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Original Article

The Effect of Anthocyanins from Purple Sweet Potato Extract In-vitro on IL-6 mRNA Expression in PBMC Cell Cultures Infected With Hepatitis B Virus

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ABSTRACT

Background: The immune system is a crucial component of the defense against viral infections. Some cytokines directly regulate the immune response by suppressing viral replication. Interleukin-6 (IL-6) is a pleiotropic cytokine that plays a crucial role in regulating the biological responses of several target cells to viral infections. Natural compounds, such as anthocyanins, possess antiviral properties that may be beneficial in treating various viral infections. The aims of this study are to analyze the effects of anthocyanins on IL-6 expression in PBMC cultures infected with the Hepatitis B virus.

Methods: This study was an in-vitro experimental study. This study used a control group and a treatment group, with 18 PBMC cell culture wells. The treatment involved adding 50 µL of purple sweet potato water extract, with an anthocyanin content of 0.01575 µg/µL, to RPMI1640 culture medium in PBMC culture wells for 24 hours. The effect on the IL-6 cytokine response was assessed by quantifying IL-6 mRNA expression in RNA isolated from PMBC cell culture samples using the quantitative real-time PCR method and interpolating from a standard curve of serial dilutions, based on cycle threshold. Data analysis was performed using statistical analysis.

Results: Statistical analysis of the effects of purple sweet potato extract exposure on the treatment and control groups was carried out using a paired t-test, showing that there was an effect of exposure to purple sweet potato extract on IL-6 mRNA expression in the treatment group with a p value of 0.001 ($p<0.05$), while no significant effect of exposure to purple sweet potato extract was found in the control group with a p value of 0.420 ($p>0.05$). The results of the independent t-test showed that in the treatment group, the mean difference in IL-6 mRNA expression was -75.87±45.77 fg/µL, whereas in the control group, it was -49.26±173.70 fg/µL. In the treatment and control groups, the mean difference in IL-6 mRNA expression was -26.61 fg/µL, with a p value of 0.66 ($p>0.05$).

Conclusion: This study showed that anthocyanins in purple sweet potato water extract did not significantly increase IL-6 mRNA expression in HBV-infected PBMC cultures compared to controls.

INTRODUCTION

The occurrence of a viral infection will cause an immune system reaction and a systemic inflammatory process and can continue to worsen to become chronic if it cannot be handled by the immune system (Yang et al., 2024). The immune response during a viral infection often involves the same immune system as that elicited by many other pathogenic agents (Steinbrink et al., 2024). The cellular immune system, including lymphocytes and macrophages, is a crucial component of defense against pathogens, including viruses. The role of the cellular immune system is complex, as it plays a key role in antiviral immunity during viral infections (Wang et al., 2024). Many studies have shown that several cytokines increase during the progression of viral infections. These cytokines are likely involved in regulating the immune response to viral infections by directly inhibiting viral replication. Some cytokines directly regulate the immune response by suppressing viral replication in cell culture (Mourtzikoua et al., 2014).

Interleukin-6 (IL-6) is a pleiotropic cytokine that plays a crucial role in regulating the biological responses of several target cells to viral infections. IL-6 inhibits viral replication, as observed in HBV infection (Kuo et al., 2009). In HBV infection, IL-6 is produced by hepatocytes, Kupffer cells, and immune cells in response to the activation of inflammatory pathways. IL-6 contributes to B cell differentiation, T cell activation, and the induction of acute-phase proteins of viral infection (Dimitriadis et al., 2023). IL-6 is a pro-inflammatory cytokine that plays a key role in the immune response to viral infections, including hepatitis B virus. In HBV infection, IL-6 contributes to B cell differentiation, T cell activation, and the induction of acute-phase proteins in the liver (Abakar et al., 2023). In-vitro studies of Peripheral blood sel mononuclear cell (PBMC) cells with several pathogens that trigger immune responses have shown that circulating leukocytes are present during infections, such as viral infections[2]. Lymphocytes and monocytes in PBMC cells play an important role in fighting infections such as viral infections and other pathogens(Nadhira et al., 2018).

Purple sweet potato (*Ipomea batatas* L.) is a type of sweet potato that comes in various shades of purple with varying degrees of intensity, due to its high anthocyanin content. Purple sweet potatoes have a high total phenol content and high antioxidant activity (Tang et al., 2023). Purple sweet potatoes are a readily available food and are frequently consumed daily. They contain a high level of the antioxidant anthocyanin. A study by Ramdan et al.(2023) showed that purple sweet potatoes, using the differential pH method, contained an anthocyanin content of 4.26 mg/liter (Ramdan & Lestari, 2023). Natural compounds such as polyphenols possess antiviral properties that are potentially beneficial in various viral infections. Polyphenols also inhibit viral enzymatic activity by inhibiting the multiplication and function of viral glycoproteins, which are key components of nucleocapsid formation in viruses (Chang et al., 2013).

Anthocyanin, a bioactive component is a natural coloring agent classified as an antioxidant. Anthocyanins have a phenolic structure, allowing them to transfer hydrogen atoms from hydroxyl free radicals. Foods such as fruits, vegetables, roots, tubers, nuts, and cereals with bluish or purplish hues contain high levels of anthocyanins (Santoso et al., 2014; Tall et al., 2004). Anthocyanins can induce the production of anti-inflammatory cytokines and significantly reduce nitric oxide (NO) production, thereby reducing oxidative stress in the anti-inflammatory process. Anthocyanins and anthocyanin-rich extracts can induce cytokine production and act as modulators of the immune response in activated macrophages (Wang et al., 2002). Anthocyanins are water-soluble flavonoid pigments found abundantly in red, purple, and blue plants and have significant activity against the immune system. Anthocyanins act as powerful antioxidants that protect immune cells such as lymphocytes, macrophages, and dendritic cells from oxidative stress. Furthermore, these compounds are capable of modulating intracellular signaling pathways such as NF-κB and MAPK, which play a role in regulating the production of pro-inflammatory cytokines IL-6 and TNF-α. Anthocyanins can also increase macrophage phagocytic activity and the balance of the Th1/Th2 immune response, thus potentially acting as natural immunomodulators in various inflammatory and infectious conditions (Ijinu et al., 2023; Noman et al., 2025). Anthocyanins have shown immunomodulatory potential against virus-infected peripheral blood mononuclear cells (PBMCs). In vitro, anthocyanins can reduce oxidative stress in PBMCs by

inhibiting ROS formation and increasing the activity of antioxidant enzymes such as SOD and catalase (Meng et al., 2025; Zhang et al., 2024).

Patients with HBV infection in the chronic phase are difficult to cure, currently available antiviral treatments cannot eliminate cccDNA and cannot eliminate the risk of liver cancer due to viral integration (Valaydon et al., 2016). Treatment for HBV infection is costly. Chronic HBV infection requires a long treatment period and high costs (Rajendra & Wong, 2007). It is necessary to find safer and more efficient methods to treat HBV infections. A study to find out whether alternative treatments using natural compounds such as anthocyanins, which are relatively safer, can be a solution for controlling HBV virus infections. The aims of this study are to analyze the effect of anthocyanins on the expression of the cytokine IL-6 in PBMC cell cultures infected with the Hepatitis B virus, with the hypothesis that administration of anthocyanins from purple sweet potato extract increases IL-6 mRNA expression in PBMC cultures of asymptomatic chronic hepatitis B patients compared to controls.

METHODS

This study was an in vitro experimental study with a pre- and post-test control group design. The samples were PBMC cell cultures taken from the blood of an asymptomatic chronic hepatitis B patient. The characteristics of the study sample, in terms of leukocyte differential counts, were determined using a complete hematology examination using an automated hematology analyzer, as shown in Table 1.

Table1. Characteristics of the Research Sample

Blood Sample Characteristics	Result	Number of Cells
Leukocyte cells	6.900 cells/µL	6.900 cells/µL
Lymphocyte cells	40,6 %	2.801,4 cells/µL
Monocyte cells	6,7 %	462,3 cells/µL
PBMC cells	47,3 %	3.263,7 cells/µL
Number of PBMC cells/well	212.140,5 cells/65µL	7.637.058 cells/36 wells

This study used two groups: a control and a treatment group, with 9 PBMC culture wells in each, for a total of 18. Nine wells in the control group were exposed to normal saline, while nine wells in the treatment group were exposed to purple sweet potato extract.

Anthocyanins were obtained from purple sweet potato extract using a maceration technique with water as the solvent. The anthocyanin content in this purple sweet potato extract was 1.05 mg/g, as determined using a spectrophotometer.

To determine the presence of HBV infection in PMBC, viral load tests were performed on 18 samples. HBV viral load was determined from isolated DNA from PMBC cell cultures using the MyGo Mini real-time PCR (IT-IS Life Science, UK) with a specific kit and reagents.

Two measurements were carried out before (pre) and after (post) treatment: IL-6 mRNA expression in PBMC cultures. The treatment involved adding 50 µL of purple sweet potato water extract to PBMC culture wells containing RPMI1640 culture medium for 24 hours. Meanwhile, the control group received 50 µL of normal saline, which was added to the PBMC culture and incubated for 24 hours. The anthocyanin content in the PBMC cell culture was 0.01575 µg/µL.

The effect on the IL-6 cytokine response was measured by measuring IL-6 mRNA expression extracted from RNA isolated from PMBC cell culture samples using a MyGo real-time PCR machine using the quantitative real-time PCR (qRT-PCR) method, using a kit from Kapa Biosystem (USA), and interpolation using a serial dilution standard curve cycle threshold (Ct). The specific primer set used is shown in Table 2.

Table 2. IL-6 mRNA Gene Primers

Target	Sequence 5'-3'
Forward	AATCTGGATTCAATGAGGAGAC
Reverse	GCATTTGTGGTTGGGTCAG

Amplification is performed on a thermal cycler machine with the protocol as in table 3.

Table 3. Amplification Protocol on the thermal cycler machine

Step	Temp	Duration	Cycles
Syntesis cDNA	42°C	5 minutes	hold
Inactivation RT	95°C	5 minutes	hold
Denaturation	95°C	3 seconds	40
Annealing	60°C	20 seconds	

Before the qRT-PCR assay, a standard curve was generated for the targeted genes. β-actin was used as a housekeeping gene (Sadeghabadi et al., 2019).

Amplification was performed to obtain Ct data and then interpolated using the Ct of a serial dilution standard curve. IL-6 mRNA expression data were obtained in numbers with units of fg/µL and then compared for analysis. Data analysis was performed using statistical analysis. This research has been approved for ethical feasibility by the ethics commission of the Faculty of Medicine, Udayana University, No.1821/UN14.2.2.VII.14/LT/2020.

RESULTS

In the control and treatment groups, viral load tests were performed before sample exposure to determine the presence of HBV infection in the PMBC cells. HBV viral load in PMBC cell cultures, in copies/µL, was measured using a real-time PCR machine with qRT-PCR and a standard curve. The viral load results for each sample are shown in Table 4**.**

Table 4. Number of Viral load in The Samples

Group	Sample code	Number of viral load (copies/µl)	Mean (copies/µl)
Control	1	88347,34	86992,72
	2	87925,19	
	3	112401,43	
	4	75982,72	
	5	84221,85	
	6	83673,28	
	7	88600,62	
	8	78085,32	
	9	88347,34	
Treatment	1	57515,01	82453,66
	2	93036,76	
	3	104009,67	
	4	73748,43	
	5	87804,90	
	6	87884,21	
	7	92604,93	
	8	68453,89	
	9	77025,12	

The results of IL-6 mRNA expression in PMBC cell culture using the qRT-PCR method using a standard curve are shown in Figure 1.

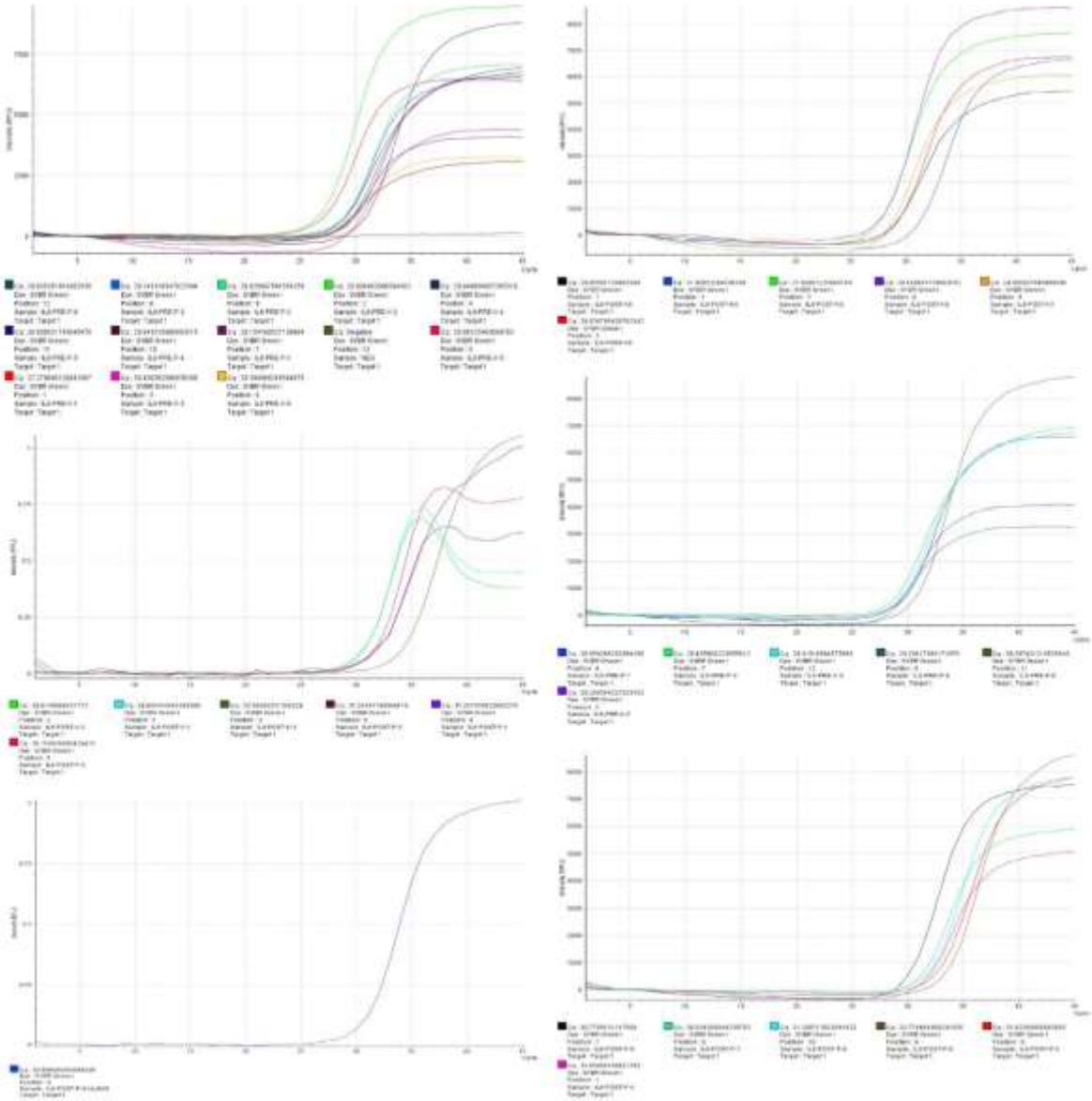

Figure 1. qRT-PCR Amplification Result Curve of IL-6 mRNA Expression

The results of the Ct and interpolarization data using the Ct serial dilution standard curve in the control and treatment groups before and after treatment are as follows:

Table 5. Results of Ct Data for the Control and Treatment Groups

	Group	N	Mean	Standard Deviation	Standard Error Mean
IL-6 fg/µl Pre	Treatment	9	116.8270	40.7220	13.57340
	Control	9	164.7231	147.51788	49.17263
IL-6 fg/µl Post	Treatment	9	40.9477	20.17612	6.72537
	Control	9	115.4551	71.79645	23.93215
Difference	Treatment	9	-75.8793	45.77232	15.25744
	Control	9	-49.2680	173.70273	57.90091

The normality and homogeneity tests for IL-6 mRNA expression data using the Shapiro-Wilk and Levene tests showed that the data in the control and treatment groups were normally

distributed (p>0.05) and homogeneous (p>0.05).

To determine the difference in the effect of treatment on IL-6 mRNA expression after observation between samples given purple sweet potato water extract treatment (Treatment) and samples that did not receive treatment (Control), statistical analysis was carried out using a paired t-test because the IL-6 mRNA expression data in the treatment group and the control group were normally distributed. The results are shown in Table 6.

Table 6. Paired t-test of Exposure Effects on Treatment and Control Groups

Group	Mean	Standard Deviation	Standard Error Mean	CI 95% (Min – Maks)		*p*
Treatment	-75.87	45.77	15.25	-111.06	-40.69	0.001
Control	-49.26	173.70	57.90	-182.78	84.25	0.420

The results showed that there was an effect of exposure to purple sweet potato extract on IL-6 mRNA expression in the treatment group, with a p value of 0.001 (p <0.05), while there was no significant effect of exposure to purple sweet potato extract in the control group, with a p value of 0.420 (p>0.05).

To determine the difference in the effect of treatment on IL-6 mRNA expression after observation between samples given purple sweet potato water extract treatment (Treatment) and samples that did not receive treatment (Control), statistical analysis was carried out using the independent t-test because the IL-6 mRNA expression data in the treatment group and the control group were normally distributed. The results of the statistical analysis test of the difference in IL-6 mRNA expression in the two groups are presented in Table 7.

Table 7. Differences in IL-6 mRNA Expression Between the Two Groups After Observation

IL-6 mRNA expression (fg/µL)					
Group	n	Mean ± SD	Mean Difference	CI 95% (Min - Max)	p
Treatment	9	-75.87 ± 45.77	-26.61	-161.82 – 108.60	0.66
Control	9	-49.26 ± 173.70			

Table 7 shows the results of the independent t-test analysis of the differences in IL-6 mRNA expression in the Treatment Group and the Control Group. In the Treatment Group, the mean difference in IL-6 mRNA expression was -75.87±45.77 fg/µL, while in the control group -49.26±173.70 fg/µL. In the Treatment Group and the Control Group, the average difference in IL-6 mRNA expression was -26.61 fg/µL. These results indicate that there was no significant difference in IL-6 mRNA expression between the treatment group and the control group, where in the treatment group, the IL-6 mRNA expression was not higher than that of the control group, with a p value = 0.66 (p>0.05).

This study showed that there was no significant difference in IL-6 mRNA expression between the treatment and control groups, with the treatment group not producing higher IL-6 mRNA than the control group. Indicate that the anthocyanin compound content in purple sweet potato water extract was not able to increase the expression of IL-6 mRNA.

DISSCUSSION

This study showed different results on the effect of exposure to purple sweet potato extract on IL-6 mRNA expression between the treatment and control groups, and showed that exposure to purple sweet potato extract was not able to increase IL-6 mRNA expression in PBMC cells.

Several studies have shown an insignificant effect of anthocyanins in increasing IL-6 expression. As shown in a study by Zhang et al. (2016) which showed that anthocyanin-rich phenolic extract not only showed strong antioxidant activity, but also significantly reduced the IL-6 cytokine in H2O2-induced Caco-2 cells. Anthocyanin supplementation can improve cellular antioxidant status, resulting in downregulation of the pro-inflammatory mediator IL-6 (Zhang et

al., 2016).

Anthocyanins from various berries, including blackcurrants, blackberries, and blueberries, can suppress the production of proinflammatory cytokines, especially IL-6, through inhibition of the NF-κB pathway in various immune cell and cancer models. Anthocyanin fractions from blackcurrants and other berries reduce IL-6 in HT29 cells and immune cells by modulating COX-2, iNOS, IL-6, and IL-10 (Maaz et al., 2025).

A study by Hartati et al. (2017) showed that black rice ethanol extract containing anthocyanins at a concentration of 100 μg/mL and black rice water extract at concentrations of 50 and 200 μg/mL reduced the relative number of IL-6 and IFN-γ-producing macrophages. Black rice water extract at concentrations of 50 μg/mL and 200 μg/mL showed anti-inflammatory activity, reducing the production of IL-6 and IFN-γ cytokines in macrophages (Hartati et al., 2017).

Research by Xu et al. (2016) showed that the concentration of anthocyanins in garden blueberry extract significantly affected IL-6 gene expression. By increasing the concentration of anthocyanins in garden blueberry extract, both levels of gene expression were completely inhibited at a concentration of 1200 μg/mL, indicating that anthocyanins in garden blueberry extract had a selective inhibitory effect on IL-6 production (Xu et al., 2016).

Natural herbal ingredients can have an immunomodulatory effect. This effect occurs by influencing several cytokines in the body. Each affected cytokine can produce a unique response in the body's immune system, depending on the synergistic effects of their composition. Many compounds in natural ingredients exhibit biphasic effects in the body; that is, a compound has different effects on the user's body depending on its blood concentration, meaning that both low and high doses can produce opposite effects (Spelman et al., 2006).

In line with the study by Xu et al. (2016) to determine the effect of anthocyanin extract of garden blueberry on the secretion of pro-inflammatory cytokine IL-6, RAW 264.7 cells were pretreated with various concentrations of anthocyanin extract of garden blueberry (400, 800, and 1200 μg/mL) and stimulated with 1 μg/mL LPS for 24 hours. IL-6 levels were significantly increased in cells stimulated with LPS compared to cells that were not stimulated. However, IL-6 levels were significantly reduced in cells treated with the anthocyanin extract of garden blueberry in a dose-dependent manner. These results indicate that anthocyanin extract of garden blueberry effectively inhibits IL-6 production in a dose-dependent manner (Xu et al., 2016).

Anthocyanins contained in natural plant extracts affect IL-6 expression. According to research results by Zhang et al. (2016), exposure of Caco2 cells to anthocyanin-rich purple carrot and sweet potato extracts showed a 50% decrease in the expression of the cytokines IL-8, IL-1β, and TNF-α, except IL-6, compared to the control. At a concentration of 50 mg/mL, purple carrot and sweet potato extracts showed no significant effect on reducing IL-6 (Zhang et al., 2016).

The effects of anthocyanins on PBMC cells are highly dose-dependent. At low to moderate doses, anthocyanins are generally protective by reducing oxidative stress, stabilizing cell membranes, and modulating signaling pathways such as NF-κB and MAPK, thus controlling the inflammatory response in PBMCs and maintaining cellular immune function. However, at high doses or inappropriately prolonged exposure, anthocyanins can exhibit paradoxical effects, acting as pro-oxidants, increasing intracellular ROS production, disrupting redox homeostasis, and triggering mitochondrial stress. These conditions can lead to decreased PBMC viability, activation of the apoptotic pathway, or dysfunctional cytokine responses. Anthocyanins at high doses or prolonged exposure can disrupt PBMC cellular homeostasis (Queiroz et al., 2017).

Many phytochemicals exhibit a biphasic dose-response, meaning they induce opposite biological effects at different doses. The most common finding is a stimulatory effect at low doses and an inhibitory effect at high doses. Different doses or concentrations can produce different effects, some positive or negative, while others may be unresponsive or clinically insignificant.

Doses tested in vitro, such as in cell culture, may not be relevant for comparison in different organs or the organism as a whole. A low or high dose cannot be defined precisely, as a high dose used in in vitro experiments may be a low dose when applied in vivo to the organism as a whole. Although the stimulatory effect caused by administering a low dose does not always have a beneficial effect. This depends on the application context and the desired effect. Researchers

conclude that the biphasic dose response is influenced by many factors. It is suspected that this biphasic dose response is related to molecular aspects involving the induction of gene expression or activation by phytochemical compounds of various signaling pathways (Jodynis-Liebert & Kujawska, 2020).

This study used crude purple sweet potato extract in vitro exposure to PBMC cell cultures. This is a limitation of this study. Doses tested in vitro, such as in cell cultures, may not be relevant for comparison across organs or the whole organism. A low or high dose cannot be clearly defined. A high dose used in an in vitro experiment may be a low dose when applied in vivo to an organ or the whole organism (Romeo et al., 2022).

CONCLUSIONS

This study shows that anthocyanins in purple sweet potato water extract do not significantly increase IL-6 mRNA expression in HBV-infected PBMC cell cultures compared to controls. This study can be further developed to investigate the effect of anthocyanins at effective doses on IL-6 in HBV infection and can be developed as an immunomodulator in HBV infection therapy in the future.

Author's Contribution Statement: Made Agus Hendrayana: Conceptualization, Validation, Resources, Data Curation, Writing - Original Draft, Visualization, Project administration. **I Gusti Kamasan Nyoman Arijana:** Methodology, Software, Resources, Formal analysis, Writing - Review & Editing.

Conflict of Interest: The authors declare no conflict of interest.

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