Blood glucose level and other biochemical changes induced in normal mice by oral Traditional Chinese Medicine complex for diabetes (TCM-D™)

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Background: A number of Traditional Chinese Medicine (TCM) preparations are being used for the treatment of diabetes mellitus. Some components of these preparations have biochemical effects other than those of lowering blood glucose and indeed have been used for other medical indications in traditional practice. The primary objective of the study was to determine the effect of the oral mixture of Traditional Chinese Medicine for diabetes (TCM-D™ complex) on blood glucose level and the biochemical changes if any, on the liver (ALT, AST, gamma-GT, albumin, globulin) and renal (blood creatinine, urea) functions in normal mice. The oral mixture is an aqueous extract of four well-known traditional Chinese medicinal herbs and consists of *Trichosanthes kirilowii* Maxim., *Paeonia lactiflora* Pall., *Glycyrrhiza uranlensis* Fisch., and *Panax ginseng* (red) CA Meyer in the proportion of 36%, 28%, 18%, and 18% respectively of the dry weight. These herbs have been shown to have blood glucose lowering activity and have been used for other traditional medicinal purposes. The safety of the combination was evaluated in the present study.

Methods: Experimental Balb/c mice were treated orally via gastric tube with the extract at daily doses equivalent to 1 and 10 times the recommended human dose for 8 weeks. Blood glucose and other biochemical profiles were monitored at pre-treatment and monthly post-treatment until killed.

Results: When compared to pre-treatment levels, the blood glucose levels were significantly lower in treated animals compared to those in the control group. At the recommended TCM-D™ dose the levels in treated animals were significantly lower than that of control animals and at pre-treatment. When compared with pre-treatment, the glucose levels were lowest at Week 8 of treatment, the mean levels being 111.23%, 83.32% and 70.33% in control, and in animals given 1 x and 10 x the recommended TCM-D™ dosage respectively. The blood glucose lowering effect was also associated with a significant weight loss in treated animals. There were transient increases in AST and ALT levels but these reverted to normal at Week 8 of treatment. The levels of bilirubin, γ-GT, albumin, creatinine and blood urea were also not significantly different at Week 8 from pre-treatment levels in all groups.

Conclusion: Even at 10 times the dosage recommended for humans, TCM-D™ did not affect the liver and renal functions of treated animals. Treated and control animals remained healthy and normal throughout the period of observation.

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Key words: ***Trichosanthes kirilowii*** *Maxim.,* ***Paeonia lactiflora*** *Pall.,* ***Glycyrrhiza uranlensis*** *Fisch., and* ***Panax ginseng*** *(red) CA Meyer, blood glucose lowering, weight loss, biochemical changes.*

Introduction

A number of Traditional Chinese Medicine (TCM) preparations are being used for the treatment of diabetes mellitus. Some components of these preparations have biochemical effects other than those of lowering blood glucose and indeed have been used for other medical indications in traditional practice.

The non-dialyzable portion of the water extract of the roots of *Trichosanthes kirilowii* was shown to reduce the plasma glucose level in mice.[1] Five glycans termed trichosan A, B, C, D and E obtained through fractionation of this portion showed hypoglycaemic activity in normal mice and trichosan A was also active in alloxan-induced hyperglycaemic mice. The leaf extracts of another species, *Trichosanthes dioica* (pointed gourd), used in Ayurvedic Medicine for diabetes treatment, have also been shown to lower the blood glucose level in normal rats and in experimentally induced diabetic rats.[2] The extracts of *T. dioica* fruits also reduced the blood glucose levels in streptozotocin induced diabetic rats.[3] Triterpenoids isolated from seeds of *T. kirilowii* inhibited Epstein-Barr virus early antigen activation and karounidiol (the most prominent triterpenoid) showed

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cytotoxic activity against various human cancer cell lines especially that of renal cancer cell lines.[4] Trichokirin, a basic glycoprotein from the seeds of *T. kirilowii* showed a strong ribosome-inactivating activity and is a selective immunotoxin against leukaemia cells expressing Thy 1.2 antigen.[5] Trichosanthin, another protein of this plant, activates complement via the alternative pathway leading to induction of neutrophil accumulation.[6] The seeds also produce a lectin which is a glycoprotein of 57kDa, and which is specific for galactose, and not mitogenic to human lymphocytes.[7]

Triterpenoids and flavonoids have been found in the roots and aerial parts of *Paeonia lactiflora*.[8] Resveratrol (*trans*-3,4',5-trihydroxystilbene) and its oligomers have been isolated from the seeds of *P. lactiflora*. This was shown to have cytotoxic properties against various cancer cell lines and anti-mutagenic effects.[9] These stilbenes were also shown to have anti-oxidative properties.[10] The glucosides (paeoniflorin and 8-debenzoylpaeoniflorin) from the root of *P. lactiflora* were shown to produce a significant blood sugar lowering effect in streptozotocin-treated rats, the maximum effect being observed at 25 minutes post-treatment.[11] As with trichosan, the hypoglycaemic activity was also induced in normoglycaemic rats at a dose of 1 mg/kg. The antihyperglycaemic effect of paeoniflorin was insulin-independent, probably due to increased glucose utilization, and was greater than that of 8-debenzoylpaeoniflorin.

Phenolic compounds and flavonoid glycosides have been isolated from licorice, the roots and rhizomes of *Glycyrrhiza uralensis*.[12,13] Raw and roasted *G. uralensis* extracts improved impaired glucose tolerance possibly through enhancing insulin sensitivity.[14] In addition, it was reported that the extracts from roasted roots and rhizomes improved insulin secretion in the mice islets; probably due to an increase in glycyrrhetinic acid in the roasted roots. Phenolic compounds including glycyrin in ethanol extracts of *G. uralensis* showed significant PPAR-γ ligand-binding activity and reduced blood glucose levels in genetically diabetic mice.[15] The methanol extracts also showed anti-oxidant activity.[16]

The ginsenosides from the root of *P. ginseng* have a wide range of pharmacological activity including significant cardiotonic and hypertensive effects in circulatory failure after blood loss, lowering of blood sugar, and promotes phagocytosis and lymphocyte blastogenesis. Water extracts of ginseng with and without heat processing improved the diabetes associated pathological conditions and diabetic nephropathy in rats.[17] Ginsenoside Rh1 isolated from *P. ginseng* inhibited histamine release from rat peritoneal mast cells and IgE-induced passive cutaneous anaphylaxis in mice.[18] These authors attributed the anti-allergic action through its cell membrane-stabilising and anti-inflammatory activities. Extracts of ginseng berry were also found to reduce the fasting blood glucose of diabetic obese mice to normal levels and significant weight loss. The fasting blood glucose levels in normal mice did not decrease significantly after treatment.[19]

The TCM-D™ Complex for diabetes (TCM-D™) produced commercially by REM Corporation Malaysia, is an aqueous extract derived from a mixture of *Trichosanthes kirilowii* Maxim., *Paeonia lactiflora* Pall., *Glycyrrhiza uranlensis* Fisch., and *Panax ginseng* (red) CA Meyer. Extracts from each of the four herbal sources have proven blood glucose lowering activities. The composition of the mixture per 330g dry weight is: *T. kirilowii* (36%), *P. lactiflora* (28%), *G. uralensis* (18%), and *P. ginseng* (18%). The final volume of the extract from the mixture is 2800 ml. The dosage 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. There are plans to incorporate TCM-D™ in health drinks in the form of aqueous juices.

Although it has been reported that the individual herbs and their active components used are safe for use, not much experimental data are available to define the safety and toxicity when they are used in combination.

Thus the present study was carried out to determine the biochemical changes if any, that are induced by this extract used in traditional Chinese medicine to lower blood glucose, in normal mice. This study forms 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 Research and Ethics Committee.

Materials and Methods

Animals

Balb/c mice (4-6 weeks old) were obtained from the Animal Resources Unit, Institute for Medical Research, Kuala Lumpur. They were maintained in controlled environment, given normal laboratory pellet, and water *ad libitum*, as per guidelines in use at the Institute.

TCM-D Complex

The main components of TCM-D™ Complex are aqueous extracts of *Trichosanthes kirilowii* (root), *Paeonia lactiflora*, *Glycyrrhiza uralensis* and *Panax ginseng* (Red). Sodium benzoate (300 ppm) was added as preservative. The preparation of the extract was carried out in the REM Corporation Malaysia Laboratory with Good Manufacturing Practice facilities.

The composite mixture (total dry weight of 330g) consisting of *T. kirilowii* (36%), *P. lactiflora* (28%), G. *uralensis* (18%), and *P. ginseng* 18%) was shredded, and added to 1200 ml of 40% ethanol at 70°C to 80°C for 2 hours. The extraction procedure was repeated thrice to yield a total volume of 2800 ml. The extract was then concentrated to a final volume of 360 ml. This aqueous TCM-D™ Complex was further concentrated 10x and used for the experimental study. A similar diluent used for preparation of the 10x concentrate of the TCM-D™ Complex was used in control animals.

Animal Study Groups

Thirty healthy Balb/c mice were randomly assigned to the experimental and control groups of 10 mice each as follows:

(a) Group 1: treated with the TCM-D™ Complex (10x concentrate) at an oral single daily dose of 3 ml/kg or 0.3 ml/100 g (diluted to 1 ml with diluent) x 8 weeks.

(b) Group 2: treated with the TCM-D™ Complex (10x concentrate) at an oral daily dose of 30 ml/kg or 3 ml/100 g (diluted to 1 ml with diluent) x 8 weeks.

(c) Group 3 (Control): given a single oral daily dose of 1 ml drug diluent x 8 weeks.

All TCM-D™ Complex or diluent were given by gastric tube and mice fed on standard laboratory pellet and given water *ad libitum*. The general condition of the mice was monitored at daily intervals, throughout the period of observation. The following biochemical parameters were monitored pre-treatment and at monthly intervals after start of treatment until the end of the observation period: (a) Blood glucose, (b) Liver function tests (AST, ALT, bilirubin, albumin), (c) Renal function (creatinine, blood urea). Animals were sacrificed 8 weeks after start of treatment using an overdose of ether and at autopsy gross abnormalities were looked for. 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 and a P level < 0.05 was considered significant. Further comparisons using paired t-tests were carried out if ANOVA results showed significant difference among group means.

Results

Biochemical profile

The various biochemical profiles at pre-treatment and at monthly intervals during the period of treatment with oral TCM-D™ were monitored until the animals were sacrificed at the end of 8 weeks treatment. The blood glucose levels taken at the same time of the day during the period of observation are as shown in Table 1.

Table 1: Mean ± SE blood glucose (mg/dl) levels in normal Balb/c mice treated with oral TCM-D™ Complex (10x concentrate) daily until killed at 8 weeks

Treatment Group*	Treatment Week		
	0	4	8
Control Group	77.91 ± 3.76	108.79 ± 2.20[a]	86.66 ± 5.55[b]
0.3 ml/100 g (Group 1)	111.42 ± 4.36[1,2]	84.11 ± 4.47[c,4,5]	92.83 ± 2.84[d]
3.0 ml/100 g (Group 2)	98.58 ± 3.94[3]	94.34 ± 2.33[6]	69.33 ± 6.96[#,e,f,7,8]

**10 animals per group unless indicated otherwise; #8 animals; [a]Significantly higher than at Week 0 (P <0.001); [b]Significantly lower than at Week 4 (P = 0.007); [c]Significantly lower than at Week 0 (P = 0.002); [d]Significantly lower than at Week 0 (P = 0.005); [e]Significantly lower than at Week 0 (P < 0.001); [f]Significantly lower than at Week 4 (P = 0.016); [1]Significantly higher than Control Group (P < 0.001); [2]Significantly higher than Group 2 (P = 0.032); [3]Significantly higher than Control Group (P = 0.001); [4]Significantly lower than Control Group (P < 0.001); [5]Significantly lower than Group 2 (P = 0.31); [6]Significantly lower than Control Group (P = 0.003); [7]Significantly lower than Control Group (P = 0.029); [8]Significantly lower than Group 1 (P = 0.004)*

The aspartate aminotransaminase (AST) levels during the period of observation are as shown in Table 2.

Table 2: Mean ± SE aspartate aminotransaminase (AST) (u/l) levels in normal Balb/c mice treated with oral TCM-D™ Complex (10x concentrate) daily until killed at 8 weeks

Treatment Group*	Treatment Week		
	0	4	8
Control Group	20.17 ± 5.50	38.33 ± 9.34	84.99 ± 27.11[3,a]
0.3 ml/100 g (Group 1)	14.50 ± 4.66	72.83 ± 12.96[1,b]	28.66 ± 12.5[6]
3.0 ml/100 g (Group 2)	38.50 ± 17.93	89.16 ± 12.14[2,c]	27.29 ± 7.18[#]

**10 animals per group unless indicated otherwise; #8 animals; [1]Significantly higher than Control Group (P < 0.044); [2]Significantly higher than control group (P < 0.004); [3]Signifcantly higher than Group 1 (P < 0.039) and Group 2 (P < 0.045); [a]Significantly higher than at Week 0 (P = 0.029); [b]Significantly higher than at Week 0 (P = 0.001) and at Week 8 (P = 0.029); [c]Significantly higher than at Week 0 (P = 0.035) and at Week 8 (P = 0.009)*

The alanine aminotransaminase (ALT) levels during the period of observation are as shown in Table 3.

Table 3: Mean ± SE alanine aminotransaminase (ALT) (u/l) levels in normal Balb/c mice treated with oral TCM-D™ Complex (10x concentrate) daily until killed at 8 weeks

Treatment Group*	Treatment Week		
	0	4	8
Control Group	66.99 ± 13.96	19.67 ± 9.24[a]	60.83 ± 18.58[4]
0.3 ml/100 g (Group 1)	17.83 ± 3.77[1,2]	75.00 ± 22.19[3,b]	20.83 ± 4.50
3.0 ml/100 g (Group 2)	108.65 ± 24.04[c]	45.16 ± 10.53	43.33 ± 15.31[#]

**10 animals per group unless indicated otherwise; # 8 animals; [1]Significantly lower than Control Group (P = 0.041) and Group 2 (P < 0.001); [3]Significantly higher than Control Group (P = 0.015); [4]Significantly higher than Group 1 (P = 0.037); [a]Significantly lower than at Week 0 (P = 0.045); [b]Significantly higher than at Week 0 (P = 0.016) and at Week 8 (P = 0.045); [c]Significantly higher than at Week 4 (P = 0.048).*

The mean ± SE levels of bilirubin, albumin and γ-glutamyl transferase (γ-GT) levels at week 8 in the three groups are as shown in Table 4.

Table 4: Mean ± SE bilirubin, albumin and γ-glutamyl transferase (γ-GT) at week 8 in normal Balb/c mice treated with oral TCM-D™ Complex (10x concentrate) daily until killed at 8 weeks

Treatment Group*	Treatment Week 8		
	bilirubin (mg/dl)	albumin (g/l)	γ-GT (u/l)
Control Group	1.93 ± 0.38	50.28 ± 1.89	52.50 ± 9.99
0.3 ml/100 g (Group 1)	0.79 ± 0.15	46.39 ± 1.89	33.33 ± 6.31
3.0 ml/100 g (Group 2)	1.84 ± 0.34	47.84 ± 3.54	47.22 ± 11.09

**10 animals per group except for Group 2 with 8 animals*

The creatinine (mg/dl) levels during the period of observation are as shown in Table 5.

Table 5: Mean ± SE creatinine (mg/dl) levels in normal Balb/c mice treated with oral TCM-D™ Complex (10x concentrate) daily until killed at 8 weeks

Treatment Group*	Treatment Week		
	0	4	8
Control Group	0.51 ± 0.09	1.34 ± 0.76	0.23 ± 0.05[a]
0.3 ml/100 g (Group 1)	0.06 ± 0.01[1]	0.32 ± 0.08[b]	1.87 ± 0.97
3.0 ml/100 g (Group 2)	0.41 ± 0.15	0.60 ± 0.12	0.34 ± 0.20[#]

**10 animals per group unless indicated otherwise; #8 animals; [1]Significantly lower than Control Group (P = 0.003) and Group 2 (P = 0.017); [a]Significantly lower than at Week 0 (P = 0.021); [b]Significantly higher than at Week 0 (P = 0.006)*

The blood urea (mg/dl) levels during the period of observation are as shown in Table 6.

Table 6: Mean ± SE blood urea (mg/dl) levels in normal Balb/c mice treated with oral TCM-D™ Complex (10x concentrate) daily until killed at 8 weeks

Treatment Group*	Treatment Week		
	0	4	8
Control Group	24.40 ± 0.71[1,a]	32.13 ± 1.63[2]	45.22 ± 1.97[b]
0.3 ml/100 g (Group 1)	30.23 ± 0.58[c]	27.37 ± 0.94	33.52 ± 3.38
3.0 ml/100 g (Group 2)	29.02 ± 1.88	29.87 ± 0.87	38.24 ± 8.08[#]

**10 animals per group unless indicated otherwise; #8 animals; [1]Significantly lower than Group 1 (P = 0.002) and Group 2 (P = 0.012); [2]Significantly higher than Group 1 (P = 0.009); [a]Significantly lower than at Week 4 (P = 0.002) and at Week 8 (P < 0.001); [b]Significantly higher than at Week 4 (P = 0.002); [c]Significantly higher than at Week 4 (P = 0.034)*

The body weight of animals was monitored at monthly intervals throughout the period of observation (Table 7).

Table 7: Mean ± SE body weight (g) in normal Balb/c mice treated with oral TCM-D™ Complex (10x concentrate) daily until killed at 8 weeks

Treatment Group*	Treatment Week		
	0	4	8
Control Group	20.80 ± 0.25	20.26 ± 0.39	19.22 ± 0.36[2]
0.3 ml/100 g (Group 1)	20.80 ± 0.25	19.76 ± 0.32	16.36 ± 0.47[3,a]
3.0 ml/100 g (Group 2)	20.80 ± 1.88	18.89 ± 0.16[1]	14.80 ± 0.61[#,b]

**10 animals per group unless indicated otherwise; #8 animals; [1]Significantly lower than that of Control Group (P = 0.004); [2]Significantly higher than that of Group 1 and Group 2 (P < 0.001); [3]Significantly higher than that of Group 2 (P = 0.032); [a]Significantly lower than that Week 0 and Week 4 (P < 0.001); [b]Significantly lower than that at Week 0 and Week 4 (P < 0.001)*

Discussion

As seen from Table 1 there is a wide fluctuation in the 'normal' pre-treatment blood glucose levels in Balb/c mice, the mean levels in the three groups ranging from 77.91 to 111.42 mg/dl. It would therefore be more appropriate if comparisons be made within groups. In the Control Group, the blood glucose levels at 4 and 8 weeks were higher than that at Week 0.

The experimental Groups were treated with the recommended human dosage (Group 1) or ten times the daily dose (Group 2). In Group 1 (treated with 0.3 ml/100g of TCM-D™ daily for 8 weeks), the blood glucose levels at 4 and 8 weeks of treatment were significantly lower than that at pre-treatment. At 4 weeks the mean reduction compared to pre-treatment was 27.31 ± 6.22 (95% C.I. 13.25, 41.38) mg/dl. At 8 weeks the mean reduction from pre-treatment was 18.59 ± 5.02 (95% C.I. 7.23, 29.95) mg/dl. However, the level at Week 8 was not significantly lower than that of controls. Similarly, in Group 2, treated with 3 ml/100g of TCM-D daily for 8 weeks, or 10x that of the recommended human dose, the level at Week 8 of treatment was significantly lower than that at Week

0 and Week 4. The mean reduction compared to pre-treatment was 27.93 ± 4.56 (95% C.I. 17.14, 38.72) mg/dl. Compared to week 4, the reduction was 25.97 ± 23.31 (95% C.I. 6.48, 45.46) mg/dl. It is also interesting to note that the lowest mean level was achieved in this group at Week 8 of treatment and which was significantly lower than that of Control and Group 1 animals.

It is seen that with the recommended human dosage, the blood glucose lowering effect though present is not very consistent when compared to that of untreated control animals.

The significant decline in blood glucose level in Group 2 animals given 10x the recommended dose of TCM-D™ is in line with previous report with *T. kirilowii*[1], *P. lactiflora*[11], and *P. ginseng*[17]. The combination of these three herbal compounds with G. *uralensis* into the TCM-D™ also reduced the blood glucose level significantly at Weeks 4 and 8 after treatment, when compared to pre-treatment levels and with the control animals. The lowest glucose levels were achieved at Week 8 in animals given 10 times the recommended dosage.

The liver function assays also showed some fluctuation in 'normal levels' of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) as seen in Tables 2 and 3. Although the AST levels at Week 4 in both the treated groups were significantly higher than that at pre-treatment, these levels had returned to levels even lower than that of the Control Group by Week 8 (Table 2). The AST level in the Control Group at Week 8 was significantly higher than that at pre-treatment.

The ALT levels showed a similar picture to that of the AST levels (Table 3). The levels at Week 8 of treatment in the treated groups were not significantly different from those at pre-treatment and were lower than those of the Control Group.

The mean levels of bilirubin, albumin and γ-glutamyl transferase (γ-GT) at 8 weeks in the treated groups were lower than that of the Control Group (Table 4). There were no significant changes in these levels at week 0 and week 4 of treatment (data not shown).

The renal function assays for creatinine and blood urea are as shown in Tables 5 and 6 respectively. There is a large variability in the mean creatinine levels among groups (Table 5). Thus a comparison at different weeks within groups showed no significant changes in the levels in treated groups when compared to pre-treatment levels. Similarly, there was no significant change in blood urea levels at Week 4 and Week 8 of treatment when compared to the pre-treatment levels (Table 6).

A surprising finding was when the mean weight of treated animals was compared to that of control animals. Animals in the treated groups lost weight progressively and were significantly less in body weight when compared to animals in the Control Group (Table 7). In fact the mean weight in Group 2 animals was significantly less than that of animals in Group 1.

Summary

The changes in biochemical profile in Balb/c mice treated orally with TCM-D™ complex daily over a period of 8 weeks were investigated. When compared to pre-treatment levels, the blood glucose levels were significantly lower in treated animals than in the control group. The glucose levels were lowest in animals given 10 times the recommended human dosage at Week 8 of treatment. There was a transient increase in AST and ALT levels but these reverted to normal levels at Week 8 of treatment. The levels of bilirubin, γ-GT, and albumin were not significantly different in treated and control animals at Week 8 of treatment. The creatinine and blood urea levels were also not significantly different at Week 8 from pre-treatment levels in all treatment groups. Treated animals were significantly lower in weight compared to control animals at Week 8 of treatment.

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