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Original Research Article

Activity of Apigenin Isolated from *Peronema canescens* Jack. on IgG Levels in Mice (*Mus musculus)* to Enhance Immunity

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ABSTRACT

Immunoglobulin G (IgG), a key component of the adaptive immune response, plays a crucial role in neutralising pathogens and affecting immune activity. This study examined the immunomodulatory properties of apigenin, a flavonoid isolated from *Peronema canescens* Jack. leaves on serum IgG in male *mice*. Twenty-four male mice (n=6/group) were used in this study, divided into 4 groups of 6 mice each. Group 1 (negative control) received oral apigenin at 0 mg/kg BW, and groups 2-4 received 25, 50, and 100 mg/kg BW daily for 14 days. Serum IgG was measured by sandwich ELISA on day 15. Data were analysed using one-way ANOVA with post-hoc Duncan tests. $p < 0.05$ was considered statistically significant. Results of the study showed that the Mean ± SD IgG (ng/mL) were: control 7596.616 ± 208.654; 25 mg/kg BW 8480.75 ± 170.656; 50 mg/kg BW 8857.22 ± 145.543; 100 mg/kg BW 9975.81 ± 127.320. One-way ANOVA showed a significant dose effect in IgG levels ($p < 0.05$). Post-hoc test indicated that the 100 mg/kg BW provided the optimal impact on increasing IgG levels. Apigenin from *P. canescens* increased serum IgG in mice, supporting its potential application in herbal therapy for immune enhancement.

Keywords: Immunoglobulin G, *Peronema canescens* Jack. Apigenin, immunomodulatory.

Introduction

The immune system plays a fundamental role in maintaining host defense against pathogenic threats through coordinated interactions between innate and adaptive immunity. Disruption of immune function increases susceptibility to infections, inflammatory disorders, and immune-related diseases, highlighting the importance of maintaining immune homeostasis [1,2]. Adaptive immunity provides long-term and antigen-specific protection, primarily mediated by humoral immune responses involving immunoglobulins. Immunoglobulins are produced by plasma cells following activation of antigen-specific B cells and function to recognize and neutralize pathogens such as bacteria, viruses, and toxins [3]. Among the immunoglobulin classes, Immunoglobulin G (IgG) is the most abundant antibody in the blood circulation. IgG plays a central role in toxin and virus neutralization, opsonization to facilitate phagocytosis, activation of the complement system, and passive immune protection through placental transfer [4]. One strategy to maintain or optimize immune function is immunomodulation, which refers to the regulation of immune responses to achieve balanced immune activity according to physiological or pathological conditions[5]. Immunomodulatory agents may act as immunostimulants to enhance immune responses in individuals with low immunity or as immunosuppressants to control excessive immune reactions [6].

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Among Indonesian medicinal plants, *Peronema canescens* Jack. has been reported to contain bioactive compounds, including flavonoids, saponins, and triterpenoids, which are associated with immunomodulatory activity [7,8]. One of the major flavonoid compounds identified in *Peronema canescens* leaves is apigenin (4′,5,7-*trihydroxyflavone*), a natural flavonoid also found in several medicinal plants. Apigenin has been reported to influence humoral immune responses by modulating immunoglobulin production and T helper cell balance [9,10]. In pathological conditions characterized by Th2-dominant immune responses, apigenin suppresses excessive IgG_1 and IgE production while enhancing IgG_{2a} levels, indicating its role in restoring immune balance [9]. In contrast, under physiological conditions, apigenin does not suppress basal immunoglobulin levels and has been reported to slightly increase IgG levels in healthy or non-sensitized animals, suggesting a stimulatory effect on baseline humoral immunity [10,11]. These findings indicate that the immunomodulatory activity of apigenin is context-dependent, acting as an immunoregulator rather than a purely stimulatory or suppressive agent. However, studies specifically evaluating the effect of apigenin on total IgG levels under physiological conditions remain limited, particularly when apigenin is isolated from local natural sources such as *Peronema canescens* Jack. Therefore, this study aimed to evaluate the effect of apigenin isolated from *Peronema canescens* Jack. on IgG levels in normal mice (*Mus musculus*) as an indicator of humoral immune modulation. The results are expected to provide scientific evidence supporting the role of apigenin as a natural immunomodulatory agent targeting adaptive immunity.

Materials and Methods

Materials

Reagents used include Na CMC, Aqua Pro Injection (Andeska Laboratory, Indonesia), and Mouse IgG (Bio Technology Lab®, Indonesia).

The study was conducted between March 2025 and August 2025 at the Immunology and Serology Laboratory of the Faculty of Pharmacy, Andalas University, and the Biomedical Laboratory of the Faculty of Medicine, Andalas University.

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Collection and preparation of Plant Material

Peronema canescens Jack. leaves were obtained in October 2024 from the Aia Pacah area in Padang City, West Sumatra, Indonesia. Botanical identification was performed by Dr Nurainas from the ANDA Herbarium, Department of Biology, Andalas University, and the plant sample was assigned voucher number 717/K-ID/ANDA/X/2024. The leaf samples were then air-dried, ground to a fine powder, and stored in an airtight container until use.

Test Animals and Ethical Clearance

The animals used were 24 male BALB/c mice, aged 2-3 months and weighing 25-35 g. The animals were acclimatised for 7 days to help them adjust to the environment, thereby reducing stress and fear during the test. Mice were placed in a spacious area, allowing them to move freely and exhibit their natural behavior, with access to standard chow and water *ad libitum*.

Ethical approval was obtained from the Ethics Committee of the Faculty of Pharmacy, Andalas University, under number 61/UN16.10.D.KEPK-FF/2025.

Extraction Procedure

Dried *Peronema canescens* leaf powder (17 kg) was macerated with 42.5 L of 96% ethanol in a material-to-solvent ratio of 2:5 (w/v). Maceration was carried out for 72 hours at room temperature, with the solvent replaced every 24 hours. The collected filtrate was then evaporated using a rotary evaporator to yield about 1.2 kg of crude ethanol extract.

Isolation of Apigenin

A 500 g ethanol extract was further fractionated (liquid–liquid fractionation) using a series of increasing solvent polarities: n-hexane, ethyl acetate, and ethanol. The ethanol-soluble fraction was then purified by vacuum liquid chromatography (VLC) using a gradient of n-hexane: ethyl acetate (100:0 to 0:100), followed by methanol. This yielded 14 fractions, which were subsequently grouped into five main fractions based on similarities in TLC staining patterns. The fraction with the highest UV staining intensity (Fraction X) was further purified by radial chromatography on 0.5 mm silica gel plates. A graded eluent system of n-hexane: ethyl acetate (6:4, 5:5, 3:7) was used. This yielded about 10 g of a yellow solid (compound **1**).

Characterisation of the Isolated Compound

Spectroscopic characterisation confirmed that compound **1** is apigenin, based on the following data: UV–Vis (MeOH): λmax at 210, 267, and 336 nm; FT–IR: characteristic absorption bands of –OH (3286 cm^{-1}), C=O (1653 cm^{-1}), aromatic ring (1587–1446 cm^{-1}), and C–O (1298–1031 cm^{-1}); ^{1}H-NMR and ^{13}C-NMR (DMSO-d_6, 500/125 MHz): show consistent flavonoid proton and carbon patterns, along with HMBC and COSY correlations supporting the structure of apigenin.

Preparation of Apigenin Suspension

The active compound isolated from *Peronema canescens* leaves was suspended in a 0.5% sodium carboxymethyl cellulose (Na CMC) solution. The Na CMC solution was prepared by weighing 250 mg of Na CMC, grinding it to a smooth paste, and adding 10 mL of hot water while stirring continuously until a homogeneous mixture formed. Distilled water was gradually added to the mortar contents until the total volume reached 50 mL. Apigenin was suspended in the Na CMC solution at the predetermined concentration.

Administration (Dosing)

Twenty-four male mice were divided into four treatment groups, each with six mice. The doses of apigenin used were 25 mg/kg body weight (BW), 50 mg/kg BW, and 100 mg/kg BW [12]. Group I (K1) negative control with healthy mice was administered only Na CMC for 14 days; Treatment Group I (P1), received apigenin suspension at a dose of 25 mg/kg BW orally once daily for 14 days; Treatment Group II (P2), received apigenin suspension at a dose of 50 mg/kg BW orally once daily for 14 days; and Treatment Group III (P3), received apigenin suspension at a dose of 100 mg/kg BW orally once daily for 14 days.

Blood Sampling and Serum Preparation

The test animals were sacrificed under chloroform anaesthesia, and blood samples were obtained by carotid artery excision using the guillotine method, yielding approximately 1 mL per mouse [13]. The collected blood was transferred into microtubes and centrifuged at 3000 rpm for 10 minutes. The serum was carefully separated, and each sample was processed in duplicate, then stored at -80°C until analysis.

ELISA and IgG Level Determination

Serum immunoglobulin G concentration was determined using the Mouse IgG ELISA kit (BT Laboratory, Cat. No. EA0027Mo) following the kit's sandwich ELISA instructions. An anti-mouse IgG antibody was pre-coated onto a 96-well plate. Standards (0-16000 ng/mL) and serum samples (100 µL, in duplicate) were added and incubated. After washing, biotinylated detection antibody and streptavidin-HRP were sequentially added. The substrate solution (TMB) initiated a color reaction, which was stopped with an acid stop solution. The absorbance was measured at 450 nm using a microplate reader (xMark, Bio-Rad, USA) at room temperature (26.1°C), with optical density proportional to the IgG concentration in the sample.

A standard curve was generated using a four-parameter logistic model (4-PL) (r^2 = 0.991, χ^2 = 0.003, RMS = 0.033), with concentrations ranging from 0 to 16000 ng/mL. The lower limit of detection (LOD) was 685 ng/mL, and intra-assay variability was below 10%. Serum IgG concentrations in experimental samples were interpolated from this curve, and all samples were analysed in duplicate for reliability.

Statistical Analysis

Data are presented as mean ± standard deviation (SD). Analysis was performed using one-way ANOVA followed by Duncan's test in SPSS. Differences were considered significant if the p-value was <0.05.

Results and Discussion

The results of IgG level testing showed an average increase in IgG levels in male mice treated with apigenin derived from *Peronema canescens* leaves (Table 1). The average IgG levels in the negative control group of mice, and at doses of 25 mg/kg BW, 50 mg/kg BW, and 100 mg/kg BW were 7596.616, 8480.75, 8857.22, and 9975.81 ng/mL, respectively (Figure 1). These average data indicate that the higher the dose of apigenin administered, the more optimal the effect on increasing IgG levels.

Table 1*:* Results of determining immunoglobulin G (IgG) levels in male white mice

Groups	Mean ± SD (ng/mL)
I (Negative Control)	7596.616 ± 208.654
II (Dose 25 mg/kg BW)	8480.75 ± 170.656
III (Dose 50 mg/kg BW)	8857.22 ± 145.543
IV (Dose 100 mg/kg BW)	9975.81 ± 127.320

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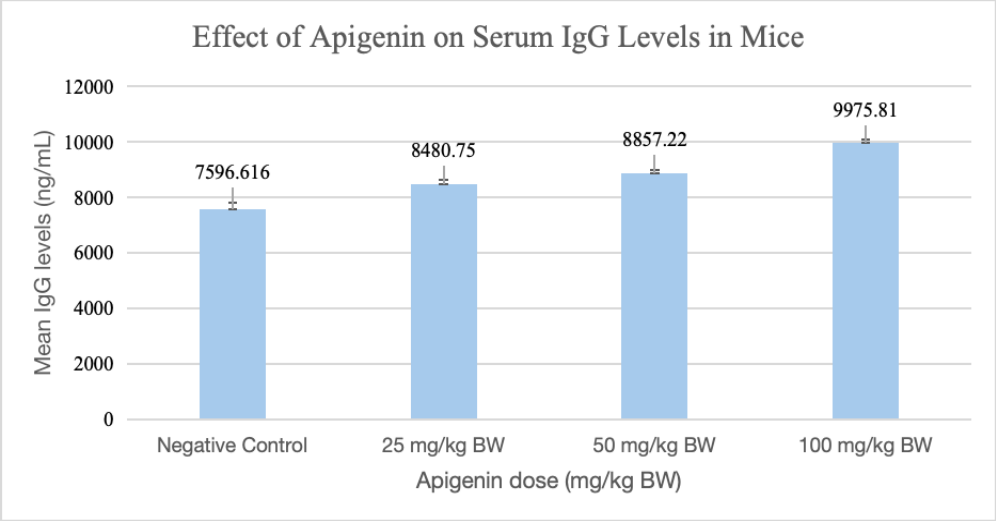

Figure 1: Average IgG levels versus plant extract concentrations

The Shapiro-Wilk test was used to assess the distribution of data in each treatment group. Based on the results, the negative control group showed a p-value = 0.989 (> 0.05); the 25 mg/kg BW dose group showed a p-value = 0.765 (> 0.05); the 50 mg/kg BW dose group showed a p-value = 0.392 (> 0.05); and the 100 mg/kg BW dose group showed a p-value = 0.878 (> 0.05), so all of the data were normally distributed (Table 2). This indicates that the data in all treatment groups were normally distributed, so that parametric statistical analysis, ANOVA, can be used.

Table 2: Shapiro-Wilk Normality Test

		Kolmogrov-Smimov[a]			Shapiro-Wilk		
Groups		**Statistics**	**df**	**Sig.**	**Statistics**	**df**	**Sig.**
Dose	Negative Control	0.139	6	0.200*	0.990	6	0.989
	Dose 25 mg/kg BW	0.155	6	0.200*	0.953	6	0.765
	Dose 50 mg/kg BW	0.226	6	0.200*	0.903	6	0.392
	Dose 100 mg/kg BW	0.176	6	0.200*	0.968	6	0.878

*This is a lower bound of the true significance
[a] Lilliefors Significance Correlation

The homogeneity of variance test was conducted to assess whether the variances between treatment groups were equal (homogeneous). The Levene test results provide several calculation approaches: Based on Mean, Based on Median, Based on Median with adjusted degrees of freedom, and Based on Trimmed Mean. In all calculation methods, the significance value obtained was above 0.05 (Based on the Mean, p = 0.664; based on the Median, p = 0.676), indicating that the variances between groups are homogeneous (Table 3). Since the data meet the assumption of homogeneity of variance, further analysis using ANOVA and Duncan's multiple range test can be carried out.

Table 3: Test of Homogeneity of Variance (Levene's Test)

		Levene Statistic			
Groups		**Statistics**	**df1**	**df2**	**Sig.**
Dose	Based on Mean	0.534	3	20	0.664
	Based on Median	0.516	3	20	0.676
	Based on Median and with adjusted df	0.516	3	12.926	0.679
	Based on the trimmed mean	0.534	3	20	0.668

The results of the one-way ANOVA test showed a significant difference in the average concentration of Immunoglobulin G between treatment groups (F(3,20) = 211.962; p = 0.000; Table 4). This finding indicates that variations in the dose of apigenin significantly affect Immunoglobulin G levels in male white mice (p<0.05).

Table 4: One-way ANOVA test

Dose	**Sum of Squares**	**df**	**Mean Squares**	**F**	**Sig.**
Between Groups	17489457.37	3	5829819.125	211.962	0.000
Within Groups	550080.514	20	27504.026		
Total	18039537.89	23			

The post hoc test indicated each group belonged to a distinct subset: negative control (7596.62 ng/mL), 25 mg/kg BW (8480.75 ng/mL), 50 mg/kg BW (8857.34 ng/mL), and 100 mg/kg BW (9975.81 ng/mL). Each dose significantly increased IgG levels (p < 0.05). These results show that the extract increased IgG levels at all doses compared to the control. The lowest increase was at 25 mg/kg BW (+884.13 ng/mL), followed by 50 mg/kg BW (+1260.72 ng/mL) and 100 mg/kg BW (+2379.19 ng/mL) (Table 5).

This pattern exhibits a dose-response relationship, where higher doses of the test compound lead to higher IgG levels compared to lower doses. This finding indicates that apigenin enhances basal humoral immune function under physiological conditions. In the adaptive immune response, IgG is produced by activated B cells with the assistance of T helper cells and plays a central role in pathogen neutralization, opsonization, and immune regulation through Fcγ receptor (FcγR) interactions [14].

Table 5: Post Hoc test

Groups	N	1	2	3	4
Negative Control	6	7596.61600			
Dose 25 mg/kg BW	6		8480.75333		
Dose 50 mg/kg BW	6			8857.34467	
Dose 100 mg/kg BW	6				9976.80583
Sig.		1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed
a. Uses Harmonic Mean Sample Size = 6.000

The observed increase in IgG levels is consistent with previous studies reporting the context-dependent immunomodulatory activity of apigenin. In pathological conditions dominated by Th2 responses, apigenin suppresses excessive IgG_1 and IgE production while promoting IgG_2a, thereby restoring Th1/Th2 balance [9,10]. Conversely, in healthy or non-sensitized animals, apigenin has been shown to maintain or slightly increase basal IgG levels, indicating a stimulatory effect on humoral immunity without inducing hyperactivation [10,11]. This bidirectional effect supports the classification of apigenin as an immunoregulator rather than a pure immunostimulant or immunosuppressant. Furthermore, IgG produced under physiological conditions can interact with FcγRs on innate immune cells, enhancing antigen presentation and reinforcing the functional link between innate and adaptive immunity while maintaining immune homeostasis [15]. Thus, the dose-dependent elevation of IgG observed in this study suggests that apigenin isolated from *Peronema canescens* acts as a humoral immunomodulator that strengthens basal adaptive immune function in normal mice.

Conclusion

Apigenin isolated from *Peronema canescens* leaves has the potential to enhance immunity in normal conditions, with a dose of 100 mg/kg body weight providing the optimal effect in increasing IgG levels. This supports the development of *Peronema canescens* leaves as a candidate for a safe immunomodulatory phytopharmaceutical raw material under normal physiological conditions.

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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