ORIGINAL ARTICLE

Tualang Honey Potentially Delay Deterioration in Haematological and Immunological Parameters in Asymptomatic, Treatment-naïve HIV-infected Patients

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ABSTRACT

Introduction: This report aimed to assess the effects of administration of Tualang honey for six months duration on the haematological and immunological parameters in treatment-naïve HIV-infected patients who were asymptomatic. **Methods:** This was a randomised, controlled, open-labelled study. A total of 95 asymptomatic HIV-positive subjects with low CD4 counts of 250-600 cells/mm^3 and not on antiretroviral therapy were recruited. Tualang honey was administered at 20 g each, once daily (HLD; total of 20 g honey), twice daily (HID; total of 40 g honey) or thrice daily (HHD; total of 60 g honey) for six months period. Control (CT) group did not receive any honey supplementation. Haematological and immunological parameters were measured at baseline, three-month and six-month follow-up. The differences within the group (time effect) and between the groups (regardless of time) for all four groups were analysed using Repeated Measures ANOVA followed by a post-hoc test. **Results:** A significant reduction in total white blood cell, neutrophil and lymphocyte counts were observed at six-month follow-up in CT and HLD groups when compared to baseline. The immunological parameters showed similar trend of reduction in the CT and HLD groups. Meanwhile, the measured parameters were relatively maintained in HID and HHD groups at six-month period when compared to baseline. **Conclusion:** Tualang honey supplementation at intermediate and high doses for six months delay the deterioration of haematological and immunological parameters in asymptomatic, treatment-naïve HIV subjects.

Keywords: Tualang honey, HIV, Asymptomatic, Haematological parameters, Immunological parameters

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INTRODUCTION

Haematological and immunological abnormalities were common in human immunodeficiency virus (HIV)-infected patients. Haematological changes that are commonly seen in HIV patients include anaemia, leucopenia, lymphopenia and thrombocytopenia which are partly contributed by defective haematopoietic functions in the bone marrow. These abnormalities can be due to various reasons (1,2) which include direct cytotoxic effect of HIV itself, opportunistic infections, malignancies as well as adverse effects from drug treatments.

Most haematological abnormalities can be observed in the later stages of HIV infection but several abnormalities including reduced platelets and haemoglobin levels have been demonstrated at early stages of the disease. For example, anaemia is the most common blood abnormality seen in HIV patients and is frequently due to malnutrition and malabsorption. It is linked to higher morbidity and mortality, decreased quality of life and hastened progression of HIV disease (3,4). As disease progresses, degree of cytopenia was more related to the level of immunosuppression (5,6).

In terms of immunological response, persistent activation of systemic immunity is considered as driving factor for progressive loss of CD4 T-cell, the important characteristic of HIV infection. The indicators of immune activation include greater T cells turnover and increased expression of T cells activation. In addition

to that, increased activation-induced apoptosis in the uninfected T cells has been observed. Furthermore, there are also increased levels of pro-inflammatory cytokines and chemokines, B cells, natural killer (NK) cells and macrophage proliferation rates (7). These dysregulations ultimately lead to immune dysfunction in HIV-infected patients.

People living with HIV (PLHIV) have been demonstrated to have higher level of inflammation in their bodies even with a normal CD4 count and reduced viral load (VL) (8). Despite having low VL and high CD4 count, PLHIV are prone to develop inflammation-related diseases such as neurodegenerative diseases and cardiovascular disorders (9). In these conditions, it is believed that residual levels of HIV presence in the body is capable to trigger immune activation and inflammation (10), leading to the above-mentioned afflictions.

In Malaysia, there was an estimated 87041 PLHIV at the end of year 2018. With regards to testing and treatment aiming to end AIDS, 86% of the PLHIV knew their status and among this, only 55% received antiretroviral treatment (ART), indicating a gap in the treatment and care (11). PLHIV can eventually develop acquired immune deficiency syndrome (AIDS) without appropriate treatment. Some factors which hinder successful treatment plan include social stigma, discrimination, willingness to receive therapy as well as poor medication adherence and/or follow-up clinic visits.

As chronic inflammation and immune disturbances contribute to the progression to AIDS, administration of Tualang honey might be beneficial in improving the outcome of the PLHIV. Tualang honey, rich in flavonoids and phenolic contents (12), was previously shown to inhibit the release of various inflammatory mediators including cyclooxygenase-2, cytokines, nitric oxide and prostaglandin E_2 (13,14). The anti-inflammatory effects of Tualang honey may reduce the immune activation and inflammatory effects induced by HIV. Moreover, Tualang honey administration was previously reported to improve CD4 count in asymptomatic HIV patients not receiving ART (15). However, its effects on other immunological and haematological parameters in asymptomatic HIV patients are not known. This report aimed to assess the effects of administration of Tualang honey for six months duration on the haematological and immunological parameters.

MATERIALS AND METHODS

Study design

This study was approved by the Malaysian Prison authority and the Human Research Ethics Committee, Universiti Sains Malaysia (Ethical Approval Number: USMKK/PPP/JEPeM [198.3(1)]) in accordance to the guidelines outlined by Helsinki Declaration. Tualang honey was supplied by the Federal Agriculture and Marketing Authority (FAMA), Malaysia. The honey was individually packed in a sachet of 20 g and subjected to gamma-irradiation (20 Gy) for sterilization.

Asymptomatic, HIV-infected patients with CD4 level ranged between 250-600 cells/mm^3 and do not received antiretroviral treatment (ART) were recruited from Pengkalan Chepa prison, Kelantan. Subjects with other chronic diseases such as diabetes mellitus, chronic liver or renal impairment, AIDS and infections such as tuberculosis were excluded from the study. The recruited subjects were randomised into four groups. The control group (CT) was not given any honey. The treatment groups were given honey once daily (HLD; total of 20 g honey), twice daily (HID; total of 40 g honey) or thrice daily (HHD; total of 60 g honey) for six months. The honey was taken an hour prior to meal and supervised by prison officer. All subjects underwent similar daily activity and diet arranged by the prison authority. The blood (3 mL) was collected during baseline visit and during follow-up visits at three-month and six-month later for evaluation of haematological and immunological parameters. The haematological parameters (haemoglobin, total white blood count, neutrophil, lymphocytes, erythrocyte sedimentation rate, platelet count) were measured using an automated haematology analyser (Model: KX-21, Sysmex Corporation, Kobe, Japan). The immunological parameters (CD45, CD3, CD4:CD8 ratio, CD19, NK cells) were measured using BD Multitest™ IMK Kit (CA, USA). The tests were performed in an ISO 15189-accredited laboratory to ensure the reported results were valid and reliable.

Statistical analysis

A Statistical Software Package (SPSS) (v22.0; IBM Corporation, USA) was used for statistical analysis. The descriptive data were presented as mean [standard error of mean (SEM)]. Repeated measures ANOVA within-group analysis was applied to investigate the differences within each group (time effect) followed by Bonferroni correction. The result for the pairwise comparison was expressed in mean difference (95% confidence interval) [MD (95% CI)] and was considered statistically significant if the two-sided p-value is less than 0.05. Repeated measures ANOVA was also utilised to investigate the differences between all the four groups followed by post hoc multiple comparisons. Level of significance was taken at $p < 0.05$.

RESULTS

A total of 95 subjects (mean age of 34.73 ± 7.07 years) were recruited in this study. Majority were male subjects (85.3%) and Malay (93.7%). Other races include Chinese (4.2%) and Indian (2.1%). The descriptive statistics of the haematological parameters were shown in Table I. The values for all parameters were normal except for higher erythrocyte sedimentation rate (ESR) (> 15 mm/

TABLE I : Descriptive statistics of haematological parameters for control and honey-treated groups.

Parameters		Groups			
		CT (n=23)	HLD (n=26)	HID (n=24)	HHD (n=22)
Haemoglobin (g/dL)	Baseline	14.28 (0.29)	13.99 (0.35)	13.71 (0.49)	13.38 (0.32)
	3 months	14.07 (0.32)	14.44 (0.25)	13.55 (0.47)	13.48 (0.31)
	6 months	13.62 (0.36)	14.58 (0.32)	13.78 (0.50)	13.45 (0.28)
TWBC ($x10^9$/L)	Baseline	7.88 (0.33)	6.75 (0.38)	6.15 (0.24)	7.05 (0.45)
	3 months	6.56 (0.24)	6.13 (0.36)	5.62 (0.15)	7.31 (0.57)
	6 months	5.79 (0.28)	5.32 (0.29)	6.10 (0.31)	6.54 (0.29)
Neutrophil ($x10^9$/L)	Baseline	4.76 (0.30)	3.75 (0.23)	3.73 (0.17)	4.22 (0.35)
	3 months	3.66 (0.19)	3.35 (0.23)	3.09 (0.17)	3.75 (0.32)
	6 months	3.32 (0.26)	2.96 (0.19)	3.62 (0.31)	3.70 (0.21)
Lymphocytes ($x10^9$/L)	Baseline	2.73 (0.18)	2.61 (0.20)	2.10 (0.10)	2.46 (0.21)
	3 months	2.59 (0.15)	2.42 (0.18)	2.12 (0.09)	2.64 (0.27)
	6 months	2.14 (0.13)	2.01 (0.15)	2.06 (0.08)	2.40 (0.19)
ESR (mm/hr)	Baseline	20.05 (3.96)	24.32 (4.93)	23.54 (4.40)	19.73 (4.25)
	3 months	17.05 (2.71)	24.72 (3.73)	21.96 (3.66)	25.36 (4.72)
	6 months	24.79 (6.27)	22.63 (4.25)	24.05 (4.54)	23.09 (4.81)
Platelet ($x10^9$/L)	Baseline	218.21 (15.76)	222.23 (17.85)	232.63 (18.15)	247.68 (19.52)
	3 months	222.70 (17.67)	199.12 (15.23)	207.48 (12.09)	218.05 (15.91)
	6 months	225.89 (18.00)	213.25 (17.66)	225.82 (15.25)	223.32 (16.78)

Data are presented as the mean (SEM). (Abbreviations: CT, control; HLD, honey low dose; HID, honey intermediate dose; HHD, honey high dose)

hr) demonstrated in all four groups.

The repeated measures ANOVA analysis for within-group and between-groups analysis were summarised in Table II. For within-group analysis, significant differences in the total white blood count (TWBC), neutrophil, lymphocytes, and platelet count were observed which indicate some changes occurred over time. Subsequent pairwise comparison for TWBC, neutrophil, lymphocytes and platelet count showed significant differences particularly in CT and HLD groups (Table III). In CT group, significant reduction of TWBC and neutrophil were seen at both three-month and six-month follow-up when compared to baseline. Significant reduction of lymphocytes count was also seen at six-month follow-up when compared to both baseline and three-month follow-up. Similarly, in HLD group, there were significant reduction in TWBC, neutrophil and lymphocytes at six-month follow-up when compared to both baseline and three-month follow-up. Meanwhile, a significant

TABLE II: Summary of the repeated measures ANOVA within and between group analyses for haematological parameters.

Parameters	Within-group		Between-group	
	F-stat (df)	p-value	F-stat (df)	p-value
Haemoglobin	0.012 (2)	0.988	1.782 (3)	0.156
TWBC	12.547 (2)	<0.001*	3.83 (3)	0.010#
Neutrophil	10.935 (2)	<0.001*	0.505 (3)	0.680
Lymphocytes	10.969 (2)	<0.001*	0.482 (3)	0.696
ESR	0.883 (2)	0.415	0.092 (3)	0.964
Platelet	4.175 (2)	0.019*	0.273 (3)	0.845

Abbreviations: df, degree of freedom; ESR, erythrocyte sedimentation rate; F-stat, F-statistic; TWBC, total white blood count
*p value obtained using repeated measure ANOVA within-group analysis showed significant differences ($p<0.05$) over time
#p-value obtained using repeated measure ANOVA between group analysis showed significant differences ($p<0.05$). Subsequent post hoc analysis showed no significant difference

reduction of neutrophil and platelet count was observed in HID group at three-month follow-up when compared to baseline. Otherwise, no other significant reduction was noted in the HID and HHD group, indicating that these measured parameters were relatively maintained in HID and HHD group after six-month period when compared to CT and HLD groups. On the other hand, the repeated measures ANOVA between-group analysis showed significant difference for TWBC, indicating that honey supplementation at different doses may cause some changes. However, subsequent post hoc analysis showed no significant difference.

The descriptive statistics for the immunological parameters were summarised in Table IV. In general, all measured parameters showed trend of reduction after six-month period. Repeated measures ANOVA within-group analysis showed significant differences in all measured parameters, indicating changes occurred over time (Table V). Similar to haematological parameters, subsequent pairwise comparison showed that immunological parameters were relatively maintained in HID and HHD group after six-month period when compared to CT and HLD groups (Table VI). However, the CD4:CD8 ratio and B cells (CD19) levels in all the groups showed no significant changes at six-month follow-up.

DISCUSSION

We previously reported that honey supplementation at intermediate and high doses helped to maintain CD4 counts. Considering CD4 counts as one of the major outcome measures in HIV prognosis, we suggest that honey supplementation at suitable doses may be beneficial to HIV subjects. This study further explores the effects of Tualang honey supplementation on general haematological and immunological parameters in HIV patients who were asymptomatic. Our study showed that Tualang honey supplementation may slow down disease progression in terms of relatively well-maintained haematological and immunological parameters at six-month follow-up when compared to baseline visit. In

TABLE III: Pairwise comparison for total white blood count, neutrophil, lymphocyte and platelet count within each group based on time (within-group analysis)

Comparison	CT		HLD		HID		HHD	
	MD (95% CI)	p-value	MD (95% CI)	p-value	MD (95% CI)	p-value	MD (95% CI)	p-value
TWBC								
Baseline-3 months	-1.31 (-2.16, -0.46)	0.002*	-0.62 (-1.55, 0.30)	0.285	-0.53 (-1.11, 0.05)	0.083	0.26 (-1.43, 1.94)	>0.999
3 months-6 months	-0.78 (-1.70, 0.15)	0.120	-0.81 (-1.49, -0.13)	0.016*	0.48 (-0.33, 1.28)	0.427	-0.78 (-2.28, 0.73)	0.580
Baseline-6 months	-2.09 (-3.37, -0.81)	0.001*	-1.44 (-2.37, -0.50)	0.002*	-0.06 (-0.81, 0.70)	>0.999	-0.52 (-1.66, 0.63)	0.758
Neutrophil								
Baseline-3 months	-1.08 (-1.96, -0.21)	0.013*	-0.26 (-0.98, 0.46)	>0.999	-0.68 (-1.15, -0.21)	0.004*	-0.47 (-1.40, 0.47)	0.619
3 months-6 months	-0.32 (-1.27, 0.64)	>0.999	-0.49 (-0.92, -0.06)	0.021*	0.55 (-0.39, 1.50)	0.362	-0.05 (-0.77, 0.67)	0.532
Baseline-6 months	-1.40 (-2.61, -0.19)	0.021*	-0.75 (-1.35, -0.16)	0.011*	-0.13 (-0.94, 0.68)	0.309	-0.52 (-1.48, 0.45)	>0.999
Lymphocyte								
Baseline-3 months	-0.22 (-0.55, 0.11)	0.286	-0.20 (-0.58, 0.17)	0.508	0.02 (-0.26, 0.30)	>0.999	0.18 (-0.28, 0.63)	0.970
3 months-6 months	-0.37 (-0.68, 0.06)	0.018*	-0.29 (-0.54, -0.04)	0.019*	-0.12 (-0.36, 0.11)	0.555	-0.25 (-0.67, 0.18)	0.436
Baseline-6 months	-0.59 (-1.04, -0.13)	0.010*	-0.50 (-0.90, -0.10)	0.012*	-0.11 (-0.35, 0.14)	0.808	-0.07 (-0.52, 0.38)	>0.999
Platelet								
Baseline-3 months	-4.28 (-33.67, 25.11)	>0.999	-14.22 (-34.82, - 6.39)	0.263	-24.52 (-48.64, -0.40)	0.045*	-29.64 (-82.81, 23.53)	0.486
3 months-6 months	-4.28 (-29.00, 20.45)	>0.999	-4.96 (24.05, 14.13)	>0.999	-9.86 (-41.15, 21.44)	>0.999	-5.27 (-35.25, 24.70)	>0.999
Baseline-6 months	0.00 (-27.04, 27.04)	>0.999	-9.26 (-26.61, 8.09)	0.542	-14.67 (-50.67, 21.33)	0.899	-24.36 (-85.22, 36.49)	0.929

Abbreviations: CI, confidence interval; CT, control; HLD, honey low dose; HID, honey intermediate dose; HHD, honey high dose; MD, mean difference

TABLE IV : Descriptive statistics of immunological parameters for control and honey-treated groups

Parameters (cells/mm³)		Groups			
		CT (n=23)	HLD (n=26)	HID (n=24)	HHD (n=22)
CD45 (leukocytes)	Baseline	2854.35 (241.96)	2587.18 (157.31)	2331.73 (99.13)	2789.70 (235.09)
	3 months	2586.68 (159.92)	2403.85 (168.48)	2150.00 (99.09)	2681.82 (268.20)
	6 months	2164.13 (128.76)	2123.93 (160.05)	2078.50 (114.33)	2472.72 (217.24)
CD3 (T cells)	Baseline	2267.24 (233.62)	1877.28 (125.54)	1791.49 (85.08)	1910.16 (167.47)
	3 months	1988.45 (122.36)	1751.69 (128.89)	1640.65 (79.74)	1821.19 (170.62)
	6 months	1639.22 (107.35)	1549.08 (116.93)	1593.96 (99.74)	1694.96 (176.04)
CD4:CD8 ratio	Baseline	0.32 (0.03)	0.33 (0.02)	0.35 (0.03)	0.42 (0.08)
	3 months	0.33 (0.03)	0.30 (0.03)	0.38 (0.03)	0.42 (0.08)
	6 months	0.35 (0.03)	0.33 (0.03)	0.37 (0.04)	0.40 (0.08)
CD19 (B cells)	Baseline	260.80 (19.76)	287.47 (19.90)	273.45 (18.85)	255.97 (23.43)
	3 months	248.02 (21.44)	292.44 (26.22)	364.01 (113.28)	236.72 (17.76)
	6 months	227.37 (22.55)	246.28 (20.56)	228.04 (23.60)	211.60 (17.88)
NK cells	Baseline	274.00 (28.25)	378.71 (64.01)	231.82 (20.69)	583.43 (163.50)
	3 months	310.50 (63.94)	319.33 (54.35)	218.55 (27.90)	584.85 (210.14)
	6 months	252.31 (45.40)	280.59 (45.83)	219.61 (24.17)	515.80 (146.86)

Data are presented as the mean (SEM). (Abbreviations: CT, control; HLD, honey low dose; HID, honey intermediate dose; HHD, honey high dose)

TABLE V: Summary of the repeated measures ANOVA within and between group analyses for immunological parameters

Parameters	Within-group		Between-group	
	F-stat (df)	p-value	F-stat (df)	p-value
CD45	12.062 (2)	<0.001*	1.696 (3)	0.173
CD3	11.048 (2)	<0.001*	1.117 (3)	0.346
CD4:CD8 ratio	0.275 (2)	0.760	0.864 (3)	0.463
CD19	11.389 (2)	<0.001*	0.881 (3)	0.454
NK cells	3.327 (2)	<0.038*	2.753 (3)	0.047#

Abbreviations: df, degree of freedom; F-stat, F-statistic; NK cells, natural killer cells
*Repeated measure ANOVA within-group analysis showed significant differences within the groups.
#Post hoc analysis showed no significant difference

the present study, all studied haematological parameters were within normal reference range with exception to higher ESR found in all groups. Nonetheless, significant reduction of TWBC, neutrophil and lymphocytes over time were observed in CT and HLD groups but relatively maintained in the HID and HHD groups.

Haematological complications significantly contribute to morbidity and mortality of HIV-infected patients. Anaemia, leucopenia and thrombocytopenia are frequently found in all stages particularly during advanced stages of the HIV infection. This HIV-associated cytopenias may be due to direct effect of HIV infection on bone marrow or related to medication-induced bone marrow suppression which leads to defective haematopoiesis (2). A cross-sectional study by Akinbami et al. (5) showed that about 20% of HIV-treatment naïve patients were cytopenic during enrolment and the degree of cytopenia was directly linked to the degree of immunosuppression (low

TABLE VI: Pairwise comparison of CD45, CD3, CD19 and NK cells within each group based on time (within-group analysis)

Comparison	CT		HLD		HID		HHD	
	MD (95% CI)	p-value	MD (95% CI)	p-value	MD (95% CI)	p-value	MD (95% CI)	p-value
CD45								
Baseline-3 months	-267.66 (-764.42, 234.10)	0.542	-183.33 (-461.49, 94.83)	0.310	-181.73 (-384.45, 21.03)	0.090	-107.88 (-509.77, 294.02)	>0.999
3 months-6 months	-422.55 (-741.37, -103.74)	0.007*	-279.92 (-677.29, 117.46)	0.248	-71.50 (-310.21, 167.21)	>0.999	-209.09 (-684.00, 265.82)	0.795
Baseline-6 months	-690.22 (-1239.84, -140.60)	0.011*	-463.25 (-859.70, -66.79)	0.018*	-253.23 (-496.05, -10.41)	0.039*	-316.97 (-893.23, 259.29)	0.502
CD3								
Baseline-3 months	-278.78 (-698.12, 140.56)	0.297	-125.60 (-334.39, 83.19)	0.406	-150.84 (-325.70, 24.02)	0.108	-88.96 (-386.69, 208.76)	>0.999
3 months-6 months	-349.24 (-604.20, -94.27)	0.005*	-202.61 (-537.04, 131.82)	0.398	-46.69 (-247.80, 154.43)	>0.999	-126.23 (-460.14, 207.67)	>0.999
Baseline-6 months	-628.02 (-1097.98, -158.06)	0.007*	-328.21 (-650.20, -6.22)	0.045*	-197.53 (-405.52, 10.47)	0.067	-215.20 (-659.67, 229.28)	0.665
CD19								
Baseline-3 months	-12.78 (-54.28, 28.73)	>0.999	4.97 (-57.99, 67.93)	>0.999	90.56 (-202.19, 383.31)	>0.999	-19.25 (-60.68, 22.17)	0.720
3 months-6 months	-20.66 (-60.89, 19.58)	0.591	-46.17 (-92.29, -0.05)	0.050	-135.97 (-423.25-151.30)	0.702	-25.11 (-61.71,11.48)	0.266
Baseline-6 months	-33.44 (-84.89, 18.02)	0.319	-41.20 (-83.50, 1.10)	0.058	-45.42 (-93.16, 2.33)	0.066	-44.37 (-91.06, 2.33)	0.066
NK cells								
Baseline-3 months	36.50 (-81.25, 154.24)	0.527	-59.38 (-118.47, -0.29)	0.049*	-13.27 (-54.84, 28.30)	0.516	1.42 (-132.21, 135.05)	0.983
3 months-6 months	-58.19 (-110.89, -5.50)	0.032*	-38.74 (-83.93, 6.45)	0.090	1.06 (-47.17, 49.30)	0.964	-69.05 (-222.24, 84.14)	0.359
Baseline-6 months	-21.70 (-100.44, 57.05)	0.574	-98.12 (-174.79, -21.45)	0.014*	-12.21 (-56.17, 31.76)	0.571	-67.63 (-193.47, 58.21)	0.276

Abbreviations: CI, confidence interval; CT, control; HLD, honey low dose; HID, honey intermediate dose; HHD, honey high dose; MD, mean difference

CD4 count). Another study by Parinithia and Kulkarni (16) also suggested that decreasing CD4 counts were associated with a significant increase in the incidence of anaemia and lymphopenia. A comparative study by Enawgaw and colleagues (6) suggested that anaemia was more common in patients not receiving ART while leucopenia and neutropenia were common in patients receiving ART.

In this study, the haemoglobin level for all patients was normal at baseline visit. The subjects investigated in the present report were asymptomatic and did not have signs of immune suppression, which may be the possible reason for the normal values. However, reduction trend was seen in the group that did not consume honey (CT) over the investigated period. In contrast, groups which received honey supplementation showed increased haemoglobin level in general. Similarly, increased haemoglobin level was previously reported in healthy subjects and in animals following honey supplementation (17–19). The improvement in haemoglobin levels in HIV patient indicates a better prognosis (20).

Leucopenia is commonly seen in HIV-infected patients and is associated with HIV disease progression. It may also reflect the toxicity of ART or presence of comorbid conditions (21). One of the earliest immunological abnormalities seen in HIV-infected patients was the reduction in lymphocyte counts, specifically the CD4+ T cells, which also served as an important prognostic indicator for disease progression and risk of getting opportunistic infections. In this study, significant reduction in the neutrophil and lymphocyte counts were seen in the CT and HLD groups but not in the HID and HHD groups after six months, suggesting that adequate consumption of honey may prevent or delay neutropenia and lymphopenia. Tualang honey was previously showed to significantly increase the white blood cells and platelet counts in postmenopausal breast cancer patients (22). Similarly, increased in haemoglobin level, TWBC, neutrophil, lymphocyte and platelet count were reported in an HIV patient consuming 80 g honey daily after 30 days (23).

Another possible complication of HIV infection which is thrombocytopenia was not found in this study. The reported prevalence of thrombocytopenia in HIV-infected patients varies from 3.74% to 26.24% (1,21,24). Different study populations, clinical conditions as well as sampling method may cause this variation. Study by Woldeamanuel & Wondimu (25) suggest that the risk of developing thrombocytopenia is higher in patients with low CD4 counts (<200 cells/μL) and older age (>50 years). In contrast, our patients are relatively young in their 30's with CD4 counts more than 250 cells/μL, hence may be the reasons for the absence of thrombocytopenia in this study.

With regards to ESR test, it is generally regarded as a

less useful parameter to predict HIV disease progression particularly in those countries with ease access to monitor CD4 level. Hence, the ESR result is rarely reported in recent large prospective cohort studies except from African countries. However, it may still serve as an important marker in resource limited centres (26). In the current study, all groups demonstrated elevated ESR values at baseline visit and six-months later regardless of honey supplementation. The raised ESR is consistent with chronic HIV infection, which involves increased turnover in whole body protein. Nonetheless, the ESR was only mildly increased in this study when compared to other study population which reported a much significantly higher mean ESR (> 80 mm/hr) in both symptomatic and asymptomatic HIV patients (27,28). Extreme increase in the ESR was shown to be associated with other underlying diseases, such as infection, malignancy and collagen vascular disease (29). Other common causes of increased ESR include advanced age, pregnancy, obesity, thyroid disease and kidney impairment, but these diseases were not present in the current report.

Meanwhile, analysis of lymphocyte subpopulations plays a vital role in the management of PLHIV. For instance, the CD4 count, together with viral load measurement, are traditionally used as a guide to monitor therapeutic responses in HIV patients. In addition, assessment of the CD4 to CD8 ratio, which reflects overall immune status more accurately, may be served as a better marker to monitor disease progression (30). In a healthy individual with strong immune system, the CD4:CD8 ratio value is normally greater than one. HIV infection, if left untreated, is associated with inversed or low CD4:CD8 ratio (<1) which is contributed by progressive loss of CD4+ T cells and persistent expansion of CD8+ T cells. In this study, six-month Tualang honey supplementation did not improve the CD4:CD8 ratio in which all groups showed low CD4:CD8 ratio. Study by Hughes et al. (31) suggests that the restoration of CD4:CD8 ratio takes long period up to 15 years after initiating ART. Moreover, although successful ART may restore the CD4:CD8 ratio, normalization (>1) seldom occurs (32).

HIV infection also causes damage to B cells and lead to dysfunction in humoral immunity, another key component of adaptive immunity. For instance, hypergammaglobulinaemia, B cells malignancies, autoimmune disorder and memory B cells reduction are among the defects of B cell reported in HIV-infected patients (33,34). In the present study, the number of CD19+ B cells was reduced after six-month periods in all groups. However, the reduction was insignificant and the CD19 counts were still within normal reference range (35). Whether honey supplementation confers protection on B-cell remains unclear in this study. Since various phenotypic alterations of B cells was previously reported in HIV patients, further detail analysis on B cells subpopulations such as immature B cells, memory B cells, exhausted B cells, plasmablasts and activated mature B cells may be useful to determine the effect of honey supplementation on various B cell subtypes.

In addition to adaptive immunity, innate immunity also plays an essential role in HIV infection. During the early stage of HIV disease, NK cells efficiently recognize HIV-infected cell and eradicate abnormal cells through its potent cytotoxic function. Activation of NK cells also stimulate production of chemokines and cytokines (particularly interferon-γ) and modulate T cells responses, thereby inhibit HIV replication and limit viral spread. However, similar to other lymphocyte populations, persistent activation in chronic HIV infection can cause exhaustion and dysfunction of NK cells (36,37). In this study, significant reduction in NK cells were observed in CT and HLD groups but not in the HID and HHD groups, suggesting adequate honey supplementation may slow down the disease progression in asymptomatic HIV patients.

Overall, deterioration in haematological and immunological parameters were seen particularly in CT and HLD groups. Supplementation of honey may be a good source of multi-nutrients which are important for haematopoiesis in PLHIV. Moreover, chronic HIV infection is associated with immune exhaustion which involve both adaptive and innate immunity. Tualang honey supplementation plausibly delayed the exhaustion in different cell populations, hence maintaining the overall immune function and delaying the disease progression. This may be useful for HIV infected patients while waiting for ART especially in those countries with low ART coverage. Considering the complex nature of honey composition, it is not surprising there were reports on both immunostimulatory and immunosuppressive effects of honey (38-40). Therefore, future investigation on T cells, B cells and NK cells' subpopulations are recommended to better understand the immunomodulatory effects of honey on these subpopulations.

This study has few limitations. Firstly, it was conducted in an incarcerated setting and the result may not reflect the general populations. Secondly, the study duration may not be sufficiently long to reflect the haematological and immunological changes at later stage of the disease.

CONCLUSION

In the present study, six-month Tualang honey supplementation at intermediate and high doses delay the deterioration of haematological and immunological parameters in asymptomatic, treatment-naïve HIV-infected subjects. These findings suggest that Tualang honey has the potential to delay HIV disease progression. A prolonged experimental period is recommended to confirm the beneficial effects of honey particularly at the later stages of the disease.

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