



Effect of Thai herbal recipes on the immune system in streptozotocin-induced diabetic retired breeder mice

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ABSTRACT

Introduction: The deterioration of the immune system occurs with increasing age. Additionally, impaired insulin secretion and decreased insulin sensitivity are associated with aging. Type 2 diabetes mellitus is related to immune dysfunction that causes the elderly with diabetes to have a higher risk of infection. Herbal-based treatments are one of the therapeutic strategies for reducing blood glucose and also rejuvenating the immune system to improve the quality of life of the elderly.

Objective: To investigate the immunomodulatory effect of two Thai herbal recipes in streptozotocin (STZ)-induced diabetic aging mouse model. Recipe 1 consisted of *M. cochinchinensis*, *C. ternatea*, and *G. inodorum* (1:1:1). Recipe 2 substituted *C. zeylanicum* for *G. inodorum*.

Methods: Thirty-week-old male mice with diabetes induced by STZ were orally administered with Thai herbal recipes at the doses of 250, 500, and 1,000 mg/kg for 28 days. Blood glucose monitoring was performed on days 7, 14, 21, and 28. After 28 days, mice were sacrificed, spleen and thymus were weighed. The peripheral white blood cell count and differential were measured.

Results: Treatment with both herbal recipes lowered blood sugar levels, however, the body weight loss could not improve in diabetic aging mice. Relative thymus weight seemed to increase in diabetic aging mice treated with a low dose of herbal recipe 1 and a high dose of herbal recipe 2. Additionally, diabetic aging mice treated with a low dose of herbal recipe 1 significantly increased relative spleen weight, whereas treatment with herbal recipe 2 at low and middle doses tended to decrease relative spleen weight. Treatment with both herbal recipes caused the diabetic aging mice to have a lower peripheral monocyte count. Nonetheless, treatment with a high dose of herbal recipe 1 significantly increased peripheral white blood cell (WBC), lymphocyte, and basophil counts.

Conclusion: This study demonstrated the immunomodulatory and hypoglycemic effects of two Thai herbal recipes in diabetic aging mice. Interestingly, Thai herbal recipe 1 tended to be able to boost the immune system.

Keywords: Thai herbal recipes; Immunomodulatory; Aging; Immune system; Mice

Introduction

According to the World Health Organization (WHO), the global population aged over 60 years will

increase from 12% to 22% between 2015 and 2050. Importantly, more than 80% of the elderly are predicted to live in low- and middle-income countries by 2050 [1].

Aging is associated with an increase in molecular and cellular damage, resulting in multiple system dysfunctions of the body including the immune system. Further, Type 2 diabetes mellitus (DM) is becoming one of the most life-threatening problems in the elderly. According to the latest estimates of the International Diabetes Federation (IDF), the diabetic people aged 65-99 years were estimated to be 123 million (around 18% of prevalence rate) in 2017 and are expected to double in 2045 [2]. Furthermore, the global population with DM aged 20-79 years were estimated to be 536.6 million (around 10.5% of prevalence rate) in 2021 [3]. Type 2 DM is linked to immune dysfunction that causes the elderly with diabetes to have a higher risk of infection. The immune system is a critical host defense system against invading pathogens. Aging-related immune changes have been investigated in several studies, and the results indicated that there were both quantitative and qualitative alterations of the immune system during senescence [4-6]. A decrease in immune functions causes the elderly to become more vulnerable to infectious diseases and other age-related diseases such as cancer and neurodegenerative diseases [5]. Therefore, preventing or boosting the immune system is the proper way that can improve the quality of life for the elderly. The previous studies have reported many species of Thai herbs such as *Momordica cochinchinensis*, *Clitoria ternatea*, and *Cinnamomum zeylanicum* that demonstrated immune-enhancing activity in both *in vitro* and *in vivo* studies [7-9]. Additionally, there are studies that have shown the antidiabetic activity of *M. cochinchinensis*, *C. ternatea*, *Gymnema inodorum*, and *C. zeylanicum* [10-13]. Nevertheless, there are no scientific reports about the effect of the recipe using these herbs on the immune system in diabetic animals. Thereby, we had invented an herbal recipe with 3 types of plants consisting of *M. cochinchinensis*, *C. ternatea* and *C. zeylanicum* that have immune-enhancing and antidiabetic activities. Since *G. inodorum* is a plant found mostly in northern Thailand and has antidiabetic properties, thus, we were interested in using it as a substitute for *C. zeylanicum* in the herbal recipe. The aims of this are to investigate the immunomodulatory effect of two Thai herbal recipes (recipe 1 consisted of *M. cochinchinensis*, *C. ternatea*, and *G. inodorum* at the ratio of 1:1:1, and recipe 2 substituted *C. zeylanicum* for *G. inodorum*.) in diabetic models of male retired breeder mice.

Methodology

Preparation of the Thai herbal recipes

All plants were collected from Chiang Mai, Thailand, and identified by Asst.Prof.Dr. Sunee Chansakaow at Department of Pharmaceutical Science, Faculty of Pharmacy, Chiang Mai University. Briefly, dried aril of *M. cochinchinensis* was ground with corn flour and Cab-O-Sil to obtain a homogeneous mixture. Flowers of *C. ternatea* in distilled water were boiled for

5 min. The extract was filtered, added with Cab-O-Sil, and then evaporated to obtain the dry extract. The aerial parts of *G. inodorum* in distilled water were boiled for 1 h. Next, the extract was filtered and repeated boiling two times. Then, Cab-O-Sil was added, and the mixture was evaporated to dry the extract. The dried stem bark of *C. zeylanicum* was ground to be a fine powder. Subsequently, two herbal recipes were prepared by mixing the three plant extracts in a ratio of 1:1:1. The first recipe consisted of *M. cochinchinensis*, *C. ternatea*, and *G. inodorum*, while the second included *C. zeylanicum* in place of *G. inodorum*.

Experimental animals and housing

ICR mice (30-week-old males weighing 40-60 g) were obtained from the National Laboratory Animal Center, Mahidol University, Salaya, Nakhon Pathom, Thailand. The mice were housed in individually ventilated cages, fed with a standard pellet diet and water *ad libitum*. Experimental animals were conducted according to the regulations of the Animal Ethics Committee of the Faculty of Medicine, Chiang Mai University, Thailand (21/2563).

Experimental design

Blood samples were collected from the tail vein by snipping the tail tip to measure the blood glucose levels at baseline by a glucometer. The mice fasted overnight for 20 h before STZ administration, a single i.p. injection of 80 mg/kg in 0.05 M citric acid buffer, pH 4.5, to induce diabetes [14]. Seven days following STZ administration, blood collections from a tail vein were performed before starting the administration of the test substance. The male ICR mice were randomly divided into eight groups (n = 5 per group). Group 1 (control group) was treated with distilled water; Group 2 (reference group) was treated with metformin (400 mg/kg body weight); Groups 3-5 were orally gavaged with herbal recipe 1 (250, 500, and 1,000 mg/kg body weight, respectively); Groups 6-8 were treated with herbal recipe 2 (250, 500, and 1,000 mg/kg body weight, respectively). All mice were orally gavaged once daily for 28 days. Blood glucose levels were measured at 60, 120, and 240 min after the first treatment. After that, blood glucose monitoring was performed on days 7, 14, 21, and 28. Body weight was measured before and after STZ injection and on days 7, 14, 21, and 28. After sacrifice, blood samples were collected for WBC counts and differential, and the spleens and the thymuses were collected and weighed. Relative organ weights were calculated as organ weight (mg)/body weight (g).

Results

Effect of Thai herbal recipes on blood glucose levels in diabetic mice during 28 days of administration

Average blood glucose levels of all groups of mice were in the range of 80 to 110 mg/dL before the STZ injection. Seven days after the STZ injection, the

average blood glucose levels of mice increased to a range of 430 to 450 mg/dL. These results demonstrated that a single injection of 80 mg/kg STZ caused the mice to have higher blood glucose levels and develop diabetes. There was no statistically significant difference in blood glucose levels between groups before and after the STZ injection. Seven days after repeated-dose treatment, the diabetic control mice showed an increasing trend in blood glucose levels, while the blood sugar level of the metformin-treated group tended to decrease but was not statistically

different when compared to the diabetic control group. Moreover, the reduction in blood sugar level after 7 days of administration was also observed in all groups of diabetic mice treated with herbal recipe 1 and the diabetic mice treated with herbal recipe 2, however, there was no significant difference when compared to the diabetic control group. The effect of both herbal recipes in lowering the blood sugar level was not dose-dependent. Nevertheless, it was worthwhile noting a progressive severity of diabetes observed after day 7 and caused some death in mice (table 1).

Table 1 Blood sugar levels after STZ injections and during administration of Thai herbal recipes. Data are represented as mean $\pm$ SEM (n=5 mice per group at the beginning and n=2-4 mice per group after day 7).

Group	After STZ injection (mg/dL)	Treatment			
		7 day (mg/dL)	14 day (mg/dL)	21 day (mg/dL)	28 day (mg/dL)
Control	447.20 $\pm$ 63.85	468.00 $\pm$ 72.58	481.40 $\pm$ 79.30	503.33 $\pm$ 80.27	578.50 $\pm$ 21.50
Met (400)	441.40 $\pm$ 58.34	381.50 $\pm$ 65.99	481.33 $\pm$ 90.11	486.67 $\pm$ 47.35	515.00 $\pm$ 85.00
R1 (250)	444.40 $\pm$ 50.70	383.75 $\pm$ 76.60	408.67 $\pm$ 34.37	373.50 $\pm$ 4.49	425.00 $\pm$ 4.00
R1 (500)	432.40 $\pm$ 54.91	407.80 $\pm$ 62.38	336.60 $\pm$ 61.31	425.00 $\pm$ 58.53	467.50 $\pm$ 46.72
R1 (1,000)	448.40 $\pm$ 31.53	425.00 $\pm$ 55.35	448.33 $\pm$ 45.06	478.00 $\pm$ 47.06	551.33 $\pm$ 20.25
R2 (250)	426.20 $\pm$ 50.10	349.40 $\pm$ 55.02	400.25 $\pm$ 112.93	522.00 $\pm$ 31.77	548.00 $\pm$ 23.00
R2 (500)	432.60 $\pm$ 54.62	434.50 $\pm$ 35.50	387.00 $\pm$ 58.65	543.33 $\pm$ 25.62	442.00 $\pm$ 79.10
R2 (1,000)	430.20 $\pm$ 58.48	381.00 $\pm$ 60.58	373.75 $\pm$ 62.75	356.33 $\pm$ 79.44	478.50 $\pm$ 4.50

Table 2 Blood sugar levels at different time points after single-dose administration of Thai herbal recipes. Data are represented as mean $\pm$ SEM (n=5 mice per group).

Group	Blood glucose level (mg/dL)			
	0 min	60 min	120 min	240 min
Control	447.20 $\pm$ 63.85	488.00 $\pm$ 51.72	496.60 $\pm$ 5.14	485.00 $\pm$ 58.86
Met (400)	441.40 $\pm$ 58.34	424.00 $\pm$ 55.66	384.60 $\pm$ 66.75	327.60 $\pm$ 87.24
R1 (250)	444.40 $\pm$ 50.70	441.80 $\pm$ 52.22	438.00 $\pm$ 27.32	354.60 $\pm$ 57.65
R1 (500)	432.40 $\pm$ 54.91	435.00 $\pm$ 47.07	437.80 $\pm$ 52.12	373.60 $\pm$ 68.40
R1 (1,000)	448.40 $\pm$ 31.53	462.80 $\pm$ 23.20	457.20 $\pm$ 23.12	407.00 $\pm$ 25.38
R2 (250)	426.20 $\pm$ 50.10	476.40 $\pm$ 24.63	422.00 $\pm$ 36.65	376.60 $\pm$ 38.98
R2 (500)	432.60 $\pm$ 54.62	455.60 $\pm$ 29.42	416.40 $\pm$ 27.53	411.40 $\pm$ 11.16
R2 (1,000)	430.20 $\pm$ 58.48	424.60 $\pm$ 87.18	395.80 $\pm$ 93.15	371.00 $\pm$ 82.82

Effect of Thai herbal recipes on blood glucose levels in diabetic mice after single-dose administration

As demonstrated in table 2, the diabetic control group had increased blood sugar levels at 60 and 120 minutes after distilled water administration and slightly decreased at 240 minutes, whereas the metformin-treated group showed a decrease in blood sugar level after 60 to 240 minutes of administration. The blood sugar levels of the diabetic mice treated with herbal recipe 1 and recipe 2 tended to reduce obviously at 240 minutes after administration. Nonetheless, there was no statistically significant difference among groups at any time point.

Effect of Thai herbal recipes on body weight in diabetic mice

The average body weights of mice before the STZ injection were in the range of 49 to 52 g and decreased to 41 to 45 g within 7 days after the STZ injection (table 3). These results indicated that a single injection of 80 mg/kg STZ resulted in the loss of body weight in mice. In the diabetic control mice, the body weight was inclined to reduce throughout the study. The body weight of the metformin-treated mice was steady during metformin administration. The same manner was observed in the groups that received herbal recipe 1 (1,000 mg/kg) and recipe 2 (500 mg/kg). However, there was no statistically significant difference in body weights among groups at any time point.

Table 3 Body weights after STZ injections and during administration of Thai herbal recipes. Data are indicated as mean $\pm$ SEM (n=5 mice per group at the beginning and n=2-4 mice per group after day 7).

Group	Body weight (g)				
	After STZ injection	7 th day	14 th day	21 st day	28 th day
Control	41.60 $\pm$ 1.72	40.00 $\pm$ 1.55	38.80 $\pm$ 1.02	37.00 $\pm$ 0.58	37.33 $\pm$ 2.91
Met (400)	42.40 $\pm$ 2.23	41.00 $\pm$ 1.00	42.67 $\pm$ 2.67	42.67 $\pm$ 1.76	42.00 $\pm$ 2.00
R1 (250)	42.40 $\pm$ 1.17	39.00 $\pm$ 1.00	39.00 $\pm$ 1.29	37.33 $\pm$ 1.76	37.33 $\pm$ 1.76
R1 (500)	44.40 $\pm$ 2.79	39.60 $\pm$ 1.47	41.60 $\pm$ 1.72	40.00 $\pm$ 3.74	41.50 $\pm$ 2.06
R1 (1,000)	43.20 $\pm$ 1.50	40.00 $\pm$ 3.90	40.50 $\pm$ 2.63	41.33 $\pm$ 2.40	42.00 $\pm$ 4.16
R2 (250)	44.40 $\pm$ 2.64	37.20 $\pm$ 2.58	38.00 $\pm$ 1.41	38.50 $\pm$ 1.50	38.00 $\pm$ 2.00
R2 (500)	42.80 $\pm$ 1.36	40.50 $\pm$ 4.11	42.00 $\pm$ 2.00	42.67 $\pm$ 2.91	42.00 $\pm$ 3.06
R2 (1,000)	41.00 $\pm$ 1.91	36.00 $\pm$ 1.63	35.00 $\pm$ 1.73	33.00 $\pm$ 2.65	34.00 $\pm$ 2.31

Effect of Thai herbal recipes on relative thymus weight in diabetic mice

After 28 days of treatment, the relative thymus weights of the diabetic mice treated with herbal recipe 1 at the dose of 250 mg/kg and herbal recipe 2 at the dose of 1,000 mg/kg tended to be increased, whereas the treatment with herbal recipe 1 at the doses of 500 and

1,000 mg/kg was similar to that of the control group. A slight decrease in relative thymus weights was found in diabetic mice treated with metformin and in diabetic mice treated with herbal recipe 2 at the doses of 250 and 500 mg/kg as shown in figure 1. Nevertheless, there was no significant difference in relative thymus weights among groups.

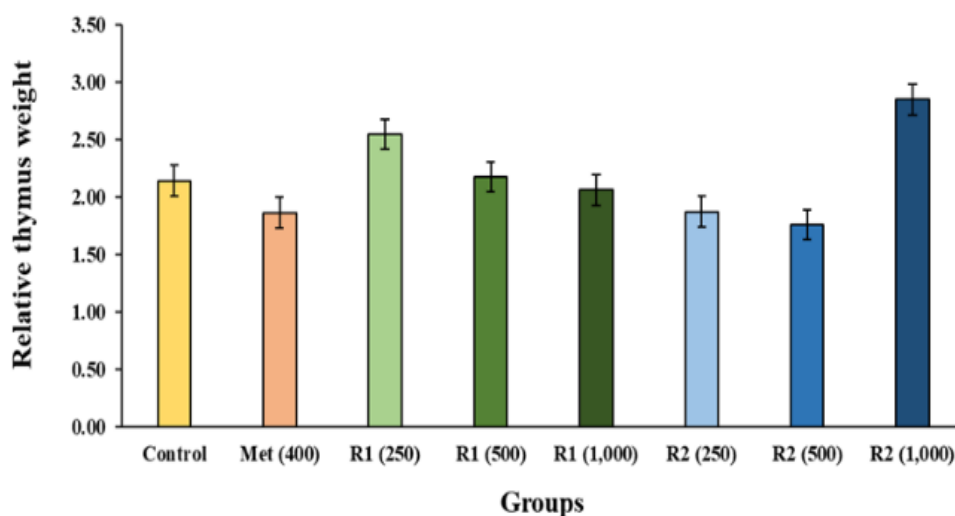


Figure 1 Relative thymus weight of diabetic mice after the administration of Thai herbal recipes for 28 days. Bar graph showed mean ± SEM (n=2-4 mice per group).

Effect of Thai herbal recipes on relative spleen weight in diabetic mice

A significant increase in the relative spleen weight was found in the group of diabetic mice treated with herbal recipe 1 at the dose of 250 mg/kg when compared with the diabetic control mice. Other groups consisted of diabetic mice treated with metformin, herbal recipe 1 at the doses of 500 and 1,000 mg/kg, and herbal

recipe 2 at the dose of 1,000 mg/kg tended to be increased in relative spleen weights but not statistically different when compared to the diabetic control group. The relative spleen weight in groups that received herbal recipe 2 at the doses of 250 and 500 mg/kg was decreased without statistically significant difference when compared to the diabetic control mice as demonstrated in figure 2.

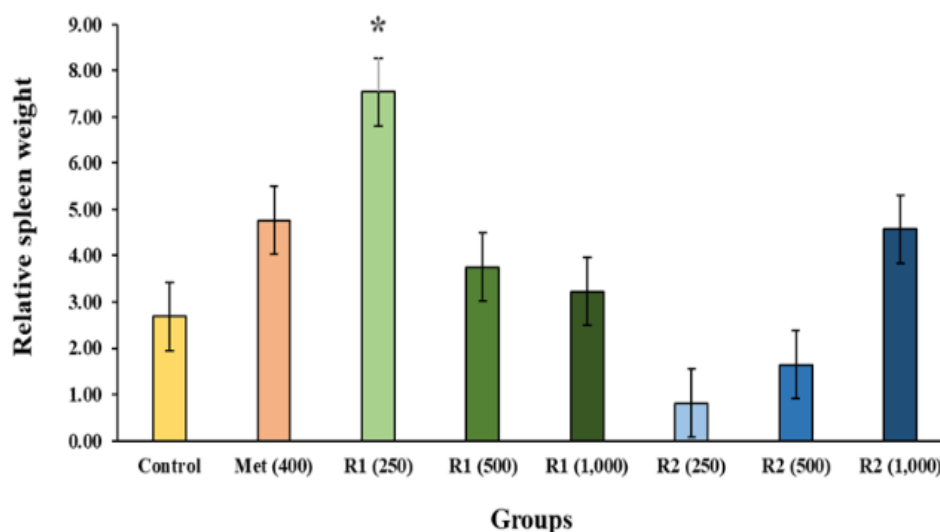


Figure 2 Relative spleen weight of diabetic mice after the administration of Thai herbal recipes for 28 days. Bar graph showed mean ± SEM (n=2-4 mice per group). *p < 0.05 vs. the control group.

Effect of Thai herbal recipes on peripheral WBC and their differentials parameter profiles in diabetic mice

The WBC count and differential in the diabetic mice are demonstrated in table 4. The treatment with herbal recipe 1 at the dose of 1,000 mg/kg significantly

increased in total WBC counts and the total number of lymphocytes and basophils in peripheral blood when compared with the diabetic control group. A significant decrease in the total number of monocytes was observed in diabetic mice treated with metformin, herbal recipe 1

at the doses of 250 and 500 mg/kg and herbal recipe 2 at the dose of 1,000 mg/kg when compared with the diabetic control mice. The total number of neutrophils

and eosinophils in diabetic mice treated with metformin and both herbal recipes were similar to the diabetic control mice.

Table 4 Peripheral WBC and their differentials parameter profiles of diabetic mice after the administration of Thai herbal recipes for 28 days. Data are displayed as mean ± SEM (n=2-4 mice per group). *p < 0.05 vs. the control group.

Group	WBC (x10 ³ /μL)	Neutrophil (x10 ³ /μL)	Lymphocyte (x10 ³ /μL)	Monocyte (x10 ³ /μL)	Eosinophil (x10 ³ /μL)	Basophil (x10 ³ /μL)
Control	2.65±0.55	1.28±0.47	0.92±0.03	0.40±0.11	0.05±0.03	0.00
Met (400)	2.04±0.93	1.64±0.97	1.00±0.39	0.18±0.07*	0.06±0.06	0.00
R1 (250)	3.08±0.45	1.27±0.51	1.62±0.38	0.12±0.05*	0.07±0.02	0.00
R1 (500)	2.47±0.69	1.59±0.47	0.65±0.19	0.18±0.06*	0.07±0.03	0.00
R1 (1,000)	6.61±2.07*	2.39±0.69	3.66±2.90*	0.46±0.08	0.09±0.07	0.02±0.02*
R2 (250)	2.97±0.92	1.48±0.70	1.15±0.12	0.24±0.03	0.10±0.07	0.00
R2 (500)	3.79±0.50	2.36±0.80	1.15±0.24	0.24±0.09	0.05±0.03	0.00
R2 (1,000)	2.07±0.72	0.68±0.13	1.32±0.70	0.05±0.01*	0.02±0.01	0.00

Discussion

Nowadays, the number of elderly people around the world is increasing [1]. Thus, the incidence of various diseases in the elderly has increased as well. Type 2 DM is one of those that has a high rate of disease in the elderly [2]. Both aging and the incidence of Type 2 DM cause progressive degeneration of various systems in the body of the elderly including the immune system. Therefore, it is important to prevent the deterioration of the immune system that occurs in the elderly. It has been confirmed that several herbs comprising *M. cochinchinensis*, *C. ternatea*, and *C. zeylanicum* have immune-enhancing effects [7-9]. Previous phytochemical studies reported the presence of carotenoids, especially lycopene and β-carotene in *M. Cochinchinensis* arils extract which these carotenoids have been confirmed to have immune-enhancing and antidiabetic properties [15-19]. Moreover, both immune-enhancing and antidiabetic activities of anthocyanins, especially ternatins, isolated from *C. Ternatea* flowers have been shown previously [20-22]. Triterpene saponins and pregnane glycosides purified from *G. Inodorum* aerial parts demonstrated antidiabetic activity, however, their immune-enhancing activity has not yet been reported [23,24]. In addition, there are reports about immunomodulatory and antidiabetic activities of proanthocyanidin and cinnamtannin B1 obtained from *C. zeylanicum* stem barks [25-28]. In addition, two Thai herbal recipes with these plants have been invented. The first herbal recipe consisted of *M. cochinchinensis*, *C. ternatea*, and *G. inodorum* while the second herbal recipe contains *C. zeylanicum* instead of

G. inodorum. The present study was undertaken to evaluate the immunomodulatory effect of these two Thai herbal recipes in diabetic models of male retired breeder mice. Antidiabetic and immunological parameters were observed in this study.

STZ, a compound derived from *Streptomyces achromogenes*, is clinically used for pancreatic β cell carcinoma treatment. Because this compound targets and damages pancreatic β cells, thereby causing subsequent hypoinsulinemia and hyperglycemia [29]. A previous study has reported that a single i.p. injection of STZ at the dose of 100 mg/kg in 8-week-old male ICR mice produced slowly progressive non-insulin-dependent diabetes mellitus (NIDDM) which increased non-fasting serum glucose levels over a 12-week study period [30]. Metformin is one of the oral antihyperglycemic agents used for the treatment of Type 2 DM. The general mechanisms of actions of metformin are the suppression of hepatic glucose production, the enhancement of glucose uptake, and the improvement of insulin sensitivity in peripheral tissues [31]. In the present study, male retired breeder mice were induced to develop diabetes by injecting 80 mg/kg STZ (i.p.) and metformin at the dose of 400 mg/kg was used as a reference drug in the diabetic positive control group.

A rise in blood glucose levels over 400 mg/dL was found in male retired breeder mice within 7 days after the STZ injection. Previous research has indicated that mouse strains with fasting blood glucose levels greater than 250 mg/dL showed common signs and symptoms of diabetes such as polyuria and polydipsia [32]. These diabetic symptoms were also observed in the present

study. After a single administration of metformin, the diabetic mice had a decrease in blood glucose level at 60 minutes after administration. It seemed that treatment with both herbal recipes could decrease blood sugar levels at 240 minutes after a single administration.

Moreover, treatment with metformin and herbal recipes showed the hypoglycemic effects in diabetic mice after repeated administration for 7 days. When comparing between the doses of herbal recipes, the low dose of both herbal recipes tended to be the most effective at lowering blood glucose levels. The hypoglycemic effect of these herbs is composed in the recipes confirmed in many studies [10-13]. Nevertheless, mice deaths occurred in all groups and the living diabetic mice had higher blood sugar levels after the 7th day of treatment, possibly as a result of higher mortality rates at older ages associated with the development of diabetes was severe in older mice. Therefore, the hypoglycemic effects of herbal recipes were dose independent.

Next, we examined the effect of two Thai herbal recipes on immunological parameters. The thymus is a primary lymphoid organ of the immune system where the process of T cell development is taking place. The reduction of functions and thymic atrophy occur with age [33]. Our study reported that the relative thymus weight appeared to increase in the diabetic mice treated with a low dose of herbal recipe 1 and a high dose of herbal recipe 2. The spleen is one of the secondary lymphoid organs of the immune system. One of the spleen's functions is involved in the initiation of innate and adaptive immune responses in which antigen-presenting cells (APCs) process antigens and present them to T lymphocytes [34]. Our study showed that the relative spleen weight was significantly increased in the diabetic mice treated with a low dose of herbal recipe 1. Meanwhile, the diabetic mice treated with herbal recipe 2 at low and middle doses seemed to decrease their relative spleen weight. The results obtained from this study demonstrated that the low dose of herbal recipe 1 and the high dose of herbal recipe 2 tended to increase the relative organs weight of thymus and spleen.

WBCs circulate in the circulatory system and function in both innate and adaptive immune responses. All granulocytes (neutrophils, eosinophils, and basophils), monocytes, and macrophages are a part of the innate immune system. Meanwhile, a vital component of the adaptive immune system is lymphocytes [35]. The present study showed that the diabetic mice treated with a high dose of herbal recipe 1 had a significant increase in total WBC, lymphocytes, and basophil counts. In addition, a significant decrease in the monocyte count was observed in the diabetic mice treated with metformin, herbal recipe 1 at low and middle doses, and herbal recipe 2 at high dose. However, a previous study demonstrated that alloxan-induced diabetic rats treated with the extract of *C. ternatea*, the component of both herbal recipes, showed a significant increase in the total number of WBCs and

lymphocytes, whereas a total number of monocytes and eosinophils exhibited a significant decrease [8]. Additionally, treatment with the *C. zeylanicum* extract, the component of herbal recipe 2, significantly increased the total number of WBCs in alloxan-induced diabetic rats [36]. The effect of herbal recipes on peripheral WBC and their differentials parameter profiles seemed to be dose independent.

Conclusion

In summary, our study shows the hypoglycemic and immunomodulatory effects of two Thai herbal recipes in diabetic aging mice. Although treatment with herbal recipes and metformin was found to reduce blood glucose levels, it could not improve body weight loss in diabetic aging mice. Treatment with herbal recipes in diabetic aging mice also caused a decrease in peripheral monocyte count. Diabetic aging mice treated with herbal recipe 1 at 250 mg/kg dose had a significant increase in relative spleen weight, whereas 1,000 mg/kg dose of herbal recipe 1 treatment significantly increased peripheral WBC, lymphocyte, and basophil counts. In addition, diabetic aging mice treated with herbal recipe 2 at 250 and 500 mg/kg seemed to decrease relative spleen weight, whereas 1,000 g/kg dose of herbal recipe 2 treated group tended to increase relative thymus weight. Altogether, herbal recipes 1 seemed to have an immune-boosting effect when administered to diabetic aging mice.

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