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Effects of Multi-Extracts of Mori Folium and of Exercise on Plasma Insulin and Glucose Levels in Streptozotocin-Induced Diabetic Rats

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This research was conducted to study the effects of the supplementation of multi-extracts of *mori folium (MF)* and of exercise on plasma insulin and glucose levels in streptozotocin (STZ)-induced diabetic rats. Eight male Sprague-Dawley rats, 4 weeks old, were assigned to each experimental group and were raised in the laboratory for 10 weeks. The animal groups consisted of a normal-control group, a STZ-control group, 3 STZ-induced diabetic groups supplemented ad libitum with various amounts of MF extracts (MF-720, MF-360, and MF-180 groups), and a STZ-induced diabetic group supplemented with MF-360 along with exercise. In the normal-control group, glucose tolerance tests resulted in the peak blood glucose level being achieved in 15 minutes and a fasting blood glucose level being achieved in 60 minutes. In the STZ-control group, the peak blood glucose level was reached after 60 minutes and, even after 90 minutes, blood glucose shown at a significantly higher level compared to the fasting levels. In the groups supplemented with MF extracts, the blood glucose level peaked after 30 minutes of glucose challenge, and returned to the fasting level after 90 minutes; the MF-360 and MF-360+exercise groups showed the best levels of glucose tolerance. Blood glucose levels in the STZ-induced diabetic groups were significantly higher compared to the normal-control group. However, after 7 weeks of supplementation with MF extracts, a significant lowering of blood glucose levels was observed in all groups supplemented with the MF extract. The best effect was observed in the group given MF extract combined with exercise. Compared to the normal-control group, blood insulin levels were significantly lower in all STZ-induced diabetic groups; however, a significantly higher level of insulin was observed in the groups given MF extracts compared to the STZ-control group. This study shows that the supplementation of MF extracts in STZ-induced diabetic rats resulted in increased blood insulin levels and lower blood glucose levels.

Key words : Mori Folium, streptozotocin, plasma insulin, plasma glucose, diabetes, rat

INTRODUCTION

Despite great improvements in modern science, diabetes mellitus, cancer and heart diseases are three major diseases which demand better cures; the increase in the incidences of these diseases is stimulating researchers' interest in finding better ways of prevention and treatment.[1),2)] Diabetes has become one of the ten major diseases causing deaths in Korea.[3)] Diabetes is a disease affected by genetic factors as well as by environmental factors such as obesity, diet, lack of exercise and stress.[4),5)] Modern medicine has not been successful in developing fundamental cures, but the best treatment option at present is to maintain blood glucose levels. The treatment methods for diabetes include drugs, exercise and diet therapies.[6),7)]

At present, sulfonylurea and biguanide are clinically used for lowering blood glucose; the side effects of sulfonylurea include a risk of hypoglycemia, increased body weight, nausea, vomiting, and indigestion,[8)] and those of biguanide include loss of appetite, nausea, and diarrhea.[8)] Furthermore, insulin treatment causes hyperinsulinemia which can lead to the development of atherosclerosis, an increased risk of obesity, hypoglycemia, allergies, and the enlargement or shrinkage of subcutaneous tissue.[8)] Of late, the focus of treatment is to maintain the blood glucose levels of diabetic patients by inducing the production of insulin-like substances in the body[9)] and to prevent the development of complications. Induction of insulin-like substances is in the experimental stage with animals and it will take a long time to actually utilize the results in the design of treatments. Treatments involving insulin or oral glucose lowering substances are not treating the cause of the problem; thus, active research in the areas of pharmaceutical treatment and functional foods is being carried out as a basis for improved prevention and treatment methods.[10),11)]

Mulberry trees are deciduous trees growing up to 7m,

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with a brownish-yellow bark, and grayish white or grayish yellow branches. The leaves are oval or irregular oval in shape, with sharp ends and asymmetrical heart-shaped lower parts; the edges of the leaves are bluntly serrated. There are approximately 130 varieties of mulberry trees grown around the world.[12] The first record of the use of mulberry leaves appeared about 2,200 years ago in a book called shin-nong-bon-cho-kyoung published by Jang Joong-Kyoung in the late Han dynasty in China. In Korea, there is a recording of the use of Mulberry in Huh Joon's "handbook of Eastern Medicine (DongEuBoGam)", from the era of King Sun-Jo in the Yi dynasty.[13]

Up to now, research has demonstrated the efficacy of mulberry leaves in lowering cholesterol, atherosclerosis and hyperlipidemia.[14] The water extracts of mulberry leaves can lower blood pressure, improve the circulation, adsorb and detoxify heavy metals, suppress cancer and aging, and act as an antioxidant.[15),16]

The present research was carried out to study the effects of the oral supplementation of mulberry leaf extracts (Mori Folium), and of exercise, on blood insulin and blood glucose levels in STZ-induced diabetic rats.

MATERIALS AND METHODS

1. Materials

Dry Mori Folium (70%), Portulacea Herba (5%), Corni Fructus (5%), Euronymus alata Siebold (5%), Maydis Stigmata (5%), Dioscoreae Rhizoma (5%) and Anemarrhenae Rhizoma (5%) was purchased in the Kyung-dong market and pulverized. The resulting powder was added to distilled water and was then placed in a water bath which was attached to a device for circulating cold air. The extract was filtered in warm temperature conditions. This process was repeated three times for three hours. 3% Mori Folium and Multiple extracts was added to distilled water. Rats were given free access distilled water and experimental diets.

2. Experimental animal, diets and diabetes inducement

Forty-eight male Sprague-Dawley rats, approximately four-weeks old, were kept on a pellet diet for 7 days in the laboratory. After one week of adjustment, animals weighing 120 to 140g were assigned to 6 groups by a randomized complete block design. There were 8 rats in each of the six groups (Fig 1). The non-diabetic (normal) control group and the STZ-induced diabetic control group were each given distilled water ad libitum. The other four STZ-induced diabetic groups were given the following treatments; three of the groups were given 720mg, 360mg, and 180mg MF extracts dissolved in distilled water ad libitum, respectively, while the fourth group was given 360mg MF extracts dissolved in distelled water at the same time as being subjected to an endurance exercise regime. The endurance exercise program was based on on the modified method of Pattengale and Holloszy[17] (Table 1) Experimental diets were manufactured on the basis given by the American Institute of Nutrition (1970).[18] (Table 2) Experimental animals were housed individually in stainless wire cages, and the condition of the room was adjusted to a constant temperature of 20-25℃, humidity level of 60-70%, and light-dark cycle of 12 hours each. The food intakes were measured at a fixed time of day and the average daily intake per animal for each week was calculated. Body weights were measured once a week by using an animal scale, and the feed efficiency ratio (FER) was calculated by dividing weight gain by the amount of food eaten.

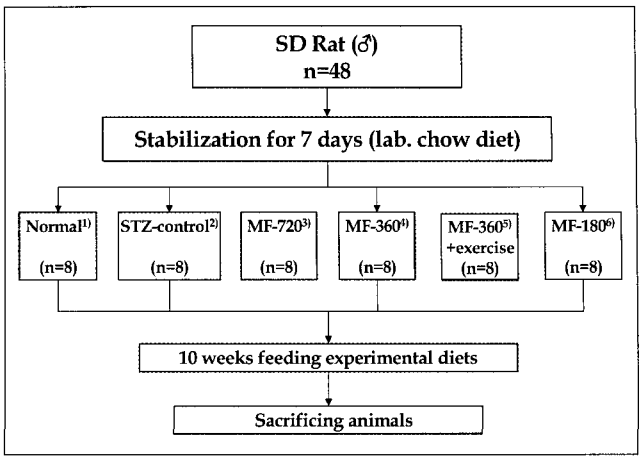

Fig 1. Design of experimental animals
1) Normal : basal diets.
2) STZ-control : basal diets + STZ(0.45mg/kg · B.W.).
3) MF-720 : basal diets + STZ(45mg/kg · B.W.) + MF 720 mg/kg/day.
4) MF-360 : basal diets + STZ(45mg/kg · B.W.) + MF 360 mg/kg/day.
5) MF-360+exercise : basal diets + STZ(45mg/kg · B.W.) + MF 360 mg/kg/day + exercise.
6) MF-180 : basal diets + STZ(45mg/kg · B.W.) + MF 180 mg/kg/day.

Table 1. Program of endurance exercise

	week	Frequency of exercise (days/week)	Exercise intensity		Duration of exercise (min)
			Speed (m/min)	Slope (%)	
Adjustment	0	3	15	6	15
	1	4	20	6	20
	2	4	20	6	20
	3	5	20	6	20
	4	5	20	6	20
Endurance exercise	5	5	20	6	20
	6	5	20	6	20
	7	5	20	6	20
	8	5	20	6	20
	9	5	20	6	20
	10	5	20	6	20

Rats were fasted for 16 hours before the inducement of diabetes by streptozotocin (STZ, Sigma Chemical Co.)[19,20] which is known to specifically act on pancreatic β-cells. Streptozotocin was dissolved in 0.01M of citrate buffer and the mixture was injected into the tail vein at a concentration of 45mg/kg body weight. The normal-control group was injected with the same amount of 0.01M citrate buffer. With the STZ injection, it is known that blood glucose is rapidly reduced and insulin concentration is increased for up to a seven hour period, then increased blood glucose and decreased insulin concentration is observed due to the destruction of pancreatic β-cells. At 24 hours after STZ injection, high blood glucose levels continue. The inducement of diabetes was confirmed when the glucose level is above 300㎎/dℓ in the blood collected from the tail vein.[21]

Table 2. The composition of experimental diets(%)

Ingredients	Basal diet	Groups[1]					
		Normal	STZ-control	MF-720	MF-360	MF-360 +exercise	MF-180
Sucrose	45.00	45.00	43.75	43.75	43.75	43.75	43.75
Casein	20.00	20.00	20.00	20.00	20.00	20.00	20.00
Corn starch	15.00	15.00	15.00	15.00	15.00	15.00	15.00
Corn oil	10.00	10.00	10.00	10.00	10.00	10.00	10.00
Cellulose	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Mineral (AIN-76)[2]	3.50	3.50	3.50	3.50	3.50	3.50	3.50
Vitamin (AIN-76)[3]	1.00	1.00	1.00	1.00	1.00	1.00	1.00
DL-methionine	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Choline bitartrate	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Cholesterol			1.00	1.00	1.00	1.00	1.00
Sodium cholate			0.25	0.25	0.25	0.25	0.25
MF[4]				720 ㎎/㎏/day	360 ㎎/㎏/day	360 ㎎/㎏/day	180 ㎎/㎏/day
Endurance exercise						20min/day, 5day/week	

1) Normal : basal diets.
STZ-control : basal diets + STZ(0.45mg/kg · B.W.).
MF-720 : basal diets + STZ(45mg/kg · B.W.) + MF 720 ㎎/㎏/day.
MF-360 : basal diets + STZ(45mg/kg · B.W.) + MF 360 ㎎/㎏/day.
MF-360+exercise : basal diets + STZ(45mg/kg · B.W.) + MF 360 ㎎/㎏/day + exercise.
MF-180 : basal diets + STZ(45mg/kg · B.W.) + MF 180 ㎎/㎏/day.
2) AIN 76 Vitamine Mixture(Modified without vitamin E and vitamin A); g/kg of mix : Thiamine hydrochloride 0.6; Riboflavin 0.6; Pyridoxine hydrochloride 0.7; Nicotinic acid 3.0; D-calcium pantothenate 1.6; Folic acid 0.2; D-biotin 0.02; cyanocobalbmine 0.001; Cholecalciferol(400,000IU/g) 0.25; Manaquinone 0.005; Ascorbic acid 0.2; Sucrose, finely powdered 992.824
3) AIN 76 Salt mixture; g/kg of mix : Calcium phosphate, dibasic 500; Sodium chloride 74; Potasium citrate, monohydrate 220; Potassium sulfate 52; Magnesium oxide 24; Manganous carbonate(43-48% Mn) 3.5; Ferric citrate(16-17% Fe)6; Zinx carbonate(70% Zno) 1.6; Cupric carbonate(53-55% Cu) 0.3; Potassium iodate 0.01; Sodium selenite 0.01; Chromium potassium sulfate 0.55; Sucrase, finely powdered 118.03
4) MF : Extract of Mori Folium (BRM-DM10)

3. Biochemical analysis

After the 10 week experimental period, the rats were fasted for 12 hours. They were then lightly anesthesized with ethyl ether, and blood was collected from the abdominal artery. Blood was kept at 4℃ for 30 minutes, and was then centrifuged at 3,000rpm for 20 minutes to obtain serum. The resulting serum was frozen until analysis.

After having been on experimental treatments for 10 weeks, the rats were fasted for 12 hours; fasting blood glucose was then collected from a capillary vein, and a glucometer (Medisense Inc, USA) was used for glucose determination. Subsequently, the rats were given an oral glucose load (75mg glucose/kg body weight) and capillary blood was collected for glucose determination after 30, 60, 90, and 120 minutes.

Blood glucose collected from capillary veins was measured by using the Precision Q.I.Dⓡ Blood Glucose Monitoring System (Medisense Inc. USA).

Serum insulin concentration was measured by using the Rat Insulin Enzyme Immunoassay (EIA) system (Amersham Pharmacia Biotech Inc. USA), and was read at an optical density of 450nm using a spectrophotometric plate reader.

4. Statistical analysis

All experimental results are expressed as mean ± S.D., and tests of significance between groups were performed by using one way analysis of variance (ANOVA). The significance of differences among the groups was tested by using Duncan's new multiple test ($p < 0.05$), using Statistical Analysis Software (SAS Institute).[22]

Table 3. Changes of body weight in diabetic rats fed on experimental diets for 10 weeks[1,2]

Groups[3]	Body Weight		
	Initial weight (g)	Final weight (g)	Weight gain (g/day)
Normar	153.30±2.45[NS4]	521.25±53.03[b]	5.24±0.21[c]
STZ-control	151.38±5.83	275.25±58.20[a]	1.77±0.23b[b]
MF-720	150.75±3.20	261.88±45.66[a]	1.59±0.35[b]
MF-360	150.88±6.85	288.25±59.22[a]	1.96±0.23[a]
MF-360+exercise	152.24±4.59	282.21±47.82[a]	1.86±0.35[a]
MF-180	153.25±4.53	290.00±46.36[a]	1.95±0.18[a]

1) Values are mean ± S.D., N=8.
2) Same alphabet doesn't mean different value significantly at $p<0.05$ by Duncan's multiple range test.
3) Normal : basal diets.
STZ-control : basal diets + STZ(45mg/kg · B.W.).
MF-720 : basal diets + STZ(45mg/kg · B.W.) + MF 720 ㎎/㎏/day.
MF-360 : basal diets + STZ(45mg/kg · B.W.) + MF 360 ㎎/㎏/day.
MF-360+exercise : basal diets + STZ(45mg/kg · B.W.) + MF 360 ㎎/㎏/day + exercise.
MF-180 : basal diets + STZ(45mg/kg · B.W.) + MF 180 ㎎/㎏/day.
4) NS : not significant at $p<0.05$ by Duncan's multiple range test

RESULTS AND DISCUSSION

1. Body weight and feed efficiency

Table 3 shows that initial body weights in all groups ranged from 150.75g to 153.30g; however, significant differences among groups were observed after 10 week experiment. In the normal-control group, the final body weight was 521.25g, resulting from a steady average gain of 5.24±0.21g/day throughout the experimental period, while the STZ-control group's final body weight was 275.25g with a much slower average gain of 1.77± 0.18g/day. All experimental groups except MF-720 group showed weight increases after 10 weeks, and the increase was the lowest in the MF-720 group. Insulin plays a role in protein metabolism by facilitating the entry of amino acids into skeletal muscles and increasing protein synthesis; in diabetes the reduction of insulin action results in lowered glucose utilization in cells and leads to a typical starvation state, thus resulting in weight loss.[23] Furuse et al[24] and Fisher & Stewart[25] reported that STZ-induced diabetic rats showed rapid growth reductions and weight reductions. Our present experiment showed that MF extracts used in the MF-360, MF-360+ exercise, MF-180 groups resulted in a smaller weight loss compared to the STZ-control group, suggesting that MF extracts can prevent a rapid weight loss in diabetic rats. The reason for a higher weight loss in the MF-720 group compared to the STZ-control group could be attributed to side effects of the excessive MF supplement over the long term. However, we cannot conclude that this was due to long-term side effects, as there has been no research on the effects of excessive MF supplements. Thus, further research is needed in this area.

Average daily food intakes were significantly higher in all diabetic groups including the STZ-control group, and this is typical in diabetes (Table 4). There was no

Table 4. Diet intake and feed efficiency ratio of diabetic rats fed on experimental diets for 10 weeks[1),2)]

weeks	Groups[3)]					
	Normal	STZ-control	MF-720	MF-360	MF-360+ exercise	MF-180
1st week	20.23 ±3.12^b	30.79 ±2.69^a	32.03 ±3.65^a	30.71 ±2.98^a	32.41 ±2.52^a	31.68 ±3.87^a
2nd week	21.63 ±2.66^b	38.73 ±3.07^a	39.32 ±2.35^a	38.41 ±3.52^a	39.74 ±3.66^a	37.85 ±3.18^a
3rt week	22.84 ±2.86^b	43.64 ±3.27^a	44.68 ±3.89^a	43.89 ±3.17^a	45.43 ±3.78^a	43.59 ±2.97^a
4th week	24.18 ±3.41^b	46.66 ±3.14^a	46.04 ±2.56^a	45.39 ±3.28^a	47.71 ±3.95^a	46.33 ±3.32^a
5th week	25.23 ±2.38^b	47.59 ±3.55^a	46.09 ±3.09^a	47.93 ±3.44^a	48.11 ±2.63^a	47.91 ±2.45^a
6th week	25.71 ±2.58^b	47.19 ±2.43^a	46.25 ±3.18^a	48.11 ±3.05^a	48.76 ±3.58^a	47.82 ±2.55^a
7th week	26.79 ±2.36^b	47.98 ±3.27^a	47.16 ±2.89^a	46.25 ±2.38^a	48.51 ±3.88^a	47.59 ±3.52^a
8th week	28.29 ±2.68^b	48.81 ±2.63^a	46.29 ±3.05^a	47.79 ±2.76^a	49.91 ±3.64^a	48.13 ±3.41^a
9th week	29.95 ±2.46^b	49.52 ±3.32^a	45.86 ±3.44^a	48.80 ±3.54^a	48.65 ±4.45^a	49.22 ±3.03^a
10th week	31.20 ±2.68^b	50.17 ±3.25^a	47.14 ±3.95^a	49.29 ±3.41^a	51.41 ±4.48^a	51.41 ±3.62^a
MEAN	25.60 ±3.47^b	45.32 ±4.47^a	43.83 ±5.38^a	44.66 ±5.42^a	46.06 ±4.94^a	45.31 ±4.86^a
FER[4)]	0.20 ±0.08^b	0.05 ±0.02^a	0.04 ±0.02^a	0.04 ±0.02^a	0.04 ±0.02^b	0.04 ±0.02^a

1) Values are mean ± S.D., N=8.
2) Same alphabet doesn't mean different value significantly at $p<0.05$ by Duncan's multiple range test.
3) Normal : basal diets.
STZ-control : basal diets + STZ(45mg/kg · B.W.).
MF-720 : basal diets + STZ(45mg/kg · B.W.) + MF 720 mg/kg/day.
MF-360 : basal diets + STZ(45mg/kg · B.W.) + MF 360 mg/kg/day.
MF-360+exercise : basal diets + STZ(45mg/kg · B.W.) + MF 360 mg/kg/day + exercise.
MF-180 : basal diets + STZ(45mg/kg · B.W.) + MF 180 mg/kg/day.
4) FER : Body weight gain/diet intake
5) NS : not significant at $p<0.05$ by Duncan's multiple range test

Table 5. Drinking Quantity of diabetic rats fed on experimental diets for 10 weeks[1),2)]

weeks	Drink intake (ml/day)					
	Normal	STZ-control	MF-720	MF-360	MF-360+ exercise	MF-180
0day	32.7 ±3.7[NS3)]	30.9 ±2.9	32.3 ±3.6	30.7 ±2.9	31.7 ±2.5	31.8 ±3.8
1st week	33.5 ±3.8^b	221.6 ±10.3^a	226.3 ±8.3^a	224.4 ±7.9^a	230.7 ±11.9^a	219.3 ±9.2^a
2nd week	35.6 ±2.6^b	236.5 ±11.2^a	235.4 ±10.5^a	241.2 ±12.0^a	238.7 ±12.9^a	229.8 ±13.4^a
3rt week	34.8 ±3.9^b	238.6 ±9.3^a	235.6 ±12.2^a	239.4 ±9.5^a	237.7 ±14.6^a	230.3 ±11.6^a
4th week	35.9 ±3.4^b	241.5 ±12.6^a	236.9 ±12.5^a	240.8 ±10.7^a	243.5 ±13.9^a	238.2 ±8.6^a
5th week	36.8 ±4.5^b	246.1 ±13.6^a	238.6 ±15.1^a	243.1 ±9.6^a	247.8 ±15.4^a	240.4 ±12.5^a
6th week	38.6 ±2.8^b	246.5 ±12.3^a	242.5 ±13.1^a	248.1 ±13.0^a	251.37 ±14.5^a	247.2 ±12.5^a
7th week	39.9 ±4.3^b	247.9 ±13.2^a	247.1 ±12.8^a	246.2 ±9.3^a	247.7 ±11.2^a	247.5 ±13.5^a
8th week	38.9 ±3.6^b	248.8 ±12.3^a	246.9 ±13.0^a	247.7 ±12.6^a	251.9 ±16.9^a	248.1 ±9.1^a
9th week	39.5 ±4.4^b	250.5 ±13.3^a	242.3 ±12.4^a	248.8 ±13.5^a	253.3 ±12.3^a	249.8 ±11.3a
10th week	41.2 ±4.8^b	251.1 ±10.2^a	245.1 ±12.9^a	249.2 ±13.4^a	254.5 ±14.2^a	251.4 ±13.6^a
MEAN	37.0 ±3.7^b	223.6 ±16.2^a	220.8 ±15.1^a	223.6 ±9.2^a	226.3 ±12.6^a	221.2 ±13.8^a

1) Values are mean ± S.D., N=8.
2) Same alphabet doesn't mean different value significantly at $p<0.05$ by Duncan's multiple range test.
3) NS : not significant at $p<0.05$ by Duncan's multiple range test

significant difference in food intake between the STZ-control and STZ-MF groups. Despite higher food intakes in the diabetic groups, these groups showed a slow weight gain compared to the control group.[26]

The average daily liquid intake in the control group was 37ml, while all diabetic groups (the STZ-control, MF-720, MF-360, MF-360+exercise and MF-180 groups), showed an average consumption of 220.8 to 226.3ml, equivalent to almost 6 times that of the normal-control group. The MF supplementation itself did not appear to affect liquid consumption (Table 5).

2. Organ weight

Relative organ weights per 100g body weight were calculated in order to study the effects of supplementation of MF extracts (Table 6). Relative weights of the kidney, liver, lungs, and heart tended to become smaller in the normal control group as they grew; however, the opposite was observed in the diabetic groups.

The relative weight of the kidney became much larger compared to other organs, in the diabetic groups. In the STZ-control group, the relative weight of the kidney was 0.76g compared to 0.35g in the normal-control group; this result is in agreement with Gallagher et al[27] as well as Hong et al,[28] who observed a rapid enlargement of the kidneys. The enlarged kidneys in STZ-induced diabetic rats are due to changes in renal metabolism.[29]

Table 6. Change of organ weights of diabetic rats fed on experimental diets for 10 weeks[1),2)]

(g/100g B.W.)

weeks	Groups[3)]					
	Normal	STZ-control	MF-720	MF-360	MF-360+exercise	MF-180
liver	3.97 ±0.32^{c}	5.44 ±0.32^{b}	4.70 ±0.36^{a}	4.65 ±0.39^{a}	4.67 ±0.31^{a}	4.88 ±0.25^{a}
kidney	0.35 ±0.11^{c}	0.76 ±0.12^{b}	0.67 ±0.09^{a}	0.66 ±0.13^{a}	0.62 ±0.11^{a}	0.69 ±0.10^{a}
lung	0.45 ±0.02^{b}	0.59 ±0.07^{a}	0.56 ±0.06^{a}	0.57 ±0.05^{a}	0.56 ±0.08c	0.58 ±0.04^{a}
pancreas	0.32 ±0.02$^{NS4)}$	0.33 ±0.02	0.32 ±0.02	0.33 ±0.02	0.31 ±0.02	0.31 ±0.02
heart	0.35 ±0.01^{b}	0.44 ±0.02^{a}	0.44 ±0.02^{a}	0.43 ±0.02^{a}	0.43 ±0.02^{a}	0.44 ±0.02^{a}
spleen	0.19 ±0.01$^{NS4)}$	0.20 ±0.01	0.20 ±0.01	0.20 ±0.01	0.19 ±0.01	0.19 ±0.01

1) Values are mean ± S.D., N=8.
2) Same alphabet doesn't mean different value significantly at $p<0.05$ by Duncan's multiple range test.
3) Normal : basal diets.
STZ-control : basal diets + STZ(45mg/kg · B.W.).
MF-720 : basal diets + STZ(45mg/kg · B.W.) + MF 720 mg/kg/day.
MF-360 : basal diets + STZ(45mg/kg · B.W.) + MF 360 mg/kg/day.
MF-360+exercise : basal diets + STZ(45mg/kg · B.W.) + MF 360 mg/kg/day + exercise.
MF-180 : basal diets + STZ(45mg/kg · B.W.) + MF 180 mg/kg/day.
4) NS : not significant at $p<0.05$ by Duncan's multiple range test

Diabetes increases excretion from the kidneys and high blood glucose increases the supply of phosphoribosyl pyrophosphate (PRPP) for RNA and DNA synthesis, which eventually increases cell division in the kidneys and enlarges the kidneys.[30),31)] Steer et al[32] reported that kidney enlargement occurs due to high concentrations of serum glucose which is metabolized to udp-galactose or glycogen that is accumulated in membranes of the mesangial cells of the glomerulus. In our experiment the significantly lower relative weight of the kidneys in the groups supplemented with MF compared to the STZ-control group could be due to flavonoids such as rutin and quercetin, which regulate the permeability of blood vessels and lipid metabolism, resulting in lower blood glucose levels.[33]

The relative weight of the liver in the STZ-control group was 37% higher that that of the normal-control group, while the STZ-MF group showed a significantly lower liver weight compared to the STZ-control group. The MF extracts may therefore have anti-diabetic ingredients.

The relative weights of lungs and hearts were significantly higher in all STZ-induced diabetic groups; this may be due to an inadequate blood supply to muscles and tissues, resulting in the enlargement of lungs and hearts due to overworking. The relative weights of the pancreas and spleen were similar in all groups, and it appears that these organs are least affected by diabetes.

3. Glucose tolerance test

The effects of MF extracts on glucose tolerance were tested (Fig 2). The STZ-control group maintained very

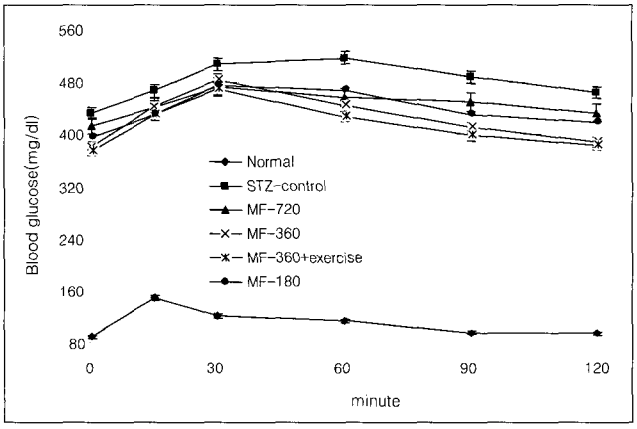

Fig 2. Glucose challenge of normal and diabetic rats fed experimental diet for 10 weeks[1),2)]

1) Values are mean ± S.D., N=8
2) Normal : basal diets.
STZ-control : basal diets + STZ(45mg/kg · B.W.).
MF-720 : basal diets + STZ(45mg/kg · B.W.) + MF 720 mg/kg/day.
MF-360 : basal diets + STZ(45mg/kg · B.W.) + MF 360 mg/kg/day.
MF-360+exercise : basal diets + STZ(45mg/kg · B.W.) + MF 360 mg/kg/day + exercise.
MF-180 : basal diets + STZ(45mg/kg · B.W.) + MF 180 mg/kg/day.

high blood glucose throughout the test period. In the normal-control group, the peak blood glucose level was reached in 15 minutes after the glucose loading, and the fasting blood glucose level was observed in 60 minutes. In the STZ-control group, blood glucose gradually increased and reached its peak at 60 minutes, followed by a slow decrease; however, even at 90 minutes blood glucose remained at a higher level than during the fasting level. On the other hand, MF supplemented groups showed the peak blood glucose level at 30 minutes followed by a gradual reduction, with the fasting blood glucose level being attained at 90 minutes; the best glucose tolerance was observed in the MF-360 + exercise group. However, the peak glucose level was 4.5 times higher in the MF groups compared to the normal-control group. Therefore, it appears that the MF extracts did not affect the increase in blood glucose level after glucose loading; however, improvements in fasting blood glucose level and in the glucose tolerance test suggest its possible function in improving glucose metabolism.

4. Blood glucose and insulin levels

Blood glucose was determined every 7 days (Table 7). All STZ-induced diabetic rats showed a significantly higher blood glucose level compared to the normal-control group. Up to 6 weeks there was no difference between the STZ-control group and MF treated groups. After 7 weeks, the MF-360, MF-360+exercise and MF-180 groups started showing a significant reduction in blood glucose, and by 10 weeks the % of reduction of blood glucose was 6.7%, 12.4%, 13.9%, 10.1% in the MF-720, MF-360, MF-360+exercise, and MF-180 groups, respectively. The best result was observed in the MF-360 +exercise group. Our present study therefore shows a potential role for MF extracts in regulating blood glucose. Myo-inositol and quercetin, present in MF extracts, are known to improve diabetes and galactosemia[34] by increasing insulin sensitivity or by improving damaged pancreatic β-cells. The more limited reduction of blood glucose in the MF-720 group compared to the MF-360 and MF-180 groups may be due to the side effects of long-term supplementation of MF-720. Future scientific investigation in more detail is needed for determining the physiologically active substances in MF extracts and the anti-diabetic effects of different concentrations of MF extracts.

Blood insulin levels were lower in all STZ-induced diabetic groups compared to the normal-control group (Table 8). The highest increase in blood insulin level was observed in the MF-360 + exercise group, and all MF supplementation groups showed higher blood insulin levels compared to the STZ-control group. The reason for the higher blood insulin concentration in the MF supplementation groups compared to the STZ-control group may be due to the roles of MF extracts in improving secretion of insulin from the β-cells of the

Table 7. Blood glucose levels in diabetic rats fed on experimental diets for 10 weeks[1),2)]

weeks	Groups[3)]					
	Normal	STZ-control	MF-720	MF-360	MF-360+exercise	MF-180
0day	107.00 ±7.60^{b}	374.88 ±45.90^{a}	371.50 ±32.01^{a}	373.75 ±55.65^{a}	370.87 ±45.64^{a}	373.50 ±47.93^{a}
1st week	100.50 ±3.42^{b}	399.50 ±36.22^{a}	388.13 ±40.03^{a}	381.75 ±42.18^{a}	376.73 ±35.15c	379.63 ±26.13^{a}
2nd week	108.88 ±10.92^{b}	410.75 ±76.50^{a}	411.38 ±60.01^{a}	387.88 ±43.35^{a}	383.85 ±43.62^{a}	386.50 ±59.77^{a}
3rt week	103.5 ±7.56^{b}	406.23 ±41.32^{a}	402.36 ±43.58^{a}	385.14 ±50.24^{a}	387.33 ±56.64^{a}	389.87 ±46.87^{a}
4th week	100.38 ±5.78^{b}	391.88 ±50.84^{a}	398.50 ±50.38^{a}	370.88 ±29.79^{a}	378.78 ±50.35c	382.25 ±52.23^{a}
5th week	102.38 ±4.44^{b}	396.38 ±36.50^{a}	402.88 ±34.73^{a}	378.63 ±34.75^{a}	374.77 ±45.05^{a}	388.75 ±30.40^{a}
6th week	99.50 ±5.37^{b}	407.38 ±27.58^{a}	386.13 ±38.36^{a}	381.50 ±47.49^{a}	383.95 ±57.65^{a}	381.88 ±34.6^{a}
7th week	100.88 ±7.30^{c}	423.00 ±18.20^{b}	397.38 ±36.70^{ab}	370.50 ±44.41^{a}	373.78 ±48.64^{a}	378.38 ±36.08^{a}
8th week	95.00 ±6.70^{c}	433.38 ±31.06^{b}	392.50 ±36.48^{ba}	375.25 ±44.66^{a}	370.11 ±51.07^{a}	399.75 ±37.50^{a}
9th week	98.00 ±5.35^{c}	447.00 ±32.68^{b}	398.38 ±42.55^{a}	390.88 ±29.32^{a}	376.74 ±58.66^{a}	410.38 ±29.81^{a}
10th week	100.50 ±10.21^{c}	430.25 ±30.27^{b}	401.63 ±34.88^{a}	376.75 ±44.05^{a}	370.39 ±48.87^{a}	387.00 ±30.10^{a}

1) Values are mean ± S.D., N=8.
2) Same alphabet doesn't mean different value significantly at $p<0.05$ by Duncan's multiple range test.
3) Normal : basal diets.
STZ-control : basal diets + STZ(45mg/kg · B.W.).
MF-720 : basal diets + STZ(45mg/kg · B.W.) + MF 720 mg/kg/day.
MF-360 : basal diets + STZ(45mg/kg · B.W.) + MF 360 mg/kg/day.
MF-360+exercise : basal diets + STZ(45mg/kg · B.W.) + MF 360 mg/kg/day + exercise.
MF-180 : basal diets + STZ(45mg/kg · B.W.) + MF 180 mg/kg/day.

Table 8. Insulin levels of diabetic rats fed on the experimental diets for10 weeks[1),2)]

Groups[3)]	Insulin(ng/dℓ)
Normal	11.81±2.18c
STZ-control	3.35±2.86b
MF-720	5.85±2.01a
MF-360	6.23±2.01a
MF-360+exercise	6.58±2.51a
MF-180	5.78±3.04a

1) Values are mean ± S.D., N=8.
2) Values with different superscript within the row are significantly different at 5% level.
3) Normal : basal diets.
STZ-control : basal diets + STZ(45mg/kg · B.W.).
MF-720 : basal diets + STZ(45mg/kg · B.W.) + MF 720 mg/kg/day.
MF-360 : basal diets + STZ(45mg/kg · B.W.) + MF 360 mg/kg/day.
MF-360+exercise : basal diets + STZ(45mg/kg · B.W.) + MF 360 mg/kg/day + exercise.
MF-180 : basal diets + STZ(45mg/kg · B.W.) + MF 180 mg/kg/day.

Langerhans isle of the pancreas and/or increases in the number of insulin receptor cells at higher blood glucose levels.

CONCLUSION

The effects of oral supplementation of 3% MF extracts, and exercise, on lowering blood glucose in streptozotocin (STZ) - induced diabetic rats for 10 seeks were determined studied.

Regarding body weight, all diabetic rats showed a significantly lower increase in body weight, and the MF-720 group showed the least body weight increase. The average food intake was significantly higher in diabetic rats compared to the normal-control, and no differences in intake were observed between the STZ-control group and MF supplemented groups. Regarding the average daily liquid intake, the STZ-treated groups consumed 6 times more than the normal-control group, and no difference was observed between the STZ-control group and the MF groups.

When the relative organ weights per 100g body weight were calculated, the weights of the kidney, liver, lungs, and heart were significantly higher in the diabetic groups (the STZ-control group and all MF groups). The relative weights of liver and kidney in the MF groups were significantly lower compared to the STZ-control group. All groups showed similar pancreas and spleen weights.

The effect of MF supplementation on glucose tolerance test results were as follows; the normal-control group showed a peak blood glucose level at 15 minutes and a return to the fasting level at 60 minutes, while the STZ-control group reached a peak at 60 minutes, and even at 90 minutes blood glucose level stayed at a much higher level than the fasting level. In the MF groups, the peak was reached at 30 minutes, and at 90 minutes a significant reduction (almost to the fasting level) was achieved. The best glucose tolerance was observed in the MF-360 group+exercise.

The blood glucose level was significantly higher in all diabetic groups compared to the normal-control. The MF supplementation groups showed similar glucose levels to the STZ-control group up to the 6 week stage; however, the MF-360, MF-360+exercise and MF-180 groups began showing lower blood glucose levels at 7 weeks, while at 10 weeks the reduction in blood glucose compared to the STZ-control group was significant. The greatest reduction was observed in the MF-360+exercise group.

Blood insulin levels were significantly lower in diabetic rats compared to the normal-control rats; however, MF supplementation resulted in a significant increase in the insulin level compared to the STZ-control group.

MF supplementation resulted in a significant increase in blood insulin level and a significant reduction in blood glucose level in STZ-induced diabetic rats. When exercise was combined with MF supplementation, the greatest reduction of blood glucose resulted. Therefore, we can conclude that MF supplementation in diabetic rats resulted in blood glucose reductions, and that the greatest blood glucose reduction was achieved when MF supplementation was combined with exercise.

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