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Effect of *Aloe vera* and *Mentha piperita* Combination Serum on CDK1 and CDC25 Expression in Chemotherapy-Induced Alopecia Rats

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ABSTRACT

Chemotherapy-induced alopecia is a prevalent side effect, prompting exploration into preventive measures. Therefore, this study aimed to investigate the effect of *Mentha piperita* (mint) leaf and *Aloe vera* serum, administered either individually or in combination,, on CDK1 (cyclin-dependent kinase 1) and CDC25 (cell division cycle 25) expression in Wistar rat hair tissue receiving cyclophosphamide (CYP) chemotherapy. A total of 42 rats were divided into six groups, with seven rats in each group. The groups consisted of: a normal control group (G1), a cyclophosphamide (CYP)-induced alopecia group (G2), and treatment groups receiving minoxidil (G3), mint leaf serum (G4), *Aloe vera* serum (G5), and a combination of mint leaf and *Aloe vera* serum (G6). CDK1 and CDC25 expression was assessed through immunohistochemistry, and One-Way Analysis of Variance (ANOVA) determined significance at $p<0.05$. The results showed that there was elevated CDK1 and CDC25 expression in G2 compared to G1. CDK1 expression in G4, G5, and G6 (20.30±4.33%, 37.43±9.86%, and 33.17±14.93%, respectively) significantly decreased compared to G2 (48.86±6.74%) ($p<0.05$). CDC25 expression in G4 (20.30±4.33%) was significantly lower than G2 ($p<0.05$). Additionally, CDK1 expression in K6 was significantly reduced compared to G3 ($p<0.05$). There were no significant differences in CDC25 expression between G4, G5, and G6 compared to G3 ($p>0.05$). The combination of mint leaf and *Aloe vera* serum effectively reduced CDK1 expression, while CDC25 remained unaffected. Mint serum alone demonstrated a reduction in CDC25 expression, underscoring the potential in preventing chemotherapy-induced alopecia.

Keywords: *Aloe vera*, *Mentha piperita*, cyclin-dependent kinase 1, cell division cycle 25, chemotherapy, alopecia, Wistar rats

Introduction

Chemotherapy is the primary treatment option for cancer in Indonesia, with both psychological and biological effects. For example, alopecia, a common side effect in various chemotherapy regimens, significantly affects mental health and quality of life.[1] It has a prevalence of 65% and leads to 8% of patients rejecting chemotherapy. Chemotherapy-induced alopecia (CIA) differs from age or hormone-related type and is more effectively prevented with scalp cooler agents.[2] Other types of alopecia can be effectively treated with minoxidil. However, the use is not recommended during chemotherapy as it may interfere with the effectiveness of the treatment.[3] Minoxidil, known for inducing angiogenesis by enhancing vascular endothelial growth factor regulation and activating prostaglandin-endoperoxide synthase 1, opposes the effects of scalp cooling. Additionally, minoxidil shortens the telogen phase and lengthens the anagen phase, making it unsuitable for use during chemotherapy, where the anagen phase is most vulnerable to drugs.[4]

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Aloe vera has the potential to enhance hair growth,[5] providing necessary nutrients that enhance the regeneration and growth of follicles.[6] Meanwhile, peppermint oil from *Mentha piperita* (mint) leaf stimulates alkaline phosphatase enzyme activity and dermal papilla hair vascularization, inducing the hair-growth anagen phase.[7] The mixed nutrient-rich *Aloe vera* and menthol compounds from mint leaf, creating a "cooling" sensation on the skin, can be utilized as a scalp cooler in serum form. Exploration of potential ingredients to prevent and treat CIA is necessary, specifically, those focused on substances that can cool and soothe the scalp (scalp cooler). *Aloe vera* contains D-mannose acetyl polysaccharides, while mint leaf contains menthol and isomenthone,[8] active ingredients with scalp-cooling properties. Scalp cooling induces vasoconstriction, potentially decreasing the reach of chemotherapy drugs to hair follicles, and mitigating the significant hair loss risk. [9–11] The synergistic effect of the combination of these two herbs for alopecia needs further investigation. Therefore, this analysis examines the effects of administering a mixed serum of *Aloe vera* and mint leaf on the expression of cyclin-dependent kinase 1 (CDK1) and cell division cycle 25 (CDC25) in the skin tissue of Wistar rats induced by chemotherapy.

Materials and Methods

This study used an experimental design to assess the effect of administering a mixed serum of Aloe vera and mint leaf on the CDK1 and CDC25 expression in the skin tissue of Wistar rats induced by chemotherapy. The experiment was performed at the Physiology Laboratory, Faculty of Medicine, Universitas Brawijaya, Malang. All procedures have obtained ethical clearance from the Ethics Committee of the Faculty of Medicine, Sultan Agung Islamic University, as

indicated by letter number No. 457/XI/2023/Bioethics Commission.

Animals

Male Wistar rats aged 2–3 months and weighing between 200–250 grams were used in this study. Prior to the experiment, the animals were acclimatized for seven days under standard laboratory conditions. The acclimatization environment included a temperature of 22 ± 2 °C, relative humidity of 50–60%, and a 12-hour light/dark cycle. During this period, the rats had free access to standard rodent chow and clean drinking water ad libitum.

Experimental procedure

A total of 42 male Wistar rats were randomly allocated into 6 groups each containing 7 members. Group 1 served as the normal control with standard feed, while Group 2 was the negative control, receiving 150 mg/kgBW cyclophosphamide (CYP) intraperitoneally for 1 day. Group 3, the positive control, received 150 mg/kgBW CYP intraperitoneally for 1 day concurrently with minoxidil for 14 days. For the treatment, Group 4 was administered 150 mg/kgBW CYP intraperitoneally for 1 day alongside a 3% topical mint leaf extract serum for 14 days. Group 5 received 150 mg/kgBW CYP intraperitoneally for 1 day alongside a 50% topical aloe vera extract serum for 14 days, and Group 6 was subjected to 150 mg/kgBW CYP intraperitoneally for 1 day alongside a topical combination serum of 50% aloe vera and 3% mint leaf for 14 days.

Plant Collection and Identification

Aloe vera and Mentha piperita (mint) leaves were collected on 1 November 2023 from the Tawangmangu Herbal Garden, located in Central Java, Indonesia (coordinates: 7.6766° S, 111.1250° E). The plants were taxonomically identified and verified by the Head of the Department of Biology, Faculty of Medicine, Universitas Islam Sultan Agung, Semarang, Indonesia. Voucher specimens were prepared and deposited at the Herbarium of the Faculty of Medicine, Universitas Islam Sultan Agung, under the specimen numbers FKSA-AV1-XI23 for Aloe vera and FKSA-MP1-XI23 for Mentha piperita.

Extraction of plant material

Aloe vera leaf was thoroughly washed, peeled, cut into small pieces, and dried in an oven until completely dry. Subsequently, blending was carried out and 80 grams was weighed for maceration through two soaking sessions with 96% ethanol, totaling 400 ml for both soakings. The filtrate was evaporated to obtain a concentrated extract. From 80 grams of dried Aloe vera, a maceration yield of 38.17 grams was obtained, resulting in a maceration yield of 47.71% w/w calculated from the weight of the concentrated extract per weight of the dried raw material multiplied by 100%.[12] Mint leaf was washed, dried to remove moisture, blended, and sifted using an 18-mesh sieve. Maceration was then carried out using 96% ethanol as the solvent.[13] The topical application of the scalp cooler would be used in a concentration of 10%.[14]

Serum Preparation

The preparation of serum started with aquadest and Tween 80 heated to approximately 50°C and then mixed using a magnetic stirrer. Mint and Aloe vera extracts were gradually added to the mixture while constantly stirring. Glycerin and preservatives (methylparaben and propylparaben) were followed by stirring with a magnetic stirrer at a speed of 1500 rpm for about 15 minutes until an emulsion was formed. The final concentrations of Aloe vera extract and mint leaf extract in the emulsion were 50% and 10%, respectively.[9]

Immunohistochemistry staining

Immunohistochemistry staining was carried out using primary antibodies (CDK1 or CDC25), secondary antibodies, and TrekavidinHRP Label. DAB was applied, followed by counterstaining with Mayer's hematoxylin. Slides were dehydrated and mounted with Entellan. Subsequently, the expression of CDK1 and CDC25 was analyzed using Imunoratio software. Data obtained were examined using the ANOVA (One-Way Analysis of Variance) test, followed by the Post Hoc LSD (Least Significant Difference), and significant difference was arranged at p-value <0.05

Results and Discussion

The CDK1 expression results in each group are presented in Table 1 and Figure 1. The healthy controls had the lowest mean of CDK1 expression at 19.42 ± 3.12, while the highest was observed in the negative control at 56.93 ± 8.25, indicating an increase due to CYP induction. CDK1 expression in G3, G4, G5, and G6 was also lower than the negative control. The combination of *Aloe vera* and mint leaf serum group caused lower CDK1 expression compared to the single application, as well as the minoxidil group. As shown in Figure 1, the administration of minoxidil, mint leaf serum, *Aloe vera* serum, and the combination of *Aloe vera* and mint leaf serum reduced CDK1 expression than the negative control ($p < 0.05$). The administration of *Aloe vera* serum had no significant effect in reducing CDK1 expression compared to minoxidil ($p > 0.05$). Furthermore, the result obtained using a combination of *Aloe vera* and mint leaf serum was not significantly different from the healthy control group ($p > 0.05$).

Table 1: Average CDK1 Expression in Each Group

Groups	Mean±SD (%)
G1	19.42±3.12
G2	56.93±8.25
G3	33.78±4.35*
G4	46.16±10.31*
G5	31.19±7.64*
G6	23.67±4.29*

SD : standard deviation; *: Significantly different from G2

The expression of CDC25 is shown in Table 2 and Figure 2. The lowest mean expression of CDC25 was found in mint leaf serum at 20.30 ± 4.33, while the highest was observed in the negative control group at 48.86 ± 6.74. Table 4.2 also shows that the mean CDC25 expression values in G3, G4, G5, and G6 are lower than the negative control. The group with mint leaf serum had lower CDC25 expression than the positive control.

Based on Figure 2, the administration of mint leaf serum reduced CDC25 expression than the negative control ($p < 0.05$). However, *Aloe vera* and mint leaf serum combination did not show a significant difference in reducing CDC25 expression compared to minoxidil ($p > 0.05$). The result obtained in rats given mint leaf serum was not significantly different from the healthy control group ($p > 0.05$).

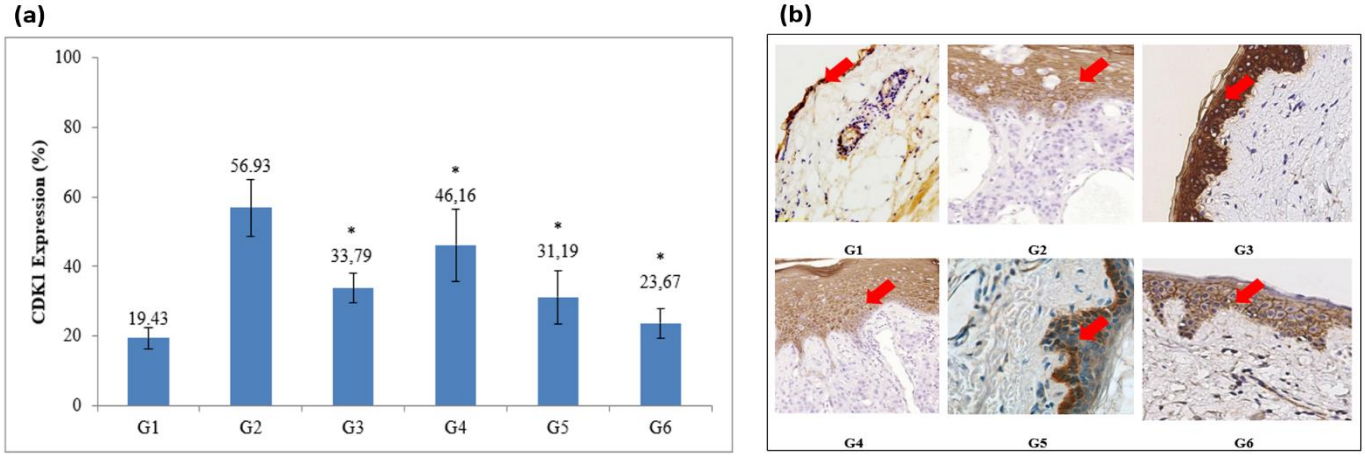

Figure 1: (a) Mean expression of CDK1 in each group, the asterisk shows there is a significant difference with G2 as negative (placebo) control; (b) CDK1 immunohistochemistry staining, the brown color shows the expression of CDK1 in the skin tissue.

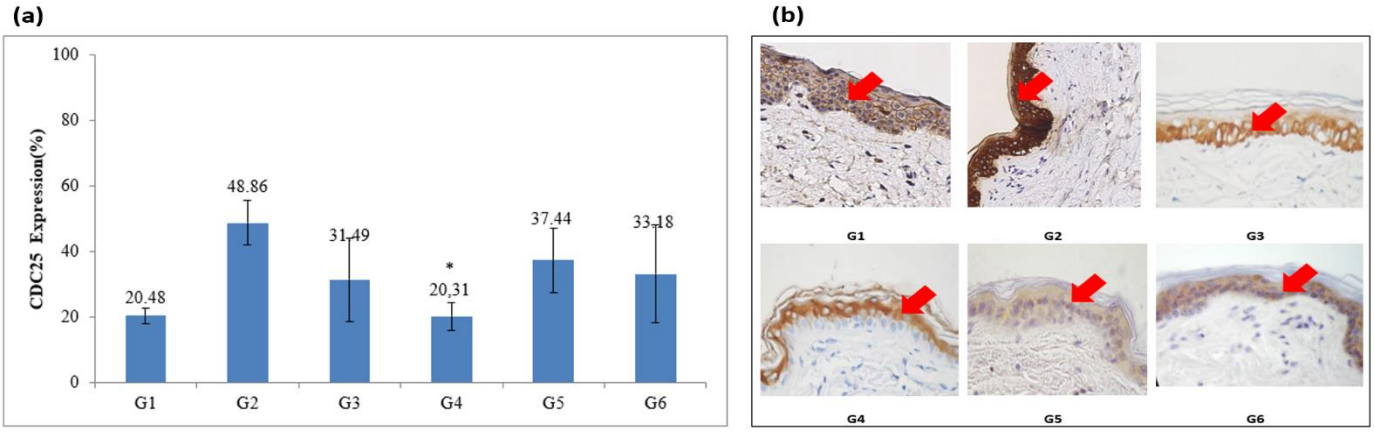

Figure 2: (a) Mean expression of CDC25 in each group, the asterisk shows there is a significant difference with G2 as negative (placebo) control; (b) CDC25 immunohistochemistry staining, the brown color shows the expression of CDC25 in the skin tissue.

Table 2: Average CDC25 Expression in Each Group

Groups	Mean±SD (%)
G1	20.47±2.5
G2	48.86±6.74
G3	31.48±12.57
G4	20.30±4.33*
G5	37.43±9.86
G6	33.17±14.93

SD : standard deviation; *: Significantly different from G2

Chemotherapy has been proven to induce alopecia. More specifically, chemotherapy with CYP reportedly triggers alopecia through the induction of apoptosis mechanisms.[14,15] According to previous studies, CYP administration leads to DNA (deoxyribonucleic acid) damage, inducing P53 expression, which in turn triggers apoptosis.[16]

Scalp cooling serves to avoid or lower chemotherapy-induced alopecia through two main mechanisms.[17] Firstly, it reduces the scalp temperature using a gel method, thereby inducing local vasoconstriction. This leads to a reduction in the flow of chemotherapy drugs to the scalp and drug concentration in hair follicles. Secondly, low temperature helps decrease cellular metabolism in hair follicles, decreasing the vulnerability to the anti-mitotic and antimetabolic effects of chemotherapy drugs. Induced low temperatures also inhibit the cell cycle at G0/G1 phase, increase HSP70 accumulation to keep cells from stress, and reduce apoptosis rates.[17] Therefore, scalp cooling has great potential as a holistic method for protecting hair follicles during chemotherapy.

This study showed that the administration of mint leaf and *Aloe vera* serum with minoxidil reduced the expression of CDK1.[18] In general, high expression indicates DNA damage, which triggers apoptosis response. CDK1 is a protein that facilitates the cell cycle from mitotic arrest to apoptosis.

Based on the results, mint leaf serum administration reduced the expression of CDC25.[19] DNA damage in G2/M phase requires two signal transduction pathways, namely CDC25 and P53. The essential function of the dual-specificity phosphatase known as CDC25 lies in regulating the cell cycle through the influence of CDK. CDC25 increases under conditions of DNA damage.[20]

Minoxidil works by increasing DNA synthesis in hair growth phase called anagen. It also stimulates secondary hair germ cells in inactive follicles (telogen), triggering a rapid transition to the anagen growth phase. Furthermore, minoxidil induces prostaglandin E2 synthesis by activating prostaglandin endoperoxide synthase-1 and conversely inhibits prostacyclin production. Minoxidil also enhances prostaglandin E2 receptor expression, a gene target in the Wnt/β-catenin pathway of dermal papilla cells. This enhancement can facilitate sustained hair

follicle growth and maintain the anagen growth phase, thereby playing a role in preventing alopecia.[21]

The results showed that *Aloe vera* and mint leaf serum administration synergistically reduced CDK1 expression.[18] Mint leaf contains menthol compounds that modulate thermoregulatory responses, binding to transient receptor potential melastatin 8 (TRPM8) and transient receptor potential ankyrin 1 (TRPA1) channels to stimulate sympathetic effects on arteries. This sympathetic effect causes vasoconstriction, inhibiting CYP compounds from reaching hair capillaries. Furthermore, mint leaf demonstrated the highest effectiveness in reducing CDC25.[22,23] Menthol compounds presumably stimulate p38, which inhibits CDC25 expression. Further study is needed to fully understand the mechanisms. *Aloe vera* activates TGF-β pathway, promoting the katagen phase in the hair cycle.[24] It also contains aloesone, which can prevent reactive oxygen species (ROS).[25,26] In general, ROS triggers diverse adaptive responses in cells, leading to cell cycle arrest, apoptosis, or necrosis, depending on the levels. Cell cycle regulation requires cyclins and CDK, and ROS reportedly affects the presence as well as the activity of these enzymes. The complex formed by cyclins and CDK controls cell cycle progression. Furthermore, aloesone compounds can reduce mRNA (Messenger Ribonucleic Acid) expression of inflammatory genes Inducible Nitric Oxide Synthase (iNOS), Interleukin-1 Beta (IL-1β), Tumor Necrosis Factor Alpha (TNF-α), and increase antioxidant enzyme expression of Glutathione Peroxidase 1 (Gpx-1) and Superoxide Dismutase 1 (SOD-1).[26] These antioxidant compounds potentially neutralize ROS, preventing alopecia.

This study has successfully demonstrated the effects of *Aloe vera* and mint leaf serum combination on CYP-induced alopecia.[1] However, further study is needed to ensure safety with an evaluation of intervention side effects. This additional information is necessary for a comprehensive understanding of the effect of serum use. Given that the experiment focused on animal models, further trials using advanced mammals are required to validate results and apply these results to humans.

Conclusion

In conclusion, this study showed that the administration of *Aloe vera*, mint leaf, and combination significantly influenced CDK1 expression in the skin tissue of Wistar rats induced by chemotherapy. The single administration of mint leaf serum also had a significant effect on CDC25 expression. However, the administration of *Aloe vera* and mint leaf did not show a significant effect on CDC25 expression.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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