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Full Length Research Paper

Treatment of canine diabetes mellitus using *Momordica charantia* capsule and a restricted-fat high-fiber diet

Kamoltip Thungrat[1], Pinit Pusoonthornthum[2], Kamonwan Fish[1] and Sirintorn Yibchok-anun[1]*

[1]Department of Pharmacology, Faculty of Veterinary Science, Chulalongkorn University, Pathumwan, Bangkok 10330, Thailand.
[2]Department of Medicine, Faculty of Veterinary Science, Chulalongkorn University, Pathumwan, Bangkok 10330, Thailand.

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The purpose of this study was to investigate effect of the Momordica charantia (MC) capsules, combined with insulin hormone and a high-fiber diet on the treatment of diabetic dogs. Twenty-five client-owned dogs with naturally occurring diabetes mellitus were entered into the study. All dogs were fed with a commercially available restricted-fat high-fiber diet and received insulin subcutaneously once a day. Twenty dogs in the treatment group received 200 mg/kg Body Weight (BW) of MC capsules orally every 12 h with meals for two months. The other 5 dogs were in the control group and received only insulin therapy. The fasting blood glucose levels, serum fructosamine concentrations, serum chemistry profiles and complete blood counts were obtained monthly. Serial blood glucose curves were performed to adjust insulin doses for an individual's requirement monthly for 2 months. After receiving the MC capsules (200 mg/kg BW/day) for 2 months, the serum fructosamine and fasting blood glucose concentrations were significantly lower than those in the control group and those before treatment. The results obtained from complete blood count and serum chemistry profiles were not significantly different between the control and treatment groups, except for the alkaline phosphatase enzyme (ALP) levels. The ALP levels in the treatment group were significantly higher than those in the control group throughout the study. In conclusion, the use of the MC capsule at 200 mg/kg BW/day combined with restricted-fat high-fiber diet improved glycemic control more efficiently than diabetic treatment with insulin alone.

Key words: *Momordica charantia*, serum fructosamine, diabetes mellitus, dogs.

INTRODUCTION

Diabetes mellitus (DM) is the most common disorder of the endocrine pancreas resulting in metabolic disorders of carbohydrate, fat and protein metabolism characterized by an absolute or relative deficiency of insulin resulting in hyperglycemia. It may cause other organ complications especially eyes, kidneys, heart and blood vessel (Kaneko, 1997). DM is classified into 2 types, type I and type II, by pathophysiology of the disease. Type I DM (insulin-dependent diabetes mellitus, IDDM) is characterized by insufficiency of insulin from the destruction of beta cells with progressive and eventually complete insulin insufficiency (Einsenbarth, 1986). Type II DM (Non-insulin dependent diabetes mellitus, NIDDM) is characterized by insulin resistance and/or impaired insulin secretion. The prevalence of DM was consistently greater over time (1970 -1999) in older compared to younger dogs with the highest prevalence occurring in dogs 10 -15 years of age (Guptill et al., 2003). Diabetes mellitus most commonly occurs in middle age to older dogs and cats, but occasionally occurs in young animals (Nelson, 1992; Feldman and Nelson,

*Corresponding author. E-mail: sirintorn.y@chula.ac.th. Tel: +662 2 2189726. Fax: +662 2 2553910.

1996). In addition, it occurs more commonly in female dogs and in male cats. All dogs virtually have IDDM at the time DM is diagnosed; therefore, insulin therapy is mandatory in diabetic dogs for their entire lives. An animal's insulin need may change over time requiring changes of insulin doses, and types or frequencies of injection (Bennett, 2002). In all diabetic dogs, several factors are used to formulate a treatment regimen for DM including adjustment in diet and exercise, correction of obesity, treatment of concurrent insulin-antagonistic disorders and avoidance of insulin-antagonistic drugs (Feldmen and Nelson, 1996). Since exogenous insulin is not as perfect as endogenous insulin that is produced from the pancreas; some diabetic dogs may develop humoral immune responses to exogenous insulin leading to failure in therapy of DM (Davison et al., 2003). In addition, overdose of insulin injection can cause hypoglycemia which has serious clinical signs including lethargy, weakness, staggering, ataxia, seizures, or just being quieter than usual (Feldman and Nelson, 2003). Alternative medicine or the use of herbal medicines may be a new therapeutic strategy which might be cheaper, safer, and more convenient for treatment of DM.

Herbal medicine, the most popular form of traditional medicine, has been used widely in Asian countries (WHO, 2007). In the United States, the lifetime prevalence of Complementary and Alternative Medications (CAM) has increased steadily since the 1950s (Kessler et al., 2001). Numerous chemical compounds from traditional plants are considered for diabetic control in many countries. *Momordica charantia* Linn. (MC) is commonly known as bitter gourd or karela. The extracts of MC have shown anti-hyperglycemic effect in animal models such as alloxan-induced diabetes (Kar et al., 2003), streptozotocin-induced diabetes (Sarkar et al., 1996) and glucose-loaded rats (Day et al., 1990). In addition, subcutaneous administration of the protein extract from fruit pulp of Thai MC exerted the hypoglycemic activity in both dogs (Tantisuwat et al., 2004), and rats (Yibchok-anun et al., 2006). The MC protein extract stimulated insulin secretion from perfused rat pancreas and increased glucose uptake into C_2C_{12} myocytes and 3T3-L1 adipocytes rats (Yibchok-anun et al., 2006). Welihinda and Karunayake (1986) also demonstrated that the fruit juice of this plant caused an increase in glucose uptake by tissues *in vitro*. Some other studies also reported the hypoglycemic activity of MC to have extra-pancreatic effects (Sarkar et al., 1996) such as increasing GLUT4 transporter protein of muscles (Miura et al., 2003; Kumar et al., 2009; Shih et al., 2009), increasing glucose utilization in the liver and muscle (Sarkar et al., 1996; Karunanayake et al., 1990) inhibiting glucose-6-phosphatase and fructose 1, 6-bisphophatase in liver and stimulation of red-cell and hepatic glucose-6-phosphate dehydrogenase activities (Shibib et al., 1993). Furthermore, MC has been shown to enhance the number of beta cells (Ahmed et al., 1998). Moreover, the extract of MC has α-glucosidase inhibitory activities at brush border of the small intestine (Matsuura et al., 2002). The α-glucosidase in the small intestine plays a physiologically significant role for the digestive process of carbohydrate diet. The inhibitory actions of α-glucosidase, as a result, could retard the glucose absorption to suppress post-prandial hyperglycemia. Some clinical trials reported the hypoglycemia effect of MC in diabetic type 2 patients with a water-soluble extract of the MC fruit (Leatherdale et al., 1981) and a homogenized suspension of the vegetable pulp of MC (Ahmad et al., 1999) which significantly decreased blood glucose concentrations. In the retro-spective study, the use of oral bitter melon for type 2 DM found that fasting plasma glucose was reduced in patients with mildly to moderately controlled diabetes (Fuangchan et al., 2009). Thus, the MC extract exerted both insulin secretagogue and insulinmimetic activities to lower blood glucose concentration. Therefore, the purpose of this study was to determine the effect of the MC capsule, combined with insulin injection on the improvement of glycemic control in naturally occurring diabetes mellitus in dogs.

MATERIALS AND METHODS

Momordica charantia capsules

Commercially available MC capsules[a] were used in this study. Each capsule contains 500 mg of dried grounded fruit of MC.

Patient selection

A randomized computer search of dogs had presented by the Veterinary Hospital of Chulalongkorn University between March 2005 and February 2007. Twenty-five client-owned dogs with previously diagnosed diabetes mellitus type 1 entered the study with client consent and approval by Chulalongkorn University Animal Care and Use Committee. Inclusion criteria included clinical signs suggestive of DM and at least 1 of the following: persistant hyperglycemia with glucosuria or persistant hyperglycemia despite insulin treatment. All dogs received physical examination and blood tests to exclude those with illnesses other than diabetes mellitus. The results of blood tested for baseline serum thyroxin, serum cortisol, serum fructosamine, serum albumin, total protein and fasting blood glucose concentrations, Complete Blood Count (CBC), blood chemistry and urinalysis were included to determine dogs' health. All dogs were non-ketotic diabetes mellitus at the time of entry into the study.

Treatment

All 25 dogs were randomly separated into two groups, control and treatment. Each dog was fed with commercially available restricted-fat high-fiber canine diet[b]. The control group consisted of 5 dogs and received only exogenous insulin therapy. The other 20 dogs were in the treatment group and got both exogenous insulin therapy and MC capsules[a]. All dogs' serum was measured serum insulin

concentration pre- and post-treatment by radioimmunoassay[c].

Control group

Each dog received physical examination and personal details, such as amount of feed, exercise and behavior, were recorded. They received lente insulin[d] therapy as recommended by the attending veterinarian based on information from serial blood glucose curves. In addition to once daily insulin injections, each dog was fed a commercially available restricted-fat high-fiber canine diet[b]. The total daily energy requirement was estimated at 40 - 80 calories/ kg BW/ day and the diet was provided in two meals fed 12 h apart. No other treatments or any food was allowed. Monthly fasting blood glucose, serum fructosamine and serum albumin concentrations, CBC and urinalysis were obtained and serial blood glucose curves were performed to adjust insulin doses for individual requirement once a month for 2 months.

Treatment group

Personal details of each dog were recorded and received the same diet and treatment with insulin as described in the control group. In addition to insulin therapy and diet, all dogs received 200 mg/kg BW of MC capsules orally with meal twice a day for two months. Blood samples were collected for measuring serum fructosamine, fasting blood glucose, and serum albumin concentrations. Serial blood glucose curve was performed to adjust insulin dose for individual requirement at monthly intervals for a period of 2 months.

Sample collection

Blood samples from all dogs were collected from the saphenous vein between 6 to 9 am after 12 h of fasting. All blood samples were collected into tubes containing EDTA for analysis of CBC. Blood samples for the measurement of fasting blood glucose and insulin concentrations were collected into fluoride heparin tubes. Samples tested for serum fructosamine and other chemical profiles were collected into plain tubes. Plasma and serum were separated by low-speed centrifugation at 4°C and stored in polyvinyl tubes at –80°C until the tests were performed.

Serial blood glucose curve

The serial blood glucose curves consisted of measuring blood glucose before and every 2 h after insulin injection for 12 h. Additional samples for determination of blood glucose were obtained at 18 and 24 h. Glucose curves were performed beginning during the morning hours, with the dog fed and given insulin. Blood glucose concentration was determined with glucose reagent strips[e] designed for use with venous blood.

Glycemic parameters

Serum fructosamine and fasting blood glucose concentrations were analyzed for each patient at the time of entry into the study (M0) and at monthly intervals for a period of 2 months. Daily exogenous insulin doses were adjusted once a month based on serum fructosamine concentration, serial blood glucose curve and resolution of clinical signs within the past month (Thoresen and Bredal, 1996).

Classification and statistics

All parameters were normally distributed and compared within group by an analysis of variance for before and after study, with repeated measures for time[f], followed by post-hoc Dunnette C multiple comparison test. Student's *t*-test was used to compare between groups.

Value of $P < 0.05$ was considered to be significant. All results are presented as mean ± standard error. Data collected from all dogs were divided into two groups, non-responders and responders. Non-responders were classified as continuing to require exogenous insulin at the same or increased dose for glycemic control, and/or exhibiting elevations in serum fructosamine concentration higher than 450 µmol/l. On the other hand, responders reduced the need of exogenous insulin dose and/or serum fructosamine concentration was lower than 450 µmol/l.

RESULTS

In this study, the dogs in treatment group enrolled were 3 castrated males, 6 entire males, 11 spayed females and 1 castrated male, 2 entire males and 2 spayed females in the control group. The range of age was seven to fourteen years old and median age was 9 years in control group and 10 years in treatment group. All dogs were diagnosed as diabetes mellitus and had received previous insulin therapy. Each dog was fed with commercially available restricted-fat high-fiber canine diet from the time of entry to the end of the study. No significant changes in body weight were observed before and after treatment in either the treatment or control group (Table 1).

In the MC capsule treated group, twelve dogs were classified as responders (serum fructosamine concentration < 450 µmol/l), and eight dogs were classified as non-responders (serum fructosamine concentration > 450 µmol/l). However, all dogs continued to require exogenous insulin therapy, but slightly decreased exogenous insulin usage compared to those before entering the study. In contrast, none of dog in the diet only control group was classified as a responder (Table 1).

Glycemic parameters

No significant difference in serum fructosamine or glucose concentrations was observed between male and female groups before or after treatment in either the study or control groups. Figure 1 demonstrated that after receiving the MC capsules at 200 mg/kg BW/day for 1 and 2 months respectively, the fasting blood glucose concentration was slightly decreased after receiving the MC capsules for one month (M1 = 249.75 ±17.74 mg/dl). The significant statistical difference was observed after receiving the MC capsules for two months (M2 = 234.70 ± 17.81 mg/dl) compared to those before treatment (M0 = 305.65 ± 31.12 mg/dl). In the control diabetic group, the fasting blood

Table 1. Age, sex, body weight, serum fructosamine concentrations and individual dose of insulin injection.

Group	R / N	Age (years)/ sex	Body weight (kg)		Serum fructosamine (µmol/L)			Pre-insulin dose (Units)	Post-insulin dose (Units)
			Pre-weight	Post-weight	Month 0	Month 1	Month 2		
C1	-	9/ M	7.4	7.6	471	522	486	6	6
C2	-	11/ M	8.7	8.2	467	525	509	5	5
C3	-	7/ FS	5.7	5.9	704	643	681	4.4	4.4
C4	-	8/ M	12.5	10	665	597	583	11	11
C5	-	10/ FS	6.9	7.5	478	487	456	5	5
T1	R	12/ M	8.9	9.4	533	422	429	6.8	3
T2	R	11/ M	11.4	12.7	559	442	378	7	7
T3	R	9/ M	6.7	5.6	478	224	316	6	5
T4	R	7/ FS	19.4	17.2	483	368	594	12	11
T5	R	9/ FS	5.3	6.0	438	423	430	5	5
T6	R	10/ FS	13.4	15.2	568	498	398	10	10
T7	R	11/ FS	22.4	20.5	763	524	325	22	22
T8	R	8/ M	3.5	3.7	562	292	225	2	2
T9	R	7/ FS	4.3	4.4	430	398	371	2	2
T10	R	9/ M	2.8	2.9	412	376	404	2	2
T11	R	8/ FS	17.5	16.4	583	439	398	11	11
T12	R	10/ M	9.2	8.8	590	487	412	14	12
T13	NR	8/ FS	7.1	8.5	256	575	570	8.4	8.4
T14	NR	13/ M	7.0	8.1	547	583	640	6.4	6.4
T15	NR	11/ FS	5.2	5.5	675	648	581	7.6	7.6
T16	NR	9/ FS	8.4	8.8	609	478	498	6	6
T17	NR	12/ M	7.9	7.1	499	515	502	6	6
T18	NR	10/ FS	4.1	4.7	681	632	527	5	5
T19	NR	14/ FS	3.1	3.5	499	578	547	3	3
T20	NR	13/ M	7.8	8.4	466	433	456	8	5

(C, control; T, MC capsule treated; R, responder; NR, non-responder; M1, after receiving MC capsule 200 mg/kg BW for 1 month; M2, after receiving MC capsule 200 mg/ BW for 2 months).

glucose concentration in the 2nd month was slightly decreased, however, no significant difference was detected when compared to those before treatment (M0 = 312.20 ± 37.47 mg/dl, M1=327.00 ± 44.68, M2=274.20 ± 33.01 mg/dl).

Serum fructosamine concentrations decreased significantly ($P < 0.05$) in the treatment group after being fed with MC capsules for 1 (M1 = 466.75 ± 24.25 µmol/l) and 2 months (M2 = 450.05 ± 23.74 µmol/l), respectively compared to those in the control group (M1 = 554.80 ± 28.35, M2 = 543.00 ± 40.38 µmol/l) and those in the month before being fed with the MC capsules (M0 = 531.55 ± 24.75 µmol/l).

Although, serum fructosamine concentration in the control group during study was also slightly decreased (M0=557.00 ± 52.45, M1=554.80 ± 28.35, 543.00 ± 40.38 µmol/l), the difference was not statistically significant (Figure 2).

Serum insulin concentration

No significant difference in serum insulin concentration was observed between the treatment and the control groups, neither before nor after treatment (Table 2).

Complete blood count and blood chemistry profiles

Selected serum chemical parameters during the two-month study following MC administration were summarized in Table 3. The CBC and serum chemistry profiles were not significantly different in comparisons between these two groups, except for the Alkaline Phosphatase enzyme (ALP) concentrations. The concentrations of ALP in the treatment group after administration with 200 mg/ kg BW/day MC capsule for 1 month (M1 = 483.60 ± 54.63 U/l) and 2 months (M2 = 519.50 ± 56.06 U/l) were significantly higher

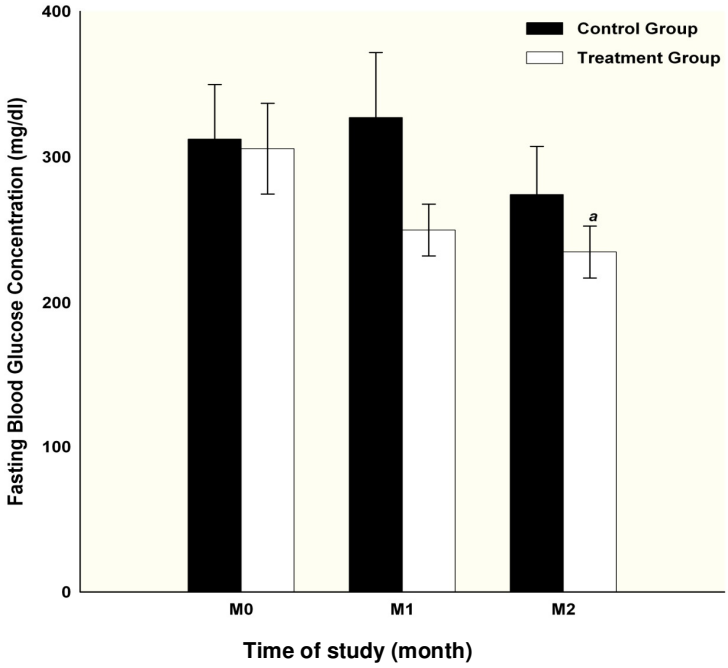

Figure 1. The fasting blood glucose concentrations of diabetic dogs in control (■) and MC capsule treated groups (□). Results shown are means with standard error. M0, the starting month of study; M1, the month after studying for 1 month; M2, the month after studying for 2 months. [a]Significant at $P < 0.05$, compared to month 0 (M0) of the same group.

($P < 0.05$) than those in the control group at the same time of the study (M1 = 385.20 ± 56.56, M2 = 364.30 ± 61.79 U/l). However, no significant difference was detected when compared to those before treatment of the same group (M0 = 448.9 ± 43.35 U/l).

DISCUSSION

The population of client-owned diabetic dogs in this study was similar to that reported by Guptill et al. (2003) in which DM is more likely to occur in older, female dogs. Although, progesterone in female dogs is cause of insulin resistance (Eignenmann et al., 1983), all female dogs in this study had been neutered. Thus, gender might not be affected in this study. Every dog in this study had been diagnosed diabetics; therefore, all dogs had been previously treated with insulin. Fructosamine is a glycosylated serum protein that is formed through irreversible non-enzymatic reactions between glucose and serum proteins. Glucose has a greater affinity for albumin in dogs (Reusch and Haberer, 2001). Fructosamine concentrations in serum directly depend on blood protein and plasma glucose concentrations. The reduction in fructosamine concentration usually occurs when blood glucose concentration decreases resulting in decreasing affinity of glucose and serum protein. One time measurement of fructosamine concentration indicates the average glucose concentration over the previous 1-2 weeks (Marca et al., 2000). Fructosamine measurements can be used for diagnosis of diabetes mellitus, monitoring the effectiveness of insulin therapy and responding of antidiabetic drug treatment. However, evaluation of efficacy of insulin is also based on the information from serial blood glucose curve for individual animal. Some changes of serum protein; such as hypoproteinemia, azotemia and hyperlipidemia, can affect the serum fructosamine concentration, in our study, serum albumin was monitored at monthly interval. In addition, dogs with hypothyroidism usually have high fructosamine concentration due to reduction in protein turnover (Reusch et al., 2002). Therefore, we also measured serum thyroxine concentrations before all dogs entered to this study. All dogs had serum thyroxine concentrations in the normal range and serum albumin concentrations were in the normal level (Plumb, 2005) over the course of study.

Several reports have demonstrated the mechanisms underlying the hypoglycemic effect of the MC which are classified into 3 pathways insulin mimetic, insulin secretagogue, and postprandial hyperglycemia suppression. The insulin mimetic and postprandial hyperglycemia

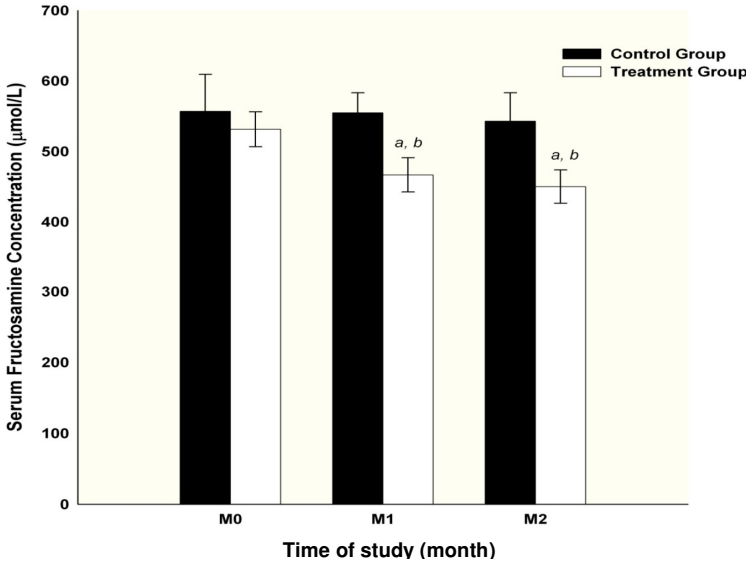

Figure 2. The serum fructosamine concentrations of diabetic dogs in control (■) and MC capsule treated groups (□). The data were shown as mean with standard error. M0, the starting month of study; M1, the month after studying for 1 month; M2, the month after studying for 2 months. [a] Significant at P < 0.05, compared to month 0 of the same group. [b] Significant at P < 0.05, compared to control group at the same month.

Table 2. Serum insulin concentration.

Diabetic dogs group	Serum insulin concentration (µIU/ml)	
	Pre-treatment	Post-treatment
Control group (n = 5)	1.64 ± 0.80	1.90 ± 0.83
Treatment group (n = 20)	1.42 ± 0.36	1.55 ± 0.44

The data were shown as mean ± S.E.

Table 3. The serum chemical profiles of diabetic dogs.

		Time of study		
		Month 0	Month 1	Month 2
Blood urea nitrogen (BUN) (mg/dl)	Control	22.00 ± 2.76	16.8 ± 2.99	19.6 ± 2.40
	Treatment	22.50 ± 2.55	19.55 ± 2.85	20.95 ± 3.43
Creatinine (mg/dl)	Control	0.88 ± 0.18	0.88 ± 0.16	0.92 ± 0.10
	Treatment	0.81 ± 0.08	0.81 ± 0.13	0.89 ± 0.08
ALT (U/L)	Control	57.60 ± 20.35	65.00 ± 15.73	63.00 ± 18.81
	Treatment	72.50 ± 14.41	105.75 ± 17.48	99.05 ± 12.70
ALP (U/L)	Control	338.20 ± 67.13	385.20 ± 56.56	364.30 ± 61.79
	Treatment	448.90 ± 43.35*	483.60 ± 54.63*	519.50 ± 56.06*
Albumin (g/ dl)	Control	3.38 ± 0.26	3.40 ± 0.17	3.22 ± 0.28
	Treatment	3.33 ± 0.08	3.44 ± 0.08	3.33 ± 0.09

The data were shown as mean ± S.E. * Significant at P < 0.05, compared to month 0 of the same group.

mechanisms are found in several studies. For example, MC promotes glucose uptake into L6 muscle cell (Cummings et al., 2004; Ahmed et al., 2004), controls glucose absorption into brush border membrane vesicle in the jejunum of streptozotocin (STZ)-induced diabetic rats (Ahmed et al., 2004), accelerates renewal process of β-cell in STZ-diabetic rats (Karunanayake et al., 1990; Ahmed et al., 1998; Ahmed et al., 2004), and increases glycogen accumulation in liver and peripheral glucose utilization (Bailey et al., 1985). Furthermore, the MC protein extract stimulates insulin secretion from perfused rat pancreas (Yibchok-anun et al., 2006). As a result, the MC capsules might act through one or more of the mentioned mechanisms, which in turn, improves the effectiveness of insulin therapy in diabetic dogs.

Since all dogs in this study had naturally occurring diabetes and were classified into IDDM, we did not observe the improvement of serum insulin concentrations neither before nor after treatments with the MC capsules. The unchanged level in serum insulin may be a result of the severity of destruction of β-cells with progressive and eventually complete insulin insufficiency. The dogs that have been fed with high fat diet for a period of time can develop insulin resistance and they cannot produce insulin from β-cell in order to compensate to glucose tolerance (Kaiyala et al., 1999). Recent study in diabetic rats also demonstrated that the MC was more effective in lowering blood glucose concentrations in mild to moderate, but not in severe or chronic diabetic rats (Jeevathayaparan et al., 1995).

In this study, all diabetic dogs had the higher serum alanine aminotransferase (ALT) and ALP concentrations over the normal reference values (ALT: 4-91; ALP: 3-60 U/l) (Plumb, 2005) for the whole course of study. This finding is consistent with those of previous study which demonstrated that high hepatic enzyme activities are commonly found in diabetic dogs. In addition, most diabetic dogs had hepatomegaly on physical examination, and hepatic fatty change from necropsy (Hess et al., 2000), which are a result of hepatic lipidosis (Leyva-Ocariz, 1993). Additionally, this study found that the ALP levels in the treatment group were significantly higher than those in the control group but the ALT levels of both groups were not different. There was a that study demonstrated that serum γ-glutamyl transferase and alkaline phosphatase concentrations were found to be significantly elevated following oral administration of both the fruit juice and the seed extract (Tennekoon et al., 1994). The most common liver enzymes used as markers of hepatic disease are ALT and ALP but ALT is relatively liver specific in dogs (Evans, 1996). Whereas, the ALP in dogs is found in many organs, including liver, bone, intestine, and kidneys (Keller, 1981; Kidney and Jackson, 1988). Since the intestinal and kidney isoenzymes have very short half-lives, the only bone, liver and corticosteroid-induced isoenzymes are found in canine serum (Kidney and Jackson, 1988). Moreover, increase ALP activities have also been shown to be associated with many chronic diseases, as a result of stress and the resultant increase in endogenous glucocorticoid secretion (Syakalima et al., 1997). In this study, increased ALP activity may not have been associated with progressive liver damage. Additionally, this phenomenon did not show any clinical signs of liver disease.

In summary, the results from our study demonstrated that the MC capsule (200 mg/kg BW/day) significantly decreased serum fructosamine and fasting blood glucose concentrations. The use of MC capsule in combination with insulin therapy and restricted-fat high fiber-diet could improve glycemic control in naturally occurring diabetes mellitus.

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APPENDIX

[a]Abaipubejh®, Prajeanburi,Thailand
[b]Diabetic Veterinary Diet, Royal Canin, Aimargues, France.
[c]Insulin RIA Coat-A-Count kit, Diagnostic Procucts Co., Los Angeles, CA, USA
[d]Lente insulin, Caninsulin®, Intervet, Netherlands
[e]Accu-Chek Advantage H, Roche Diagnostics Corp, Indianapolis, IN, USA
[f]SPSS 15.0 for Windows Evaluation Version, Chicago, IL, USA