Effect of Traditional Chinese Medicine Complex for diabetes (TCM-D™) on experimentally induced diabetic mice

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Background: We previously evaluated the biochemical changes induced by the local product TCM for diabetes (TCM-D™) on blood glucose levels and other biochemical changes in normal mice fed orally with the recommended human dose (30 ml/kg daily) and ten times this dose for eight weeks. TCM-D™ is an aqueous extract of the roots of *Trichosanthes kirilowii* Maxim, *Paeonia lactiflora* Pall, *Glycyrrhiza uranlensis* Fisch. and *Panax ginseng* Meyer (red) combined at the dry weight proportions of 36%, 28%, 18% and 18% respectively. The study showed that at these dosages the blood glucose levels as well as the body weights in treated mice were significantly reduced when compared with pre-treatment values and control animals. The present study evaluated the effect of the extract in a mouse model of Type 1 diabetes mellitus.

Methods: TCM-D™ extract was prepared as a 10x concentrate and given orally at 0.3 ml/100 g and 1.5 ml/100 g to mice which were experimentally induced diabetic with intraperitoneal injections of streptozotocin (5 mg/100g) in sodium citrate (pH 4.5). Control diabetic mice were dosed with extract diluent (distilled water).

Results: At the doses studied the compound did not show any significant lowering of the glucose levels in a mouse model of Type 1 diabetes. There were significant increases in the alanine aminotransferase (ALT) and creatinine levels which were most likely due to the treatment with the compound. There were no significant changes in the aspartate aminotransferase (AST) and blood urea levels due to the treatment. Neither was there any significant effect on the weight of the treated animals due to the treatment.

Conclusions: It is concluded that TCM-D™ did not have any significant blood glucose lowering effect on streptozotocin induced diabetic mice when fed orally at 1-5 times the recommended human dose. Further work is needed to determine if the extract has any significant effect in a mouse model with Type 2 diabetes mellitus.

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Key Words: Traditional Chinese Medicine; experimental diabetes in mice; Type 1 diabetes mellitus, ***Trichosanthes kirilowii*** *Maxim;* ***Paeonia lactiflora*** *Pall;* ***Glycyrrhiza uranlensis*** *Fisch;* ***Panax ginseng*** *Meyer (red)*

Introduction

Traditional Chinese Medicine (TCM) is frequently used for the treatment of chronic diseases including diabetes. Some of the compounds used for the treatment of diabetes may have adverse effects but these have not been studied adequately. We have evaluated the biochemical changes induced by a local product TCM for diabetes (TCM-D™) on blood glucose levels and other biochemical changes in normal mice fed orally with the recommended and ten times the human dosage for eight weeks.[1] The study showed that at these doses there was a significant reduction in the blood glucose levels as well as the body weights of treated mice when compared with pre-treatment values and control animals. There were no significant changes in the liver and renal functions and the treated and control animals remained healthy throughout the eight weeks of observation.

TCM-D™ is a mixture of aqueous extracts from the roots of *Trichosanthes kirilowii* Maxim, *Paeonia lactiflora* Pall, *Glycyrrhiza uranlensis* Fisch. and *Panax ginseng* Meyer (red). The extracts from each of these traditional herbs have proven blood glucose lowering activities in normal or diabetic animals in experimental studies.[2-6] The dosage of TCM-D™ used in traditional Chinese practice for the lowering of blood glucose is 630 ml before meals thrice daily. This works out to be about 30 ml/kg daily in a 65 kg body weight adult. There are plans to incorporate TCM-D™ in health drinks in the form of aqueous juice.

The objectives of the present study were to determine the effect of oral TCM-D™ on blood glucose level and adverse effects, if any on liver and renal functions in mice experimentally induced with Type 1 diabetes mellitus.

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Glucosides (paeoniflorin and 8-debenzoylpaeoniflorin) from the root of *Paeonia lactiflora* were shown to produce a significant blood sugar lowering effect in streptozotocin-treated rats, the maximum effect being observed at 25 min post-treatment.[3] The anti-hyperglycaemic effect of paeoniflorin was insulin-independent, probably due to increased glucose utilisation and was greater than that of 8-debenzoylpaeoniflorin.

What is not known is whether the aqueous extracts from the roots of plants which are the sources of these compounds when combined, will have antagonistic or synergistic effect on their blood glucose lowering activity. The study also monitored the changes in biochemical profiles induced by TCM-D™ as part of the important on-going process of evaluating the potential unwanted side effects associated with traditional herbs used for therapy or incorporated in health drinks.

This study was approved by the International Medical University Joint Committee (IMU-JC) for Research and Ethics.

Materials and Methods

Induction of diabetes in mice

Balb/c mice (4-6 weeks old) were made diabetic by four daily intra-peritoneal (i.p.) injections of streptozotocin (5 mg/100g; Sigma Chemical Co., St Louis, MO.) in sodium citrate (pH 4.5) following the method of Ziegler *et al.*[7] as modified by Waters & Ntambi.[8] The mice were monitored for blood glucose level 14 days after the first injection. As the levels of the blood glucose were not sufficiently high, a second course of four i.p. injections of streptozotocin at the same dosage were given. All animals were maintained in environmentally controlled conditions according to guidelines as laid down in the Animal Resources Unit, Institute for Medical Research, Kuala Lumpur.

TCM-D Complex (TCM-D™)

The main components of TCM-D™ are aqueous extracts of *Trichosanthes kirilowii* (root), *Paeonia lactiflora*, *Glycyrrhiza uranlensis* and *Panax ginseng* (Red) prepared from 330 g in the dry weight proportion of 36%, 28%, 18% and 18% respectively. The total volume of the extract was 360 ml and sodium benzoate (300 ppm) was added as preservative. The preparation of the extract is as described previously.[1] A 10x concentrate of the aqueous TCM-D™ was used for the experimental study. The same vehicle (distilled water) used for preparation of the 10x concentrate of the TCM-D™ was used in control animals.

Animal Study Groups

Experimentally induced diabetic mice were randomly assigned to experimental and control groups of 10 mice each as shown below.

a. Group 1 – Diabetic mice treated with the TCM-D™ (10x concentrate) at an oral single daily dose of 3 ml/kg or 0.3 ml/100 g (diluted to 1 ml with distilled water) for 8 weeks.

b. Group 2 – Diabetic mice treated with the TCM-D™ (10x concentrate) at an oral single daily dose of 15 ml/kg or 1.5 ml/100 g (diluted to 1 ml with distilled water) for 8 weeks.

c. Group 3 (Control) – Diabetic mice given a single oral daily dose of 1 ml distilled water for 8 weeks.

All TCM-D™ or diluent were given by gastric tube. The mice were given standard laboratory pellet and water *ad libitum*. The general condition of the mice was monitored daily.

The following haematological and biochemical parameters (between 9-10 am) were monitored during pre-treatment and at monthly intervals after the start of treatment until the end of the observation period: (a) haemoglobin, red cell count, total leukocyte & differential count, (b) blood glucose, (c) the liver function tests, aspartate aminotransferase (AST) and alanine aminotransferase (ALT), (d) renal function (creatinine and blood urea).

The animals were sacrificed 8 weeks after the start of treatment, using an overdose of ether. At autopsy all organs were examined for gross abnormalities. The liver, spleen, kidneys, lungs, heart and skeletal muscles were preserved in 10% buffered formalin for archival purposes.

Statistical Analyses

Results were expressed as means ± SE. One-way ANOVA was used to test for differences between group means. Groups showing differences were further analysed by Student's *t* test and a *P* value < 0.05 was considered significant.

Results

Blood Glucose Levels

All animals showed an increase in the blood glucose level after the second induction of diabetes using i.p. injections of streptozotocin (5 mg/100g; Sigma Chemical Co., St Louis, MO.) in sodium citrate (pH 4.5) i.e. after two courses of 5 mg/100g. The mean blood glucose levels at post-induction were significantly higher in all groups of animals when compared to baseline levels at pre-induction (Table 1). All groups of animals had significantly higher mean levels of blood glucose at Weeks 0, 4, and 8 than at pre-induction. In Group 1 animals (treated with 0.3 ml/100g), there was a significant drop in the mean blood glucose level at Week 4 of treatment when compared to that at Week 0, but the level increased again at week 8 of treatment. Similarly in Group 2 animals (treated with 5 times the dose as in Group 1), there was a significant decline compared to the glucose level at Week 0 but again the mean level increase to a higher level at Week 8 of treatment. This decline at Week 2 in the glucose level in the two treatment groups must be seen in the context of the significant decline in the glucose level from pre-treatment level (Week 0) at Week 8 even in the Control Group (Table 1). It is therefore concluded that treatment at the doses given in the study did not have any glucose lowering activity in diabetic mice.

Table 1. Blood glucose level in diabetic Balb/c mice treated with oral TCM-D™

Treatment Group*	Mean ± SE Blood Glucose (mg/dl) Treatment Week			
	-2	0	4	8
Control Group	79.23 ± 2.62[a, 1]	116.47 ± 5.78	99.42 ± 4.66	89.67 ± 3.05[2]
0.3 ml/100g (Group 1)	63.40 ± 1.83[3]	122.67 ± 4.59	83.73 ± 8.83[b, 4]	105.36 ± 3.25[#, c]
1.5 ml/100g (Group 2)	70.50 ± 2.41[5]	112.49 ± 2.84[#]	94.61 ± 6.24[6]	105.08 ± 4.48[#, c]

**10 animals per group unless indicated otherwise; [#]8 animals; [a]Significantly higher than Group 1 (P < 0.001) and Group 2 (P = 0.013) at Week -2; [b]Significantly lower than Control Group (P = 0.036) at Week 4; [c]Significantly higher than Control Group (P = 0.005) at Week 8; [1]Significantly lower than at Week 0 (P < 0.001), Week 4 (P = 0.011) and Week 8 (P = 0.038); [2]Significantly lower than at Week 0 (P = 0.001); [3]Significantly lower than at Week 0 (P < 0.001), Week 4 (P = 0.001) and Week 8 (P < 0.001); [4]Significantly lower than at Week 0 (P = 0.001) and at Week 8 (P = 0.020); [5]Significantly lower than at Week 0 (P = 0.001), Week 4 (P = 0.006) and Week 8 (P = 0.001); [6]Significantlty lower than at Week 0 (P = 0.002).*

Body Weight of Animals

There was no significant difference in mean body weight of animals between groups at pre-treatment and during the treatment period of 8 weeks (Table 2). The animals in the control and treatment groups had mean weights which were higher at Week 8 of treatment when compared with pre-treatment. It is concluded that in diabetic mice, treatment with TCM-D™ at the doses given did not have any appreciable effect on the body weight.

Table 2. Body weight in diabetic Balb/c mice treated with oral TCM-D™

Treatment Group*	Mean ± SE Body Weight (g) Treatment Week		
	0	4	8
Control Group	19.26 ± 0.25	20.96 ± 0.46[1]	20.28 ± 0.59
0.3 ml/100g (Group 1)	19.73 ± 0.30	20.61 ± 0.28	19.87 ± 0.70[#]
1.5 ml/100g (Group 2)	19.53 ± 3.35[#]	20.54 ± 0.32[§, 2]	21.29 ± 0.47[†, 3]

**10 animals per group unless indicated otherwise; [#]8 animals; [§]9 animals; [†]7 animals.*
[1]Significantly higher than at Week 0 (P = 0.009); [2]Significantly higher than at Week 0; [3]Significantly higher than at Week 0 (P = 0.007).

Liver Function Assays

The tests used to monitor possible changes in liver function during treatment were aspartate aminotransferase (AST) and alanine aminotransferase (ALT) and the levels at pre-treatment and at 4 and 8 weeks of treatment with the TCM-D™ are as shown in Tables 3 and 4. The mean AST levels increased in animals of treatment and control groups during the 8 weeks of observation, the highest increase being seen in the Control Group and lowest in animals of Group 2 (treated with 5x higher dose of TCM-D™); the difference between these two groups being not significant (P = 0.18). It therefore appears that the increase in the AST levels during the period of observation is not likely to be due to treatment with TCM-D.

Table 3. Aspartate aminotransferase (AST) levels in diabetic Balb/c mice treated with oral TCM-D™

Treatment Group*	Mean ± SE AST (U/L) Treatment Week		
	0	4	8
Control Group	40.42 ± 13.48	61.51 ± 20.70	123.64 ± 26.88[1, a]
0.3 ml/100g (Group 1)	35.10 ± 10.49	30.31 ± 5.34	119.29 ± 20.49[#, b, 2]
1.5 ml/100g (Group 2)	14.42 ± 3.94[§]	28.10 ± 5.11[3]	45.67 ± 11.64[#]

**10 animals per group unless indicated otherwise; [#]8 animals; [§]9 animals.*
[a]Not significantly higher than Group 2 (P = 0.18) at Week 8; [b]Not significantly higher than Group 2 (P = 0.32) at Week 8; [1]Significantly higher than at Week 0 (P = 0.048); [2]Significantly higher than at Week 0 (P = 0.034) and at Week 4 (P = 0.002); [3]Significantly higher than at Week 0 (P = 0.003).

Table 4. Alanine aminotransferase (ALT) levels in diabetic Balb/c mice treated with oral TCM-D™

Treatment Group*	Mean ± SE ALT (U/L) Treatment Week		
	0	4	8
Control Group	43.76 ± 9.43	27.55 ± 7.78	82.21 ± 29.12
0.3 ml/100g (Group 1)	42.32 ± 14.46	83.33 ± 66.13	184.90 ± 84.37[#]
1.5 ml/100g (Group 2)	27.52 ± 4.36[§]	21.66 ± 4.62	238.45 ± 49.47[#, 1]

**10 animals per group unless indicated otherwise; [#]8 animals; [§]9 animals.*
[1]Significantly higher than at Week 0 (P = 0.004) and at Week 4 (P = 0.004).

The ALT showed a similar increase as that of AST. The levels of ALT were highest in control and treatment groups at Week 8. The difference in levels were not significant between groups at Week 0 (F = 0.694, P = 0.508), Week 4 (F = 0.780, P = 0.469), and Week 8 (F = 2.382, P = 0.116). However, in Group 2, the mean AST level at Week 8 was significantly higher Week 0 and Week 4 (P = 0.004). The increase in ALT levels was 8.66 and 11.00 times higher than that at Week 0 and Week 4. Although the ALT level at Week 8 in Group 2 animals was 2.90 and 1.29 times higher than the levels of the Control Group and Group 1 animals at Week 8, the differences seen were not significant (F = 2.382, P = 0.116). When these results are taken together, it is concluded that TCM-D™ treatment could have contributed to the increase in ALT levels, especially when given at the higher dose for 8 weeks.

Renal Function Assays

The renal function assays employed were blood urea nitrogen and creatinine levels and these were monitored at pre-treatment and during the treatment period of 8 weeks. These levels are shown in Tables 5 and 6.

Table 5. Blood urea level in diabetic Balb/c mice treated with oral TCM-D

Treatment Group*	Mean ± SE Blood Urea (mg/dl) Treatment Week		
	0	4	8
Control Group	28.08 ± 1.81	24.34 ± 1.14[a]	29.44 ± 1.06[b, 1]
0.3 ml/100g (Group 1)	28.20 ± 1.74[2]	20.22 ± 1.19	31.65 ± 0.51[#, c, 3]
1.5 ml/100g (Group 2)	28.39 ± 1.68[4]	20.23 ± 0.89	25.43 ± 1.27[#, 5]

**10 animals per group unless indicated otherwise; #8 animals. [a]Significantly higher than Group 1 (P = 0.012) and Group 2 (P = 0.012) at Week 4; [b]Significantly higher than Group 2 (P = 0.01) at Week 8; [c]Significantly higher than Group 2 (P < 0.001) at Week 8; [1]Significantly higher than at Week 4 (P = 0.003); [2]Significantly higher than at Week 4 (P = 0.004); [3]Significantly higher than at Week 4 (P < 0.001); [4]Significantly higher than at Week 4 (P = 0.001); [5]Significantly higher than at Week 4 (P = 0.013).*

There was no significant difference in blood urea levels between groups at Week 0 (F = 0.008, P = 0.992) but significant group differences at Week 4 (F = 4.833, P = 0.016) and at Week 8 (F = 8.893, P = 0.001). Although there appears to have a significant decline in levels at Week 4, this eventually increased to Week 0 levels in all groups by Week 8. Thus it is unlikely that TCM-D™ causes any significant changes in blood urea levels in the doses used in diabetic mice.

Table 6. Creatinine level in diabetic Balb/c mice treated with oral TCM-D™

Treatment Group*	Mean ± SE Creatinine (mg/dl) Treatment Week		
	0	4	8
Control Group	1.10 ± 0.45	0.56 ± 0.14	0.76 ± 0.44
0.3 ml/100g (Group 1)	1.22 ± 0.26[§]	1.27 ± 0.32	0.96 ± 0.22[#]
1.5 ml/100g (Group 2)	0.89 ± 0.12[§]	2.53 ± 0.50[a, 1]	2.54 ± 0.33[#, b, 2]

**10 animals per group unless indicated otherwise; §9 animals; #8 animals. [a]Significantly higher than Control Group 1 (P < 0.001) and Group 1 (P = 0.017) at Week 4; [b]Significantly higher than Control Group (P = 0.002) and Group 1 (P = 0.007) at Week 8; [1]Significantly higher than at Week 0 (P = 0.011); [2]Significantly higher than at Week 0 (P = 0.007).*

The creatinine levels were not significantly different between groups at Week 0 but there were significant differences in levels between groups at Week 4 (F = 8.047, P = 0.002) and at Week 8 (F = 7.006, P = 0.004). The highest increase was seen in Group 2 and these levels were significantly higher than those of the Control Group and Group 1 animals at Week 4 and Week 8 respectively. Thus it is concluded that there is a significant increase in creatinine levels in diabetic mice treated with the higher dose of TCM-D in the present study.

Discussion

The present study was to determine the possible anti-diabetic effect of TCM-D™ at 0.3 ml/100 g

and 1.5 ml/100 g in mice which were experimentally induced with Type 1 diabetes mellitus through i.p. injections of streptozotocin. It is well known that streptozotocin (2-deoxy-2-(3-methyl-3-nitrosourea)1-d-glucopyranose)(α & β) induces permanent diabetes through the irreversible destruction of pancreatic β-cells if nicotinamide is not used to prevent the total destruction of islet cells.[9] Thus this study was designed to see if the extract had any effect on experimentally induced diabetes where insulin secretion was deficient or absent through chemical ablation of the islet cells.

At the doses studied the compound did not show any significant lowering of the glucose levels in Type 1 diabetic mice. Thus it can be concluded that in the absence or minimal residual insulin secretion, TCM-D™ was not able to lower the glucose level at the doses tested.

There were significant increases in the ALT and creatinine levels which were most likely due to the treatment with the compound. There did not seem to be any changes in the AST and blood urea levels due to the treatment. There was no significant effect on the weight of the treated animals due to the treatment. Further work is needed to determine if TCM-D™ has any effect in Type 2 diabetes mellitus.

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