to pro-oxidant agents. Malondialdehyde (MDA) is a secondary product of oxidative stress resulting from lipid peroxidation; it is cytotoxic to melanocytes and can inhibit the enzyme tyrosinase, which plays a role in melanogenesis and vitiligo pathogenesis. Previous studies have demonstrated a significant positive correlation between MDA levels and vitiligo disease activity and severity (Sudarsa et al., 2020; Mulianto, 2020).

Serum MDA levels reflect systemic oxidative and inflammatory stress, whereas MDA levels in skin tissue represent local oxidative and inflammatory stress in affected areas (Juan et al., 2023; El-Domyati et al., 2022; Abdelmonem et al., 2024). A study by Vineetha and Palakkal (2020) comparing serum MDA levels in vitiligo patients and healthy controls showed that MDA levels were significantly higher in patients with unstable and generalized vitiligo compared to controls (significant p-value). A study by Yildirim et al. (2020), examining the role of oxidants and antioxidants in generalized vitiligo at the tissue level, demonstrated significantly increased tissue MDA levels compared to controls ($p < 0.001$).

Papaya leaves (*Carica papaya L.*) possess antioxidant activity that may be beneficial for skin repigmentation. They contain bioactive compounds such as papain, alkaloids, flavonoids, tannins, saponins, steroids, glycosides, and vitamin C. Research by Sihombing et al. (2022) showed that flavonoids—particularly quercetin and kaempferol—in papaya leaves can reduce the area of vitiligo lesions, increase the percentage of pigmentation, and enhance melanin production in affected areas. Quercetin and kaempferol are known to have antioxidant and anti-inflammatory effects that play important roles in vitiligo therapy (Mishra et al., 2025). Therefore, the researchers were interested in investigating the effect of papaya leaf extract on vitiligo model mice by examining its impact on serum and tissue MDA levels.

Despite these findings, studies specifically evaluating the effect of oral papaya leaf extract on both serum and skin tissue MDA levels in vitiligo model mice remain very limited. Most previous studies have focused on clinical lesion improvement, pigmentation outcomes, or general antioxidant properties, whereas the biochemical effects of papaya leaf extract on oxidative stress markers in vitiligo—particularly in comparison with standard therapy and combination therapy—have not been sufficiently explored. This represents the novelty of the present study, namely the investigation of oral papaya leaf extract as an antioxidant intervention in a monobenzone-induced vitiligo mouse model by assessing its effects on both systemic oxidative stress (serum MDA) and local oxidative stress (skin tissue MDA), including comparison with standard topical clobetasol therapy and combination treatment.

Based on this background, this study aims to analyze the effect of oral papaya leaf extract on serum and tissue MDA levels in vitiligo model mice. Specifically, it seeks to evaluate whether papaya leaf extract can reduce oxidative stress, as reflected by MDA levels, and whether combination therapy with 0.05% clobetasol propionate cream provides greater benefit than monotherapy. This study is expected to provide both theoretical and practical benefits. Theoretically, it may enhance scientific understanding of the role of natural antioxidants in vitiligo management and contribute to the growing body of evidence on oxidative stress biomarkers in depigmenting disorders. Practically, the findings may serve as a basis for

developing adjunctive or complementary therapeutic approaches using papaya leaf extract in vitiligo treatment. In addition, this study may provide a foundation for future translational and clinical research on plant-based antioxidant therapies that are potentially affordable, accessible, and relevant in dermatological practice.

METHODS

This study is an in vivo experimental study design using a pretest and posttest control group design approach to measure blood serum MDA levels and a posttest only control group design to assess skin tissue MDA levels. This study was conducted on C57BL/6 mice induced as vitiligo model mice with the aim of comparing serum MDA levels before and after therapy, as well as analyzing tissue MDA levels after therapy. The research was conducted at the Laboratory of the Center for Food and Nutrition Studies of Gajah Mada University (PSPG UGM) from November 2025 to January 2026.

The population of this study was a C57BL/6 male mouse test animal. The sample size was determined using Federer's formula and the minimum number of mice for each group was 5 tails. The addition of 10% of samples was carried out to anticipate a drop out so that the number of samples used by each group was 6 mice. The study was divided into 5 groups, namely the K- group (negative control), which is a group of normal mice that were not given 40% monobenzone cream induction or therapy, used as a standard value for the origin of the parameters of the healthy mice group. The K+ (positive control) group, which is a group of vitiligo models that were induced by monobenzone cream 40% but not given therapy, was used to see the progression of the disease without being given therapy. The P1 group (standard therapy) was a group of vitiligo mice induced by monobenzone cream 40% and then given topical 0.05% clobetasol propionate cream therapy. The P2 group (papaya leaf extract therapy) was a group of vitiligo mice induced by monobenzone cream 40% and then given oral papaya leaf extract therapy. The P3 group (combination therapy) was a group of vitiligo mice induced by monobenzone cream 40% and then given combination therapy with 0.05% topical clobetasol propionate cream and oral papaya leaf extract.

Inclusion criteria: Mice of the C57BL/6 strain are 7 to 9 weeks old, male, weight ranges from 20-30 grams before treatment, normal behavior and activity, mice are kept in cages with good lighting conditions, get food and drink ad libitum, placed in the same conditions and situations. Exclusion criteria: Mice with anatomical and skin tissue abnormalities from clinical examination, mice that suffered from pain during the adaptation process in the first 7 days before induction of the vitiligo model, mice that died during the course of treatment.

Data analysis in this study used parametric tests using pretest and posttest control group design by comparing serum MDA levels before and after therapy and posttest only with control group design by assessing MDA and CXCL10 tissue with ELISA. Data processing is carried out using the SPSS 21 program version. The data normalization test used the Saphiro-wilk test technique and the homogeneity test was carried out with the Levene test. One-way ANOVA data analysis was used when a normal data distribution ($p>0.05$) was obtained followed by the Post Hoc Tukey LSD test. Normal but non-homogeneous data will be processed using the Welch test then continued with the Post Hoc Dunnet T3 test and if the data is abnormal then the data processing uses the nonparametric Kruskall Wallis with the Post Hoc Dunn test. This was done to evaluate serum MDA levels between groups.

The significance level of p<0.05 can be concluded that administration of papaya leaf extract has a significant effect in lowering MDA levels in the serum of vitiligo model mice, compared to the control group. The same is also true if p<0.05, then the examination of tissue MDA levels in vitiligo model mice is lower in combination therapy of 0.05% clobetasol propionate cream and papaya leaf extract compared to single papaya leaf extract therapy or standard topical therapy. Oral papaya leaf extract likely has a noticeable effect on antioxidants measured through MDA levels

RESULTS AND DISCUSSION

Effect of papaya leaf extract on blood serum MDA in vitiligo model mice

Before the analysis of the different serum MDA tests based on the treatment preparations in the study, normality and homogeneity tests were carried out. Testing of the analysis requirements for the normality and homogeneity of the distribution of each dataset is required before performing the analysis. The normality test of this study is to find out whether the data is distributed normally or not with the Shapiro-Wilk technique and to find out whether the data is homogeneous or not by using the Levene test. The normality test shows that the data is not normally distributed if the value is p>0.05, as well as the homogeneity test shows that the data variant is homogeneous if the value is p>0.05. Data that are normally and homogeneously distributed will use the ANOVA test, then continue with the Post Hoc LSD test. Normal but non-homogeneous data will be processed using the Welch test then continued with the Post Hoc Dunnet T3 test and if the data is abnormal then the data processing uses the nonparametric Kruskall Wallis with the Post Hoc Dunn test.

The data results from the normality and homogeneity test of each variable in this study explained that the serum MDA level was abnormally distributed and inhomogeneous (Table 1), so the statistical analysis used was the Kruskal Wallis test then continued with the Post Hoc Dunn test (Table 2 and Table 3).

Table 1. Serum MDA normality and homogeneity test

Variable	Groups	Shapiro-Wilk			Levene	
		Statistics	df	p-value	Statistics	p-value
MDA post-intervention	K-	0,957	6	0,799	3,089	0,034
	K+	0,866	6	0,211		
	P1	0,769	6	0,030		
	P2	0,975	6	0,922		
	P3	0,949	6	0,735		
MDA *post* therapy	K-	0,973	6	0,913	2,854	0,045
	K+	0,766	6	0,029		
	P1	0,906	6	0,412		
	P2	0,925	6	0,542		
	P3	0,958	6	0,801		

Source: Primary research data processed by the authors, 2026

Remarks: K- = normal mice that were not given monobenzone induction or therapy; Group K+ = 40% monobenzone cream-induced vitiligo mice but not given therapy or treatment; Group P1 = 40% monobenzone cream-induced vitiligo mice were then given 0.05%

clobetasol propionate cream therapy; Group P2 = 40% monobenzone-cream induced vitiligo mice were then given papaya leaf extract therapy; Group P3 = 40% monobenzone cream-induced vitiligo mice were then given topical combination therapy of 0.05% clobetasol propionate cream and papaya leaf extract. Saphiro wilk= normality test, Levene test= homogeneity test. The data meet the assumptions of normality and homogeneity if the value of p>0.05. Df = degrees of freedom; MDA: Malondialdehyde

Table 2. Simultaneous difference test of blood serum MDA based on treatment preparation

Groups	MDA serum post-intervention		MDA serum *post* therapy	
	Mean ±SD	**p-value**	**Mean ±SD**	**p-value**
K-	1,75 +0,03	<0,001*	1,78 +0,03	<0,001*
K+	6,13 +0,08		6,17 +0,08	
P1	6,07 +0,12		5,13 +0,10	
P2	6,17 +0,13		2,07 +0,07	
P3	6,09 +0,08		2,00 +0,02	

Source: Primary research data processed by the authors, 2026

Remarks: K- = normal mice that were not given monobenzone induction or therapy; Group K+ = 40% monobenzone cream-induced vitiligo mice but not given therapy or treatment; Group P1 = 40% monobenzone cream-induced vitiligo mice were then given 0.05% clobetasol propionate cream therapy; Group P2 = 40% monobenzone-cream induced vitiligo mice were then given papaya leaf extract therapy; Group P3 = 40% monobenzone cream-induced vitiligo mice were then given topical combination therapy of 0.05% clobetasol propionate cream and papaya leaf extract. Kruskal Wallis test (abnormal distributed data); *significant at p<0.05. MDA: Malondialdehyde

Based on Table 2, the Kruskal Wallis test found a statistically significant difference between groups in both observation phases (p < 0.001). In the post-intervention phase, it was seen that all post-intervention groups (K+, P1, P2, and P3) had higher levels of MDA than the K-group, suggesting that vitiligo induction procedures successfully triggered systemic oxidative stress conditions.

There was a decrease in serum MDA levels after 28 days of therapy. The K+ group that did not receive constant therapy showed high MDA levels of 6.17 ± 0.08 nmol/mL. In the P2 and P3 groups, it showed a decrease in serum MDA levels of 2.07 ± 0.07 nmol/mL and 2.00 ± 0.02 nmol/mL, respectively.

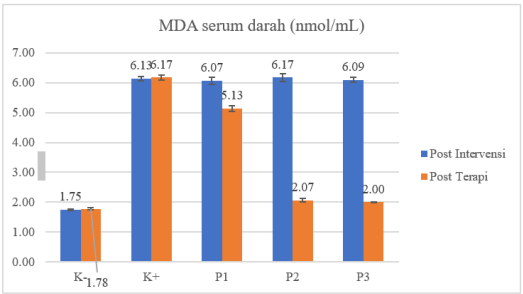

Figure 1. Comparison bar chart of blood serum MDA between treatment groups

Source: Primary research data processed by the authors, 2026

K- = normal mice that were not given monobenzone induction or therapy; Group K+ = 40% monobenzone cream-induced vitiligo mice but not given therapy or treatment; Group P1 = 40% monobenzone cream-induced vitiligo mice were then given 0.05% clobetasol propionate cream therapy; Group P2 = 40% monobenzone-cream induced vitiligo mice were then given papaya leaf extract therapy; Group P3 = 40% monobenzone cream-induced vitiligo mice were then given topical combination therapy of 0.05% clobetasol propionate cream and papaya leaf extract. MDA: Malondialdehyde.

Table 3. Partial difference test of blood serum MDA based on treatment preparation

Groups	MDA serum post-intervention		MDA serum *post* therapy	
	Mean diff	**p-value**	**Mean diff**	**nilai p**
K- vs, K+	-4,38	<0,001*	-4,40	<0,001*
K- vs, P1	-4,32	0,022*	-3,36	<0,001*
K- vs, P2	-4,42	<0,001*	-0,30	0,025*
K- vs, P3	-4,34	0,012*	-0,22	0,195
K+ vs, P1	0,06	0,300	1,04	0,238
K+ vs, P2	-0,04	0,693	4,10	0,013*
K+ vs, P3	0,04	0,420	4,18	<0,001*
P1 vs, P2	-0,10	0,152	3,06	0,195
P1 vs, P3	-0,02	0,818	3,14	0,025*
P2 vs, P3	0,08	0,230	0,08	0,341

Source: Primary research data processed by the authors, 2026

Remarks: K- = normal mice that were not given monobenzone induction or therapy; Group K+ = 40% monobenzone cream-induced vitiligo mice but not given therapy or treatment; Group P1 = 40% monobenzone cream-induced vitiligo mice were then given 0.05% clobetasol propionate cream therapy; Group P2 = 40% monobenzone-cream induced vitiligo mice were then given papaya leaf extract therapy; Group P3 = 40% monobenzone cream-induced vitiligo mice were then given topical combination therapy of 0.05% clobetasol propionate cream and papaya leaf extract. Dunn Test (abnormal distributed data); *significant at p<0.05. MDA: Malondialdehyde.

Based on Table 3, in the post intervention phase, the 40% group of mice induced by monobenzone cream (K+, P1, P2, and P3) showed significant differences in MDA levels when compared to the K- group with a p< value of 0.05. Comparisons between groups of mice in the vitiligo model (K+ vs P1, K+ vs P2, and so on) showed no significant difference ($p > 0.05$). This proves that the vitiligo induction protocol carried out succeeded in increasing MDA levels uniformly and equally in all study subjects before the start of therapy.

In post therapy, the results of the analysis showed a change in serum MDA levels. The K+ group that was not given therapy had a significant difference in MDA levels compared to the P2 group with a p value = 0.013 and the P3 group with a p value of < 0.001. MDA levels in the K+ group were not significantly different from those in the P1 group ($p = 0.238$). These findings indicate that the administration of papaya leaf extract, both single therapy and combination therapy of clobetasol propionate and papaya leaf extract has a greater effect in reducing oxidative stress than a single therapy, namely clobetasol propionate cream 0.05%.

Further analysis in the final phase of therapy showed that the P3 group had significant differences compared to the P1 group with a p=0.025 value. In the P3 group with the K- group, no significant difference was found with a value of p = 0.195. This shows that combination therapy of clobetasol propionate and papaya leaf extract is able to lower blood MDA levels to a level almost equivalent to normal mice without treatment.

To compare blood MDA levels between post-intervention and post-therapy for each group, a pair differential test was used (Table 4). All groups showed statistically significant changes in MDA levels ($p < 0.05$). In the K- and K+ groups, there was a slight increase in MDA levels (1.7% and 0.7%, respectively). This suggests that in the absence of therapeutic interventions, oxidative stress conditions in the vitiligo (K+) model tend to persist or increase slightly. All treatment groups (P1, P2, and P3) experienced a significant decrease in MDA levels. The P1 group showed a decrease of -15.5% ($p = 0.027$). The P2 group showed a decrease in serum MDA levels of -66.5% ($p = 0.028$), while the P3 group showed the largest decrease reaching -67.2% ($p < 0.001$).

Table 4. A pre-post test of MDA blood serum in each treatment group

Groups	MDA serum post-intervention	MDA serum *post* therapy	% Changes	p-value
	Mean ±SD	Mean ±SD		
K-	1,75 +0,03	1,78 +0,03	1,7%	<0,001*a
K+	6,13 +0,08	6,17 +0,08	0,7%	0,001*a
P1	6,07 +0,12	5,13 +0,10	-15,5%	0,027*b
P2	6,17 +0,13	2,07 +0,07	-66,5%	0,028*b
P3	6,09 +0,08	2,00 +0,02	-67,2%	<0,001*a

Source: Primary research data processed by the authors, 2026

Effect of papaya leaf extract on skin tissue MDA in vitiligo model mice

Based on the results of the Shapiro Wilk test in Table 5, the MDA data of the network of all groups is normally distributed. However, the results of the Levene homogeneity test showed that the data variants between groups were inhomogeneous. Furthermore, a Welch analysis and a T3 dunnet post hoc test were carried out.

Table 5. Normality and homogeneity test of MDA skin tissue

Variable	Groups	Shapiro-Wilk			Levene	
		Statistics	df	p-value	Statistics	p-value
MDA Skin Tissue *Post* Intervention	K-	0,925	6	0,540	13,470	0,000
	K+	0,839	6	0,128		
	P1	0,968	6	0,880		
	P2	0,967	6	0,869		
	P3	0,874	6	0,242		

Source: Primary research data processed by the authors, 2026

Remarks: K- = normal mice that were not given monobenzone induction or therapy; Group K+ = 40% monobenzone cream-induced vitiligo mice but not given therapy or

treatment; Group P1 = 40% monobenzone cream-induced vitiligo mice were then given 0.05% clobetasol propionate cream therapy; Group P2 = 40% monobenzone-cream induced vitiligo mice were then given papaya leaf extract therapy; Group P3 = 40% monobenzone cream-induced vitiligo mice were then given topical combination therapy of 0.05% clobetasol propionate cream and papaya leaf extract. Saphiro wilk= normality test, Levene test= homogeneity test. The data meet the assumptions of normality and homogeneity if the value of p>0.05. Df = degrees of freedom; MDA: Malondialdehyde

Table 6. Simultaneous MDA differential test of skin tissue based on treatment preparation

Groups	MDA tissue *post* therapy	
	Mean ±SD	p-value
K-	1,94 +0,03	<0,001
K+	12,77 +0,33	
P1	3,48 +0,06	
P2	3,26 +0,09	
P3	2,17 +0,08	

Source: Primary research data processed by the authors, 2026

Remarks: K- = normal mice that were not given monobenzone induction or therapy; Group K+ = 40% monobenzone cream-induced vitiligo mice but not given therapy or treatment; Group P1 = 40% monobenzone cream-induced vitiligo mice were then given 0.05% clobetasol propionate cream therapy; Group P2 = 40% monobenzone-cream induced vitiligo mice were then given papaya leaf extract therapy; Group P3 = 40% monobenzone cream-induced vitiligo mice were then given topical combination therapy of 0.05% clobetasol propionate cream and papaya leaf extract. Welch test (normal but non-homogeneous distributed data); *significant at p<0.05. MDA: Malondialdehyde

Based on Table 6, the results of the Welch test showed a significant difference in tissue MDA levels between treatment groups with a p< value of 0.001. The K+ group showed the highest level of tissue MDA, which was 12.77 ± 0.33 nmol/g. All therapy groups showed decreased tissue MDA levels compared to the K+ group. Groups P1 and P2 had tissue MDA levels of 3.48 ± 0.06 nmol/g and 3.26 ± 0.09 nmol/g, respectively. The lowest decrease in tissue MDA levels was found in the P3 group with a value of 2.17 ± 0.08 nmol/g.

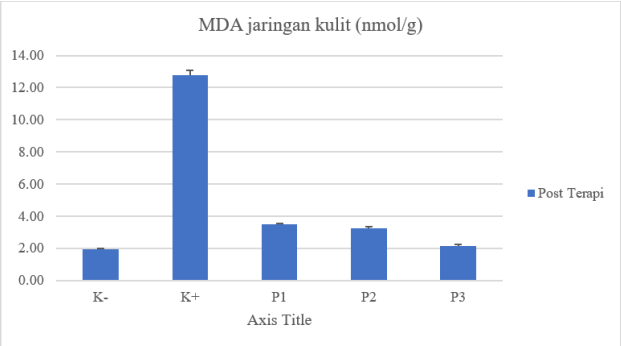

Figure 2. Bar chart of MDA levels of skin tissue between treatment groups

Source: Primary research data processed by the authors, 2026

K- = normal mice that were not given monobenzone induction or therapy; Group K+ = 40% monobenzone cream-induced vitiligo mice but not given therapy or treatment; Group P1 = 40% monobenzone cream-induced vitiligo mice were then given 0.05% clobetasol propionate cream therapy; Group P2 = 40% monobenzone-cream induced vitiligo mice were then given papaya leaf extract therapy; Group P3 = 40% monobenzone cream-induced vitiligo mice were then given topical combination therapy of 0.05% clobetasol propionate cream and papaya leaf extract. MDA: Malondialdehyde

Table 7. Partial differential test of MDA skin tissue based on treatment preparation

Groups	MDA tissue *post* therapy	
	Mean diff,	**p-value**
K- vs, K+	-10,8	<0,001*
K- vs, P1	-1,54	<0,001*
K- vs, P2	-1,32	<0,001*
K- vs, P3	-0,23	0,004*
K+ vs, P1	9,3	<0,001*
K+ vs, P2	9,51	<0,001*
K+ vs, P3	10,6	<0,001*
P1 vs, P2	0,215	0,009
P1 vs, P3	1,31	<0,001*
P2 vs, P3	1,09	<0,001*

Source: Primary research data processed by the authors, 2026

Remarks: K- = normal mice that were not given monobenzone induction or therapy; Group K+ = 40% monobenzone cream-induced vitiligo mice but not given therapy or treatment; Group P1 = 40% monobenzone cream-induced vitiligo mice were then given 0.05% clobetasol propionate cream therapy; Group P2 = 40% monobenzone-cream induced vitiligo mice were then given papaya leaf extract therapy; Group P3 = 40% monobenzone cream-induced vitiligo mice were then given topical combination therapy of 0.05% clobetasol propionate cream and papaya leaf extract. Dunnet T3 (normal but not homogeneous distributed data); *significant at $p<0.05$; MDA: Malondialdehyde

Based on Table 7, it was found that the K- group had significant differences with all other groups ($p < 0.05$), including the treatment group. This suggests that even though therapy has been given, tissue MDA levels have not fully returned to baseline levels identical to normal mouse groups. Judging from the average difference, the P3 group showed the smallest difference to K- which was 0.23, compared to P1 (1.54) and P2 (1.32).

Comparisons between the K+ group and the rest therapy groups (P1, P2, and P3) showed significant differences ($p < 0.001$). These findings prove that the administration of 0.05% chlorbetasol propionate cream, papaya leaf extract, as well as combination therapy of 0.05% chlorbetasol propionate cream and papaya leaf extract was effective in reducing the level of oxidative stress in skin tissue in vitiligo mice. The P2 group showed significant differences compared to the P1 group ($p = 0.009$). The P3 group showed significant differences compared to the P1 and P2 groups ($p < 0.001$). This shows a synergistic effect between clobetasol

propionate 0.05% cream and papaya leaf extract in reducing oxidative stress in vitiligo model mice.

Vitiligo is a disease of skin depigmentation caused by the loss of melanocyte cells that form skin pigment. Vitiligo can appear on the skin, mucous membranes and hair with characteristic features of a milky white macula (depigmentation), amelanotic that is firmly bounded (Bergqvist and Ezzedine, 2020; Liao and Chen, 2023). Vitiligo lesions can involve any part of the body, usually with a symmetrical distribution (Bergqvist and Ezzedine, 2020; Joge et al, 2022). The disease can start in any part of the body, the face, acre and genitals are often the initial predilection in vitiligo (Bergqvist and Ezzedine, 2020).

Monobenzone induces oxidative stress in pigment cells and lysosomal degradation of melanin through the process of autophagy. This directly induces the activation of CD8+ T cells that will target melanocyte cells. The cellular stress that occurs excretes inducible HSP70, the same mechanism occurs in the theory of oxidative stress in vitiligo (Dong et al., 2023). Malondialdehyde is a secondary product of oxidative stress due to lipid peroxidation which is cytotoxic to melanocytes and can inhibit the tyrosinase enzyme which plays a role in the pathogenesis of vitiligo (Sudarsa et al, 2020; Mulianto, 2020).

Oxidative stress on the skin causes a significant increase in MDA levels in the vitiligo group (Luo et al., 2024). There is a positive correlation between plasma MDA levels and vitiligo severity (Seneschal, 2023; Widayati et al., 2017). The results of this study are in accordance with the findings of another study by Vineetha and Palakkal (2020) regarding the comparison of blood serum MDA of vitiligo patients and healthy controls showed that high MDA levels in patients with vitiligo were unstable and generalist compared to the control group (significant p-value). Another supporting study by Yildirim et al (2020) showed that the patient's tissue MDA levels increased significantly compared to the control ($p<0.001$).

Effect of papaya leaf extract on blood serum MDA in vitiligo model mice

The results showed an increase in serum MDA levels after 40% monobenzone cream administration for 16 days. The value of serum MDA levels in the normal mouse group was significantly different from the vitiligo model group (diff mean = -4.38; p=<0.001) which means that significantly the vitiligo model mice had higher blood serum MDA levels compared to non-vitiligo mice, thus the vitiligo model mice successfully triggered systemic oxidative stress conditions that caused a significant increase in serum MDA levels.

From the results of the study, it is known that overall the lowest serum MDA level is in the K- group, which is an average of 1.75±0.03 and the highest MDA level in the K+ group with an average of 6.3±0.08, while the P1 group gets an average of 6.07±0.12, the P2 group gets an average of 6.17±0.13 and the P3 group gets an average of 6.09±0.08. The value of MDA levels in the K+ group without treatment was significantly different from the P2 group of papaya leaf administration (diff mean=4.10; p=0.013) which means that papaya leaf extract can significantly reduce the amount of MDA levels.

The value of serum MDA levels in the P1 group was significantly different from P3 (diff mean = 3.14; p=0.025) with the lowest MDA level found in the P3 group of 2.00±0.02 which means that vitiligo given a combination of 0.05% clobetasol propionate cream therapy and papaya leaf extract had lower serum MDA levels compared to vitiligo who only received a single therapy in the form of 0.05% clobetasol pripionate cream. The value of serum MDA levels in the P2 group did not differ significantly from the P3 group (diff mean=0.08;

p=<0.341), the lowest serum MDA levels were found in the P3 group of 2.00±0.02, which means that the vitiligo mice given the combination of 0.05% clobetasol propionate cream therapy and papaya leaf extract had no lower serum MDA levels compared to the group of vitiligo model mice that received only a single therapy of papaya leaf extract. The value of serum MDA levels in the P1 group was not significantly different from P2 (diff mean=3.06; p=0.195) with the lowest serum MDA levels found in the P2 group of 2.07±0.07, which means that vitiligo mice given a single therapy of clobetasol propionate cream 0.05% or a single therapy of papaya leaf extract had equally low serum MDA levels. From the results of the study, it can be concluded that single therapy, either clobetasol propionate cream 0.05% or single therapy of papaya leaf extract, has the same effectiveness in reducing the oxidative stress products of MDA in the pathway of vitiligo pathogenesis with a greater reduction in serum MDA levels in the papaya leaf extract therapy group.

Decreased serum MDA levels showed a decrease in oxidative stress. The results of this study used 1500 mg papaya leaf extract in accordance with the study of Kong et al (2021) which showed that aqueous extract of papaya leaf leaves at concentrations of 0.75 grams/100 mL and 1.5 grams/100 mL showed antioxidant properties by increasing the production of nitric oxide (NO) thereby reducing ROS production. Aqueous leaf extract can reduce MDA levels by 0.031 umol/L and increase glutathione peroxidase (GPx) levels by 0.246 U/mg protein (Kong et al., 2021). The type of flavonoid quercetin in papaya leaves can neutralize free radicals and reduce oxidative stress. This reduces the impact of ROS damage on cellular structures such as lipids, proteins. The content of terpenoids in the form of carenoids has strong antioxidant properties. In addition, the phenolic component of papaya leaves is also antioxidant.

Differences in tissue MDA levels in vitiligo model mice on combination therapy of clobetasol propionate 0.05% cream and papaya leaf extract compared to single therapy

The results showed that tissue MDA levels between groups differed significantly (p=<0.001). The lowest tissue MDA level in the K- group was 1.94±0.03 on average and the highest tissue MDA level in the K+ group with an average of 12.77±0.33, then the P1 group obtained an average of 3.48±0.0, the P2 group obtained an average of 3.26±0.09 and the P3 group obtained an average of 2.17±0.08. The tissue MDA levels in the P2 group differed significantly from the K+ group (diff mean=9.51; p=<0.001) which means that the administration of papaya leaf extract can significantly reduce tissue MDA levels.

The value of tissue MDA levels in the P1 group was significantly different from P3 (diff mean=1.31; p=<0.001) with the lowest tissue MDA levels found in the P3 group of 2.17±0.08, which means that the vitiligo mice given a combination of 0.05% clobetasol propionate cream therapy and papaya leaf extract had lower tissue MDA levels compared to the vitiligo model mice who only received a single therapy in the form of 0.05% clobetasol pripionate cream. The value of tissue MDA levels in the P2 group differed significantly from the P3 group (diff mean=1.09; p=<0.001), with the lowest tissue MDA levels found in the P3 group of 2.17±0.08, which means that the mendit group of the vitiligo model given a combination of 0.05% clobetasol propionate cream therapy and papaya leaf extract had lower levels of skin tissue MDA compared to the group of vitiligo model mice who received only a single therapy of papaya leaf extract. The value of tissue MDA levels in the P1 group differed significantly from P2 (diff mean = 0.215; p=0.009) with the lowest levels of skin tissue MDA found in the P2

group of 3.26±0.09 which means that the vitiligo model mouse group given papaya leaf extract had lower tissue MDA levels compared to the vitiligo model mouse group that received a single therapy of clobetasol propionate cream 0.05%. The results of the study on the MDA level of this tissue are different from the MDA levels of blood serum where papaya leaf extract is able to reduce the MDA level of skin tissue higher than clobetasol propionate cream 0.05%. The results of the study answer the hypothesis that the combination therapy of 0.05% clobetasol propionate cream and papaya leaf extract can reduce tissue MDA levels lower than a single therapy, namely 0.05% clobetasol propionate cream or papaya leaf extract alone.

Decreased levels of MDA in skin tissue showed a decrease in oxidative stress. The results of this study used 1500 mg papaya leaf extract in accordance with the study of Kong et al (2021) which showed that aqueous extract of papaya leaf leaves at concentrations of 0.75 grams/100 mL and 1.5 grams/100 mL showed antioxidant properties by increasing the production of nitric oxide (NO) thereby reducing ROS production. Aqueous leaf extract can reduce MDA levels by 0.031 umol/L and increase glutathione peroxidase (GPx) levels by 0.246 U/mg protein. The type of flavonoid quercetin in papaya leaves can neutralize free radicals and reduce oxidative stress. This reduces the impact of ROS damage on cellular structures such as lipids and proteins. The content of terpenoids in the form of carenoids has strong antioxidant properties. In addition, the phenolic component of papaya leaves is also antioxidant.

CONCLUSION

Papaya leaf extract lowers serum MDA levels in vitiligo model mice. Tissue MDA levels in vitiligo model mice were lower in combination therapy of 0.05% clobetasol propionate cream and papaya leaf extract compared to single papaya leaf extract therapy or standard topical therapy. Based on these findings, it is recommended that future studies explore the molecular mechanisms underlying the antioxidant effects of papaya leaf extract, including its interaction with inflammatory pathways and melanocyte regeneration. Further research with larger sample sizes and clinical trials in humans is also necessary to validate its safety, efficacy, and potential as an adjunctive therapy in vitiligo management. In addition, optimization of dosage and formulation of papaya leaf extract should be considered to enhance its therapeutic applicability in clinical settings.

REFERENCES

Bergqvist, C., & Ezzedine, K. (2020). Vitiligo: A review. *Dermatology, 236*(6), 571–592. https://doi.org/10.1159/000506103

Dong, J., Lai, Y., Zhang, X., Yue, Y., Zhong, H., & Shang, J. (2023). Optimization of monobenzone-induced vitiligo mouse model by the addition of chronic stress. *International Journal of Molecular Sciences, 24*(8). https://doi.org/10.3390/ijms24086990

El-Domyati, M., El-Din, W. H., Rezk, A. F., Chervoneva, I., Lee, J. B., Farber, M., & Alexeev, V. (2022). Systemic CXCL10 is a predictive biomarker of vitiligo lesional skin infiltration, PUVA, NB-UVB, and corticosteroid treatment response and outcome. *Archives of Dermatological Research*, 1–10.

Faraj, S., Kemp, E. H., & Gawkrodger, D. J. (2022). Patho-immunological mechanisms of vitiligo: The role of the innate and adaptive immunities and environmental stress factors. *Clinical and Experimental Immunology, 207*(1), 27–43.

Haulrig, M. B., Al-Sofi, R., Baskaran, S., Bergmann, M. S., Løvendorf, M., Dyring-Danersen,

B., & Loft, N. (2024). The global epidemiology of vitiligo: A systematic review and meta-analysis of the incidence and prevalence. *JEADV Clinical Practice, 3*(5), 1410–1419.
He, S., Xu, J., & Wu, J. (2022). The promising role of chemokines in vitiligo: From oxidative stress to the autoimmune response. *Oxidative Medicine and Cellular Longevity, 2022*(1), 8796735.
Inoue, S. (2026). Pathogenesis of vitiligo: Integrating immune and non-immune cell crosstalk. *The Journal of Dermatology, 53*(2), 188–199.
Kong, Y. R., et al. (2021). Beneficial role of *Carica papaya* extracts and phytochemicals on oxidative stress and related diseases: A mini review. *Biology*. https://doi.org/10.3390/biology10040287
Liao, D. M., & Chen, C. (2023). Vitiligo: A mini review. *Tungs' Medical Journal, 17*(1), 6–10.
Lim, X. Y., et al. (2021). *Carica papaya* L. leaf: A systematic scoping review on biological safety and herb-drug interactions. *Evidence-Based Complementary and Alternative Medicine*.
Mulianto, N. (2020). Malondialdehid sebagai penanda stres oksidatif pada berbagai penyakit kulit. *Cermin Dunia Kedokteran, 47*(1), 39–44.
Saiboo, A. A., Prakoeswa, C. R. S., Indramaya, D. M., Hidayati, A. N., Utomo, B., & Eliza, F. (2023). Characteristics and clinical profile of vitiligo patients in dermatology and venereology outpatient clinic unit at Dr. Soetomo General Academic Hospital Surabaya. *Berkala Ilmu Kesehatan Kulit dan Kelamin, 35*(1), 1–5.
Shiu, J., Zhang, L., Lentsch, G., Flesher, J. L., Jin, S., Polleys, C., Jo, S. J., Mizzoni, C., Mobasher, P., & Kwan, J. (2022). Multimodal analyses of vitiligo skin identify tissue characteristics of stable disease. *JCI Insight, 7*(13), e154585.
Sihombing, R. A. (2022). *Pengaruh pemberian ekstrak daun pepaya (Carica papaya L.) terhadap pigmen melanin kulit mencit (Mus musculus L.) hitam model vitiligo* (Doctoral dissertation, Universitas Sumatera Utara).
Speeckaert, R., Caelenberg, E. V., Belpaire, A., Speeckaert, M. M., & van Geel, N. (2024). Vitiligo: From pathogenesis to treatment. *Journal of Clinical Medicine, 13*(17), 5225.
Speeckaert, R., et al. (2023). A meta-analysis of chemokines in vitiligo: Recruiting immune cells towards melanocytes. *Frontiers in Immunology*. https://doi.org/10.3389/fimmu.2023.1112811
Sudarsa, P. S. S., Praharsini, I. G. A. A., & Karna, R. V. (2020). Correlation between malondialdehyde levels and disease activity in vitiligo. *Bali Medical Journal, 9*(3), 569–572. https://doi.org/10.15562/bmj.v9i3.2020
Touni, A. A., Shivde, R. S., Echuri, H., Abdel-Aziz, R. T. A., Abdel-Wahab, H., Kundu, R. V., & Le Poole, I. C. (2023). Melanocyte–keratinocyte cross-talk in vitiligo. *Frontiers in Medicine, 10*, 1176781.
Vineetha, M., & Palakkal, S. (2020). Comparison of serum malondialdehyde levels in vitiligo patients and healthy controls. *Journal of Evolution of Medical and Dental Sciences, 9*(6), 343–346. https://doi.org/10.14260/jemds/2020/78
Xuan, Y., Yang, Y., Xiang, L., & Zhang, C. (2022). The role of oxidative stress in the pathogenesis of vitiligo: A culprit for melanocyte death. *Oxidative Medicine and Cellular Longevity, 2022*(1), 8498472.
Yildirim, M., Baysal, V., Inaloz, H. S., & Can, M. (2004). The role of oxidants and antioxidants in generalized vitiligo at tissue level. *Journal of the European Academy of Dermatology and Venereology, 18*(6), 683–686. https://doi.org/10.1111/j.1468-3083.2004.01080