

COMPARATIVE STUDY ON THERAPEUTIC EFFICACY OF MINOXIDIL AND PLATELET-RICH PLASMA EXOSOME IN ANDROGENETIC ALOPECIA MICE MODELS

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ABSTRACT

Objective: Androgenetic alopecia (AGA) is a common type of hair loss worldwide. Currently, available treatment options for AGA show inconsistent results and may cause various undesirable side effects. Platelet-rich plasma exosomes (PRP-Exo) is one of the therapeutic modalities that has shown satisfactory results to treat hair loss. This study aims to assess the effectiveness of minoxidil and PRP-Exo for AGA.

Methods: An experimental investigation was conducted using alopecia BALB/c mice to examine hair follicle characteristics. Fourteen mice were divided into two groups, one group received daily topical application of 5% minoxidil, meanwhile, the other group received 0.1 ml injections of PRP-Exo on days 11, 18 and 25. Follicle density and the anagen: telogen ratio were evaluated histopathological.

Results: The administration of PRP-Exo resulted in significantly different follicle density (68.61 ± 7.64 vs. 40.14 ± 11.60 ; $p = 0.007$) and anagen: telogen ratio (0.34 ± 0.04 vs. 0.22 ± 0.02 ; $p = 0.009$) compared to minoxidil.

Conclusion: This investigation demonstrates that PRP-Exo effectively enhance both hair follicle density and the anagen: telogen ratio in the AGA mice model when compared to minoxidil 5%.

Keywords: Androgenetic alopecia, Platelet-rich plasma exosome, Minoxidil, Hair follicle density, Anagen: telogen ratio

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INTRODUCTION

Androgenetic alopecia (AGA), a highly prevalent type of hair loss, poses a significant challenge in the field of dermatology due to the limited availability of effective treatment options [1]. The global prevalence of AGA is estimated at 2%, with a tendency to increase with age and affected both men and women [2, 3]. In Indonesia, the incidence of AGA ranges from 15 to 30 cases per 100 instances of alopecia [4, 5]. Various risk factors contribute to AGA, including genetic predisposition, occupational stress, and emotional strain [6]. This condition arises from an increased sensitivity of hair follicles to androgens, potentially causing psychological and social distress by altering hair appearance and affecting an individual's self-esteem [7, 8].

Current treatment approaches, such as minoxidil, primarily focus on inhibiting testosterone activity in accordance with the prevailing understanding of testosterone hypersensitivity [9]. Minoxidil, a well-established therapy for androgenetic alopecia, has been proven to be effective in promoting hair growth and prolonging the anagen phase of the hair growth cycle [2]. Despite its efficacy, responses to minoxidil treatment can vary among individuals, and prolonged use may lead to adverse effects, such as scalp irritation, hypertrichosis and unwanted facial hair growth [10, 11].

Recent research, however, suggests that AGA is not solely driven by testosterone sensitivity but also involves microinflammation within the hair follicles orchestrated by immune cells in affected individuals [12]. This emerging understanding opens up opportunities for alternative interventions, such as platelet-rich plasma exosomes (PRP-Exo) [13]. PRP-Exo is obtained from the patient's own blood, minimizing side effects [14]. The side effects that can arise tend to be mild, such as itching of the scalp and mild pain at the injection site. The effects of PRP-Exo therapy can also last longer so that it can provide a sense of satisfaction to patients [15]. Study by Nilforoushzadeh found that the administration of PRP-Exo to human dermis papilla cells can increase the proliferation and survival of dermis papilla cells [16]. PRP-Exo also has less side effects given that it is obtained from the patient's own blood sample. Side effects from PRP-Exo administration are generally mild, such as pain and redness at the injection site [17].

Therefore, we conducted a study aimed to investigate the effectiveness of PRP-Exo against AGA in mice induced into AGA model.

MATERIALS AND METHODS

Study design

This study employs a laboratory-based experimental design utilizing a post-test-only methodology with a control group. The primary objective of this investigation is to assess the density of hair follicles and the anagen-to-telogen ratio in the skin of BALB/c mice. There were several reasons we chose to use BALB/c mice, such as a more synchronized hair cycle, thinner dorsal skin that facilitates easier evaluation of hair follicle cycle and morphology, and more immunotolerance. All animal procedures were ethically sanctioned by the Research Ethics Committee of Dr. Moewardi Hospital Surakarta under protocol number 1.853. B/X/HREC/2023.

AGA model rat induction

Fourteen male BALB/c mice, each aged eight weeks, were induced with an androgenetic alopecia model by receiving daily subcutaneous injections of 0.1 ml of testosterone on their dorsal skin over a period of 10 d. We chose the dose and duration of testosterone administration based on preliminary study that we have conducted. Before the injection, we first cleaned the hair on the mice's back using an electric hair clipper. Subsequent to this induction, the mice were segregated into two groups: the minoxidil 5% group and the PRP-Exo group.

PRP-exo preparation

Exosomes were extracted from platelet-rich plasma using size exclusion chromatography with a qEV2 column from Izon Science. A precise volume of 2 ml of platelet-rich plasma was applied to the column. During elution, fractions were collected using 0.9% sodium chloride as the mobile phase, totaling 44 ml. The initial 14 ml was denoted as the void volume, with the subsequent 30 ml systematically divided into 15 distinct fractions, each containing 2 ml of exosomes and 0.5% sodium chloride solution. Nanoparticle tracking analysis

(NTA) (viewsizer 3000® Horiba, Japan) was employed specifically on fractions demonstrating significant exosomal presence to ascertain exosome concentrations. The particle concentration determined is in the exosome size range, which is 30-150 nm.

Treatment administration

The minoxidil 5% group underwent two daily applications of 2 sprays each for a duration of 3 w on the AGA mice's dorsal skin that has been shaved. 2 sprays of minoxidil 5% were about 2 ml. Meanwhile, PRP-Exo group given the PRP-Exo solution injections administered intradermally at a specified location on the dorsal skin using that has been marked by waterproof marker, with a volume of 0.1 ml. PRP-exo injection were given once a week for a total of 3 w.

Hair follicle density and anagen: telogen ratio

After the study period, skin tissue samples were biopsied from the treated areas on the dorsal region of the mice, with a 2 cm diameter reaching the subcutaneous layer, and preserved in a 10% formalin solution. Animals were anesthetized with chloroform before the biopsy to ensure their well-being and minimize discomfort. The skin samples, collected 21 d after testosterone induction, were fixed in 10% paraformaldehyde, embedded in paraffin blocks, and stained with hematoxylin and eosin (HE). Following this, the assessment of hair follicles and the anagen to telogen ratio began. Each skin sample was examined in four distinct fields of view, and the resulting

averages were calculated. The assessment of hair follicle density and anagen: telogen ratio was conducted by two anatomical pathologists. Observations were made using a microscope at 40x magnification. Hair follicle density was determined by counting the number of hair follicles in an area of 1 mm². Anagen: telogen ratio was assessed by calculating the ratio between the number of hair follicles in anagen and telogen phases in an area of 1 mm². Anagen follicles are located deeper in the dermis and exhibit a well-defined outer and inner root sheath, while telogen follicles are situated more superficially in the epidermis with a desiccated or wrinkled outer root sheath.

Statistical analysis

Statistical analysis was conducted using IBM SPSS Statistics 25.0 software. Results are articulated as mean±standard deviation (SD) and were examined through an independent samples T-test.

RESULTS AND DISCUSSION

Following the research, histopathological examinations were performed on the skin tissues of the animal model (fig. 1 and fig. 2). Fig. 1 sequentially showed an increasing number of hair follicles, with fig. 1B, the PRP-Exo group exhibiting a higher density of hair follicles. Fig. 2 showed the histopathology examination result of anagen: telogen ratio from both minoxidil and PRP-Exo group. The histopathology examination from the PRP-Exo group showed higher anagen: telogen ratio compared to the minoxidil group.

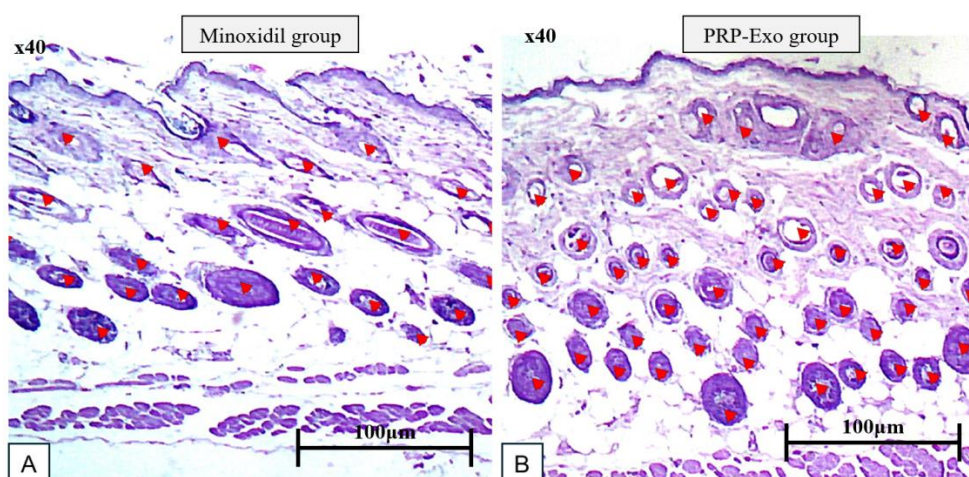


Fig. 1: Histopathology of hair follicle density in each group was examined using HE stains at a magnification of 40x. A. 5% minoxidil treatment group; B. PRP-Exo treatment group
Legend: red triangle = hair follicle

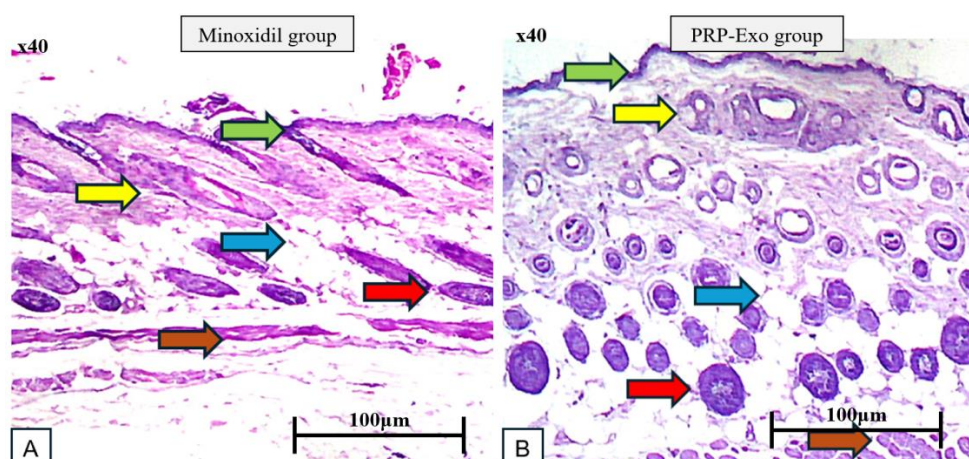


Fig. 2: Histopathology of the anagen: telogen ratio in each group with hematoxylin and eosin staining at a magnification of 40x. A. 5% minoxidil treatment group; B. PRP-Exo treatment group
Legend: green arrow = epidermis; yellow arrow = telogen; red arrow = anagen; blue arrow = sebaceous gland; brown arrow = muscle tissue

The mean values, standard deviations, as well as the minimum and maximum values of hair follicle density comparisons in the two groups can be observed in table 1. The 5% minoxidil treatment group has a mean of 40.14 ± 11.60 with a range between 29.25 to

57.25, while the PRP-Exo treatment group has a mean of 68.61 ± 7.64 with a range between 58.50 to 79.50. There was a significance difference of hair follicle density between minoxidil and PRP-Exo group with large effect size ($p = 0.0007$; $\eta^2 = 0.778$) (table 1).

Table 1: Hair follicle density in various treatment groups

Groups	Hair follicle density	Minimum	Maximum	p-value
5% Minoxidil	40.14 ± 11.6	29.25	57.25	0.0007
PRP-Exo	68.61 ± 7.64	58.5	79.5	

The mean values, standard deviations, as well as the minimum and maximum values of the anagen: telogen ratio comparisons in both groups can be observed in table 2. The 5% minoxidil treatment group has a mean of 0.22 ± 0.02 with a range between 0.20 to 0.25,

while the PRP-Exo treatment group has a mean of 0.34 ± 0.04 with a range between 0.27 to 0.38. There was a significance difference of anagen: telogen ratio between minoxidil and PRP-Exo group with large effect size ($p = 0.0009$; $\eta^2 = 0.895$) (table 2).

Table 2: Anagen: telogen ratio in various treatment group

Groups	Anagen: telogen ratio	Minimum	Maximum	p-value
5% Minoxidil	0.22 ± 0.02	0.2	0.25	0.0009
PRP-Exo	0.34 ± 0.04	0.27	0.38	

DISCUSSION

The first findings indicate that PRP-Exo represents the most effective therapy for increasing hair follicle density compared to monotherapy with 5% minoxidil. To the best of the researchers' knowledge, no other studies have utilized PRP exosomes as a therapy for treating AGA through hair follicle density improvement. However, several studies have employed PRP as a therapy for AGA by enhancing hair follicle density. These research findings are consistent with investigations carried out by Li *et al.* in 2024 in Jinan. The study conducted by Li *et al.* in 2024 in Jinan illustrated that PRP treatment led to an increase in hair density among individuals with AGA. Within this investigation, ten trials comprising a total of 555 treatment units were identified. The hair density within the PRP group exhibited a significant elevation compared to the control group [18]. It is widely recognized that platelets release a variety of growth factors and cytokines as part of the wound-healing process. Within PRP therapy, the plasma liberates growth factors that stimulate hair growth upon injection into the scalp [19]. Key growth factors, such as platelet-derived endothelial growth factor (PDGF), TGF- β , fibroblast growth factor-2 (FGF-2), VEGF, epidermal growth factor (EGF), insulin-like growth factor-1 (IGF-1), and glial cell line-derived neurotrophic factor (GDNF), are pivotal in activating fibroblasts, synthesizing collagen, promoting the extracellular matrix, and boosting the production of endogenous growth factors. The growth factors released by platelets in PRP are thought to encourage cell proliferation, differentiation, angiogenesis, and chemotaxis, all essential processes for initiating new hair growth [20]. Additionally, plasma encompasses dense granules containing bioactive factors that heighten membrane permeability [21]. These dense granules house serotonin, histamine, dopamine, calcium, and adenosine, which can elevate membrane permeability and regulate inflammation [22]. Based on the description above, there is a possibility that the treatment group of PRP-Exo most significantly increases hair follicle density in testosterone-induced AGA mice in comparison to minoxidil 5%.

The next finding showed illustrate that PRP-Exo emerges as the most efficacious therapy for augmenting the anagen-to-telogen ratio compared to 5% minoxidil. A study conducted by Elena and Irina in 2022 in Saint Petersburg yielded congruent results, indicating that the combined use of PRP-Exo and 5% minoxidil enhanced the anagen to telogen hair ratio by 80-fold compared to individuals solely receiving 5% minoxidil treatment. Conversely, the combined application of PRP exosomes and 5% minoxidil also amplified the anagen to telogen hair ratio by 8 times in contrast to those undergoing PRP therapy alone [23]. Minoxidil does not incite alterations in testosterone levels or androgen secretion from the adrenal glands, nor does it modify the genetic susceptibility of hair

follicles to androgens [24]. Utilizing PRP therapy alongside minoxidil extends the anagen, spurs proliferation, prolongs the lifespan of dermal papilla cells by impeding apoptosis processes during the hair growth cycle and synergistically enhances their effects when combined [25]. Nonetheless, the intricate mechanisms involving minoxidil and PRP remain incompletely understood. Platelet-rich plasma proves effective in treating individuals who have not responded to minoxidil therapy for cases of AGA [26]. Minoxidil sulfate, an active metabolite of minoxidil activated by sulfotransferase enzymes, is accountable for stimulating hair growth [27].

Exosomes encompass a plethora of bioactive molecules, including growth factors, cytokines, and nucleic acids [28]. These molecules demonstrate the capacity to spur proliferation, differentiation, and survival of hair follicle cells, regulate hair follicle cycles, and accelerate the transition of telogen-phase follicles into a new growth phase sooner [29]. Furthermore, exosomes also prompt Maugham cells to proliferate anew, hasten melanoblast division, and incite fresh hair growth [30]. The distinct advantages of exosomes, such as their ability to transport multiple molecules within a single vesicle, target specific cells, facilitate engineering, exhibit low immune reactivity, lack drug dependency, offer high biocompatibility, entail minimal side effects, and allow for repeated drug administration, have attracted considerable attention within the medical domain [31]. Although investigations on PRP exosomes for AGA treatment are scarce, the application of other exosomes in AGA management has been documented. The application of PRP-Exo in humans should not pose a significant challenge because the use of PRP by injection in humans is quite common, such as for wound healing of diabetic foot ulcers and osteoarthritis [32, 33]. Although minoxidil is much more affordable compared to PRP-Exo [34], given its benefits and safety, the use of PRP-Exo as hair loss management in humans should be considered.

CONCLUSION

While this study provides valuable insights into the comparative efficacy of PRP-Exo and minoxidil in a BALB/c mouse model of AGA, several limitations must be acknowledged. First, although the mouse model effectively mimics certain aspects of human hair cycle dynamics, there are inherent anatomical and physiological differences between mice and human scalp skin and hair follicles. For instance, mice's hair cycles are more synchronized and regionally specific, whereas human hair growth is asynchronous and influenced by more complex hormonal and environmental factors. Second, the delivery methods and dosages used in mice may not directly translate to clinical applications in humans due to differences in skin structure, follicular density, and immune responses. Finally, while histological and macroscopic assessments

were used to evaluate hair regrowth, molecular studies such as gene expression or signaling pathway analysis were not conducted, which could provide deeper insights into the underlying mechanisms of action. Therefore, while promising, these findings should be interpreted with caution until validated in clinical trials involving human subjects. In summary, this study concluded that the treatment with PRP-Exo demonstrates superior efficacy compared to monotherapy with minoxidil 5% in a mice model of androgenetic alopecia.

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AUTHORS CONTRIBUTIONS

All authors made significant contributions to the study's conception and design, data acquisition, or the analysis and interpretation of data. They actively participated in drafting the manuscript or critically revising it to enhance its intellectual depth. Additionally, they consented to submit the work to the current journal, granted final approval for the published version, and accepted full accountabilities for all aspects of the research.

CONFLICT OF INTERESTS

Declared none

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