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Regenerative Innovation from Fortified Mangosteen Extract

Pichaet Wiriyachitra[1]*, Preeya Leelahagul[1], Ramida Watanapokasin[2], Sirithip Wiriyachitra[1], Jannatthabhorn Janprasert[1] and Sakda Sreesangkom[1]

[1]*Asian Phytoceuticals Public Company Limited, Thailand.*

[2]*Department of Biochemistry, Faculty of Medicine, Srinakharinwirot University, Bangkok, Thailand.*

***Correspondence:**
Wiriyachitra P, Asian Phytoceuticals Public Company Limited, Thailand, Tel: +662 646 4882.

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ABSTRACT

This article describes a clinical study on a newly developed formulation, Fortified Mangosteen Extract, involving 19 participants, with the specific aim of investigating alterations in their T cells, cytokines, telomeres and autophagy. The results show that the formulation had no effect on the number of white blood cells related to immunity but caused a decrease in pro-inflammatory cytokines, TNF-α, IL-6, IL-17A, IL-12/IL-23 (p40), and IFN-γ, while increasing the level of IL-10 that regulates other pro-inflammatory cytokines. It also slowed telomere shortening in 6 participants and lengthened telomeres in 13 participants, demonstrating its anti-aging and age-reversal effects, respectively. Most importantly, the formulation induced autophagy-related gene expressions, LAMP1, LC3A, ATG5 and BECN1, during the study. In conclusion, this study clearly demonstrates the health benefits of Fortified Mangosteen Extract for individuals with autoimmune diseases, particularly its potential in the prevention and treatment of Alzheimer's disease.

Keywords

Age reversal, Alzheimer's disease, Autoimmunity, Autophagy, Black sesame, Cancer, CD4, CD8, Dietary supplement, Guava, IFN-γ, IL-6, IL-10, IL-12/IL-23 (p40), IL-17A, Immunity, Killer T cell, Mangosteen, Mylife100A®, MylifeR®, MylifeSC®, Pennywort, Regenerative, Soybeans, T-cell, Telomeres, TNF-α.

Background

Our previous publications demonstrated the efficacy of Mylife®/ Mylife100®, a dietary supplement, in improving the quality of life of cancer patients in Thailand for over a decade as a result of increased immunity from an increase in T cells, particularly killer T cells. This supplement, which is made from mangosteen aril, pennywort leaves, guava fruit, black sesame seeds, and soy protein, has also been shown to significantly promote telomere lengthening in both existing and newly increased T cells, contributing to the high efficacy of the cancer treatment. This formulation has also demonstrated that telomere elongation without cancer risk can be achieved [1-4].

However, the formulation of Mylife®/Mylife100® is not suitable for autoimmune subjects. We have therefore adjusted the extraction processes and the ingredients' proportion in order to achieve a formulation that promotes telomere elongation without causing undesirable effects in autoimmune subjects. This new formulation, **Fortified Mangosteen Extract,** has been registered with the Thai FDA as Mylife100A®/ MylifeR®/ MylifeSC®.

This article describes the clinical study of **Fortified Mangosteen Extract** in 19 participants, with the specific aim of investigating the alteration of their cytokines, T cells, telomeres and autophagy.

Materials and Methods

Preparation of Fortified Mangosteen Extract from Five Edible Plants

The dietary supplement **Fortified Mangosteen Extract** is formulated based on Mylife®/Mylife100®, but with a different concentration of active ingredients derived from five edible plants, namely pennywort leaves, black sesame seeds, soybeans, guava fruit, and mangosteen aril. Mangosteen aril juice powder is prepared by grinding, centrifuging, filtering, and spray-drying the juice. Pennywort leaf powder is obtained by heating and centrifuging an extract from the dried leaves. Guava juice powder is produced by grinding, filtering, and spray-drying the juice, while black sesame and isolated soybean protein powders are processed similarly through grinding, centrifuging, and drying steps. Each

capsule contains specific doses of these powders, and the product is registered with the Thai FDA [4].

Subjects

The study included 19 participants (2 males and 17 females) with an age range of 60 to 82 years. All participants were generally healthy, non-drinkers, non-smokers, and had no chronic illnesses requiring regular medication. Their diet, exercise, and daily routines remained consistent throughout the 8-week study period.

Study Design

The study spanned 8 weeks, with each participant attending three scheduled visits: at week 0 (first visit), week 4 (second visit), and week 8 (third visit). Body composition data and blood samples were collected at each visit. Participants were instructed to maintain their usual lifestyle, including their daily dietary intake and exercise habits, throughout the entire 8-week period.

Blood Sample Collection

Blood samples were collected by venipuncture after a 12-hour fasting period to measure various biomarkers analyzed using an automated blood BS-400 chemistry analyzer for hemoglobin, fasting plasma glucose, blood urea nitrogen (BUN), creatinine, serum glutamic oxaloacetic transaminase (SGOT) and serum glutamate pyruvate transaminase (SGPT). Serum was obtained from clotted blood to assess Interleukin (IL) - 6, IL-10, IL-12/IL-23 (p40), IL-17A, tumor necrosis factor- α (TNF-α), and interferon-γ (IFN-γ). An aliquot of EDTA blood was used for a complete blood count and to assess T-lymphocyte subpopulations. Another EDTA blood sample was used to isolate peripheral blood mononuclear cells (PBMCs) for measuring absolute telomere length and autophagy gene expression.

Measurement of Body Composition

Body weight, height, body mass index (BMI: kg/m^2), bodyfat (% of body weight), fat mass (kg), fat-free mass (FFM: kg), muscle mass (kg), total body water (%), bone mass (kg), and visceral fat were measured using a Tanita BC-420MA body composition analyzer (Tanita Corporation, Tokyo, Japan) [4].

Measurement of Leukocyte Telomere Length

Peripheral blood mononuclear cells (PBMC) were isolated from whole blood by density gradient centrifugation. Genomic DNA was extracted from 2-5 million PBMCs using the DNeasy kit (Qiagen), and its purity and quantity were checked via UV spectrophotometry. PBMC telomere length was measured by quantitative PCR, comparing the telomere repeat copy number to the single-copy gene 36B4, based on the qPCR method of O'Callaghan and Fenech. Each 10-µL qPCR reaction included 20 ng DNA, PowerUp SYBR Green, and telomere primers, with 40 cycles and a dissociation curve for verification. A standard curve ensured reaction linearity ($R^2 > 0.99$) and allowed conversion to absolute telomere length (aTL) in kb per diploid genome. Triplicate runs were used, with a 1% interassay variation [4].

Measurement of Lymphocyte Subpopulations

Total leukocytes and lymphocyte count were obtained from complete blood count analysis. The proportion and absolute number of lymphocyte subsets from whole blood were analyzed by flow cytometry using commercially available procedures (The BD Multitest IMK kit: BD Multitest™).

Briefly, 100µL of whole blood was incubated with 10µL of BD Multitest™ reagent solution in the dark at room temperature for 15 minutes. The erythrocytes were lysed by FACS lysing solution. After 15 minutes of incubation, stained cells were analyzed by BD FACSCalibur™ (Becton Dickinson, San Jose, CA, USA). The percentages and absolute counts of T cells: $CD4^+$ T cells and $CD8^+$ T cells were analyzed using FlowJo software (Tree Star, Ashland, OR, USA) [4].

Measurement of Cytokines

Using the enzyme-linked immunosorbent assay (ELISA) technique, the anti-inflammatory and immunostimulatory properties of the compounds isolated from serum were determined. Serum was separated from whole blood using a gel clot activator tube (MMS Medical and Laboratory Supplies, Philippines) and used to determine the concentrations of IL-6, IL-10, IL-12/IL-23 (p40), IL-17A, TNF-α, and IFN-γ using the ELISA MAX™ Deluxe kit (BioLegend, USA). ELISA was performed as recommended by the manufacturer. Human IL-6, IL-17A and TNF-α standards were diluted to reach the concentrations of 0–500 pg/mL. The absorbance at 450 nm and 540 nm was measured by using the SpectraMax® iD3 multi-mode microplate readers (Molecular Devices, LLC, USA). The absorbance was compared to the standard curve to determine the concentrations of IL-6, IL-17A and TNF-α and reported in pg/mL. [ELISA MAX™ Deluxe Set Human IL-6 (Product cat no. 430504), ELISA MAX™ Deluxe Set Human IL-17A (Product cat no. 433914), ELISA MAX™ Deluxe Set Human TNF-α (Product cat no. 430204), ELISA MAX™ Deluxe Set Human IL-10 (Product cat no. 430604), ELISA MAX™ Deluxe Set Human IFN-γ (Product cat no. 430104), ELISA MAX™ Deluxe Set Human IL-12/IL-23 (p40) (Product cat no. 430704), BioLegend, USA].

Measurement of Autophagy Gene Expression

Real-Time PCR (qPCR)

1. RNA Preparation

Total RNA was extracted from PBMCs using TRI Reagent® according to the manufacturer's protocol (cat. no. TR 118, Molecular Research Centre Inc.). The RNA concentration and purity were assessed using a NanoDrop spectrophotometer (Thermo Fisher Scientific, USA).

2. cDNA Synthesis

One microgram of total RNA was converted into cDNA using the iScript™ Reverse Transcription Supermix for RT-qPCR, following the manufacturer's protocol (cat. no. 1708841, Bio-Rad). The reaction was performed at 46°C for 20 minutes, followed by enzyme inactivation at 95°C for 1 minute.

3. Real-Time PCR analysis

Real-time PCR was performed using the KAPA SYBR® FAST qPCR Master Mix (2X) Kit (KAPA Biosystems, South Africa)

and the QuantStudio 3 Real-Time PCR System (Thermo Fisher Scientific, USA), following the manufacturer's protocol. The primer sequences for the
LAMP1 gene were F: CTCTAATGT CTGCAGCTCAAGG and R: TGTACACAGCGCAGA ACAGG.
BECN1 gene were F: GCTCCCGAGGGATGG and R: AGTAATGGACCTGTGAGTTC.
LC3A gene were F: CATGAGCGAGTTGGTCAAGAT and R: TCGTCTTTCTCCTGCTCGTAG.
ATG5 gene were F: TTTGCATCACCTCTGCTTTC and R: TAGGCCAAAGGTTTCAGCTT [5].

Statistical Analysis

The statistical analyses were performed using GraphPad Prism 6.0, with a significance level of 5% ($p < 0.05$) for all analyses.

Results

The initial body composition of the 19 participants is shown in Table 1. The sample group consisted of 19 participants (2 males and 17 females), aged 60-82 years (average age: 69 ± 7 years). Their average body mass index (BMI) was 23.3 ± 2.8 kg/m². All participants were generally healthy, non-drinkers, non-smokers, and had no chronic conditions requiring regular medication. Their daily living activities (diet, exercise, and routine) remained consistent throughout the 8-week data collection period.

Table 1: Initial Body Composition of 19 Participants.

No	Sex	Age	Height, cm	Weight, kg	BMI, kg/m²
1	F	60	153	67.6	28.9
2	F	63	146	45.8	21.5
3	F	63	150	53.9	24.0
4	F	63	155	45.2	18.8
5	F	64	147	49.4	22.9
6	F	65	155	48.5	20.2
7	F	66	149	47.1	21.2
8	F	67	146	45.3	21.3
9	F	67	156	60.7	24.9
10	F	69	155	57.4	23.9
11	F	73	153	63.9	27.3
12	F	73	151	47.5	20.8
13	F	76	148	60.4	27.6
14	F	76	154	61.0	25.7
15	F	80	145	48.5	23.1
16	F	82	143	47.0	23.0
17	F	82	144	41.4	20.0
18	M	60	170	75.1	26.0
19	M	68	165	59.5	21.9

During weeks 1-8, 3 capsules of **Fortified Mangosteen Extract** were taken before breakfast and 3 before dinner. All participants experienced no side effects. Food intake and energy expenditure remained consistent throughout the study, as assessed by body mass index (BMI), which remained stable during the entire period. It should be noted that at weeks 4 and 8, their percentage of body fat and body fat mass were significantly lower than those at week 0. This study also found that blood glucose levels were reduced at weeks 4 and 8 compared to week 0, but the reduction was significant only at week 4 compared to week 0 (Table 2).

Table 2: BMI, Body fat, Blood Pressure, and Blood Glucose of 19 Participants During the Study.

Parameter	Week	Mean ± SD
BMI, kg/m²	0	23.3 ± 2.8
	4	23.3 ± 2.8
	8	23.3 ± 2.8
Body Fat, % of bw	0	32.4 ± 5.6
	4	31.7 ± 5.8 [a1]
	8	31.4 ± 6.0 [a2]
Body Fat, kg	0	17.6 ± 4.9
	4	17.2 ± 5.0 [a4]
	8	17.1 ± 5.1 [a3]
Systolic BP, mmHg	0	146 ± 22
	4	144 ± 23
	8	143 ± 19
Diastolic BP, mmHg	0	82 ± 10
	4	80 ± 9
	8	80 ± 9
Blood Glucose, mg/mL	0	105 ± 17
	4	97 ± 14 [a1]
	8	102 ± 15

Significant difference from week 0: [a1] $p < 0.0005$, [a2] $p < 0.005$, [a3] $p < 0.02$, [a4] $p < 0.05$

Safety of Fortified Mangosteen Extract

Effects of Fortified Mangosteen Extract on Liver and Renal Functions

Kidney function was assessed by measuring BUN and creatinine levels, while liver function was evaluated using SGOT and SGPT enzyme levels. Throughout the study, the levels of serum BUN, creatinine, SGOT, and SGPT, which indicate liver and kidney function, remained within normal ranges (Table 3). Notably, the serum creatinine level at week 8 was significantly lower than those at weeks 0 and 4.

Table 3: Liver and Renal Function Tests of 19 Participants During the Study.

Parameter	Week	Mean ± SD	Normal Value
BUN, mg/dL	0	14.5 ± 4.4	7.8 – 20.3
	4	13.3 ± 3.5	
	8	15.4 ± 3.6	
Creatinine, mg/dL	0	0.82 ± 0.12	Female: 0.65 – 1.08
	4	0.81 ± 0.12	Male: 0.81 – 1.43
	8	0.78 ± 0.13 [a, b]	
SGOT, U/L	0	24 ± 4	Female: 0 – 31
	4	24 ± 5	Male: 0 – 35
	8	24 ± 5	
SGPT, U/L	0	24 ± 8	Female: 0 – 34
	4	26 ± 10	Male: 0 – 45
	8	24 ± 9	

Significant difference from week 0: [a] $p < 0.02$
Significant difference from week 4: [b] $p < 0.0001$

Effects of Fortified Mangosteen Extract on T Cells

Table 4 shows the absolute WBC, lymphocyte, CD4 and CD8

T-cell counts of 19 participants during the study. There were no changes in those parameters throughout the study.

Table 4: Absolute WBC, Lymphocyte, CD4 and CD8 T-Cell Counts of 19 Participants During the Study.

Parameter	Week	Mean ± SD
White Blood Cells, cells/uL (normal ≥ 4,000 cells/uL)	0	6,025 ± 1,470
	4	6,063 ± 1,722
	8	5,897 ± 1,626
Lymphocyte, cells/uL (normal ≥ 1,500 cells/uL)	0	2,007 ± 650
	4	1,916 ± 592
	8	1,985 ± 555
CD 4, cells/uL (normal ≥ 470 cells/uL)	0	801 ± 294
	4	785 ± 249
	8	807 ± 261
CD 8, cells/uL (normal ≥ 360 cells/uL)	0	497 ± 254
	4	462 ± 224
	8	476 ± 184

Effects of Fortified Mangosteen Extract on Cytokines

The results of the clinical immunology study of **Fortified Mangosteen Extract** are shown in Table 5.

TNF-α levels at week 8 were significantly lower than those at weeks 0 and 4.

IL-6 and IL-17A levels at weeks 4 and 8 were significantly lower than those at week 0. Additionally, levels at week 8 were significantly lower than those at week 4.

IL-12/IL 23 (p40) and IFN-γ levels at weeks 4 and 8 were significantly lower than those at week 0.

It is noteworthy that the levels of IL-10, which regulates other pro-inflammatory cytokines, were significantly higher at week 8 compared to weeks 0 and 4.

Table 5: IL-6, IL-10, IL-12/IL 23 (p40), IL 17A, TNF-α, and IFN-γ of 19 Participants During the Study.

Parameter	Week	Mean ± SD
TNF-α, pg/mL	0	5.02 ± 3.27
	4	4.04 ± 3.39
	8	3.31 ± 2.90 [a2, b3]
IL-6, pg/mL	0	4.84 ± 6.58
	4	2.89 ± 3.15 [a4]
	8	2.60 ± 2.84 [a4, b2]
IL-17A, pg/mL	0	2.89 ± 1.31
	4	1.65 ± 1.26 [a2]
	8	1.46 ± 1.00 [a1, b4]
IL-12/IL-23 (p40), pg/mL	0	158.8 ± 104.9
	4	140.6 ± 96.9
	8	130.9 ± 92.1 [a2, b1]
IFN-γ, pg/mL	0	1.75 ± 2.27
	4	1.45 ± 1.79
	8	1.18 ± 1.65 [a3, b2]
IL-10, pg/mL	0	6.59 ± 1.68
	4	5.34 ± 1.24 [a1]
	8	8.05 ± 1.68 [a1, b1]

Significant difference from week 0: [a1] $p < 0.005$, [a2] $p < 0.01$, [a3] $p < 0.02$, [a4] $p < 0.05$
Significant difference from week 4: [b1] $p < 0.0005$, [b2] $p < 0.005$, [b3] $p < 0.01$, [b4] $p < 0.05$

Effects of Fortified Mangosteen Extract on Leukocyte Telomere Length

An increase in absolute telomere length of 70 base pairs (bp) is equivalent to a one-year reversal in biological age [3]. As shown in Table 6, the mean ± SD of absolute telomere length for all 19 participants was 5,615 ± 820 bp at week 0, increasing to 5,688 ± 790 bp at week 4 and 5,730 ± 819 bp at week 8. The telomere lengths at both week 4 and week 8 increased significantly from week 0.

The mean absolute telomere lengths at weeks 4 and 8 increased by 73 and 115 bp from week 0, respectively, equivalent to age reversals of 1.0 and 1.6 years.

Table 6: Leukocyte Telomere Length of 19 Participants During the Study.

Parameter	Week	Mean ± SD
Total 19 Participants		
Telomere, bp	0	5,615 ± 820
	4	5,688 ± 790 [a6]
	8	5,730 ± 819 [a5]
Telomere Percentile	0	42 ± 15
	4	44 ± 14 [a5]
	8	45 ± 15 [a4]
13 Participants Had Longer Telomeres		
Telomere, bp	0	5,457 ± 820
	4	5,584 ± 836 [a2]
	8	5,664 ± 855 [a1, b1]
Telomere Percentile	0	38 ± 13
	4	41 ± 14 [a3]
	8	42 ± 14 [a1, b2]
6 Participants Had No Change in Telomere Length		
Telomere, bp	0	5,958 ± 778
	4	5,914 ± 692
	8	5,875 ± 788
Telomere Percentile	0	51 ± 14
	4	51 ± 13
	8	52 ± 17

Significant difference from week 0: [a1] $p<0.0001$, [a2] $p < 0.001$, [a3] $p < 0.002$, [a4] $p < 0.005$, [a5] $p < 0.02$, [a6] $p < 0.05$
Significant difference from week 4: [b1] $p<0.02$, [b2] $p < 0.05$

The percentiles of telomere lengths at weeks 0, 4, and 8 were 42 ± 15, 44 ± 14, and 45 ± 15, respectively. The percentile telomere lengths at both weeks 4 and 8 increased significantly from week 0.

At week 4, 13 participants experienced significantly longer telomeres compared to week 0. At week 8, their telomeres were significantly longer compared to both weeks 0 and 4. The increase in their telomere length ranged from 68 to 507 bp, equivalent to age reversals of 1.0 to 7.2 years. However, the other 6 participants experienced no change in their telomere length during the study period.

Effects of Fortified Mangosteen Extract on Autophagy Induction

The results demonstrated that **Fortified Mangosteen Extract** promotes autophagy in participants by upregulating LAMP1, LC3A, ATG5 and BECN1 (Beclin 1) expression at week 4. However, at week 8, the expression levels of LC3A, ATG5, and BECN1were reduced, while LAMP1 showed a slight decrease (Figure 1).

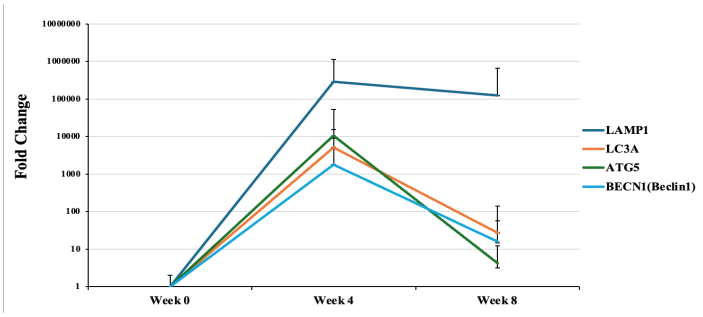

Figure 1: Expression of autophagy-related genes in participants. Gene expression was validated using RT-qPCR, with relative quantification performed using the 2^-ΔΔCt method. The GAPDH gene was used as an internal control for normalization. The data are presented as the means of 19 participants.

Discussion

The results of this study clearly show that there was no change in the number of white blood cells related to immunity. This suggests that **Fortified Mangosteen Extract** is therefore suitable for long-term consumption by individuals with autoimmune symptoms.

Fortified Mangosteen Extract reduced all pro-inflammatory cytokines [6-19], with particularly marked decreases in TNF-α [14,15], IL-6 [6,7], and IL-17A [12]. Interestingly, IL-10 [8,9], which regulates other pro-inflammatory cytokines, was significantly higher at week 8 compared with weeks 0 and 4. From an immunological point of view, this formulation demonstrates a combined effect that is beneficial in reducing autoimmunity, particularly Alzheimer's disease [11,16,18].

Fortified Mangosteen Extract slowed telomere shortening in 6 participants and lengthened telomeres in 13 participants, demonstrating its anti-aging and age-reversal effects, respectively. Long-term consumption of this formulation, which provides both telomere protection and elongation, is therefore beneficial in reducing autoimmunity.

Regarding autophagy, clear evidences of all gene expressions, LAMP1, LC3A, ATG5 and BECN1, were detected until week 4, while LAMP1 remained detected until week 8. These results suggest that **Fortified Mangosteen Extract** can induce autophagy, a process that is beneficial in alleviating autoimmune symptoms, particularly those associated with Alzheimer's disease [19].

The investigation on alterations in cytokines, T cells, telomeres and autophagy in this study clearly indicates the health benefits of **Fortified Mangosteen Extract**, particularly its potential in the prevention and treatment of Alzheimer's disease.

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