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Oral Hypoglycaemic Activity of Three Ayurvedic Drug Formulations Marketed in Sri Lanka for Diabetes

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Abstract

Most Ayurvedic drug formulations marketed in Sri Lanka for the treatment of diabetes mellitus have not been scientifically evaluated and their therapeutic efficacy remains unknown. The effect of three such formulations, 'Madumeha Harani' syrup, 'Dilini Madumeha Suwaya' syrup, and B' Kapu' tablet, on the fasting blood glucose levels of rats, was investigated. The efficacy of these preparations was evaluated by comparing with a reference hypoglycaemic drug, tolbutamide. The serum glucose level of both 'control' and 'treated' rats was determined by the glucose hexokinase UV procedure, immediately prior to dose administration, and hourly intervals, for 4 h post administration. A significant ($P<0.05$) reduction in the fasting blood glucose level of rats was observed in the first 3 h post administration for 'Madumeha Haranai' and during the last 3 h, for 'Dilini Madumaha Suwaya'. Madumeha Harani' and 'Dilini Madumeha Suwaya' syrups exhibited 49.9% and 57.6% of oral hypoglycaemic activity of tolbutamide, respectively, and thus merit further investigation on their mode of action.

Keywords : diabetes, hypoglycaemic activity, Ayurveda, Madumeha, B-Kapu, tolbutamide, fasting blood glucose.

1. Introduction

Non Insulin Dependent Diabetes Mellitus (NIDDM) is a major public health problem on global scale effecting both the developed world and developing countries. It is expected that there would be about 65 million people affected with diabetes mellitus by the year 2000 (1). The prevalence of diabetes mellitus in the general population is greater than 6% (2) and it is claimed that there is a high prevalence (5.1%) of NIDDBM in Sri Lanka too (3,4).

In Sri Lanka, especially in the rural areas, Ayurvedic medicine had been prescribed for diabetes since ancient times and approximately 40 plant species have been used in such treatment (5). While a majority of these plant species have not been subjected to any scientific investigation (6), the hypoglycaemic activity of a few plant species such as *Salacia reticulata* (7,8), *Momordica charantia, Aegle marmelos* (7), *Ficus benghalensis* (9,10,11), *Bambusa vulgaris* (12), *Momordica dioica* (13) has already been established.

From recent times, many Ayurvedic drug companies in Sri Lanka produce commercial quantities of herbal preparations in different formulations either as syrups, tablets, capsules or powders, Many of these preparations are commercially available in the market as anti-diabetic agents and can be obtained without any prescription. Some of these are the syrups, 'Madumeha Harani', 'Dilini Madumeha Suwaya', Madmeha panaya' and Kothlahimbutu panaya' the tablet 'B-kapu' and the capsule 'Karvila' Although the principal constituents of these Ayurvedic herbal formulations are believed to be extracts of one or many of the reputed plants with oral hypoglycaemic activity, in most cases, the ingredients remain a trade secret except in the Karavila capsule which is made from *Momordica charantia.* Moreover, inspite of their wide use, most of these formulations have not received any scientific evaluation for their therapeutic efficacy as anti-diabetic agent, except for a powder marketed by William Grinding Mills, Dehiwala, Sri Lanka.(14)

The aim of this investigation was to analyse the effect of three commercially available Ayurvedic drug formulations; 'Madumeha Harani' (MH), 'Dilini Madumeha Suwaya' (DMS) and 'B-Kapu' (BK), on the fasting blood glucose levels of rats. The oral hypoglycemic effect of these formulations was also evaluated with respect to tolbutamide (TB) a standard hypoglycaemic drug, in tablet form (15).

2. Materials and methods

Three Ayurvedic drug formulations, MH, DMS, and BK were purchased from two Ayurvedic drug stores in Colombo, Sri Lanka. The three drugs are each produced and marketed by the State owned Sri Lanka Ayurvedic Drug Corporation. Nawinna, Ratna Labortory, Malabe, and Sugatha Laboratory, Horana, Sri Lanka respectively. TB (Hoechst Marian Rousel Ltd., Mumbai, India) was purchased from a pharmacy in Colombo.

Healthy, adult, male, Sprague-Dawley rats of body weight 250 ± 25g were used in all experiments. They have been bred in the animal house of the Department of Zoology of the University of Colombo and were kept in wire meshed cages under standardised animal house conditions (temperature; 28-31^{0}C, photoperiod; approximately 12 hours natural light per day, humidity; 50-55%). All rats were fed

with commercial food (Oil & Fats Co. Ltd., Seeduwa, Sri Lanka) with free access to tap water.

Rats were fasted overnight for 14-16 hours (MH n=24, DMS n=20 and TB n=20) before commencement of all experiments. Blood samples (0.1 ml) were withdrawn from each rat into 1.5 ml microcentrifuge tubes to determine fasting blood glucose levels. The rats were then randomly divided into two, the control' group and the 'treated group. The surface area ratio chart was used to calculate the daily human equivalent dosage of all drug formulations for a rat of body weight 250g. Accordingly, treated groups were given 2.00 ml of MH syrup, 0.8 ml of DMS syrup, 0.11g of BK tabelts dissolved in 3.00 ml of 0.05 M NaoH. and 0.06g of TB tabelts dissolved in 3.00 ml of 0.05 M NaoH. The control group of HM and DMS were given 2.00 ml and 0.8 ml of distilled water, respectively, and the control groups of BK and TB were given 3.00 ml of 0.05 M NaOH. All the drugs and control solutions were administered orally via a stomach tube. Blood samples (0.1 ml) were withdrawn under aseptic conditions post administration at hourly intervals for 4 h from both groups to determine the blood glucose levels. All blood samples were withdrawn from the cut tail vein, under light diethyl ether anesthesia.

The blood samples collected in microcentrifugs tubes were allowed to clot at room temperature and the serum was separated within half an hour by centrifugation (Eppendorf 5415C, Eppendorf-Netheler-Hinz GmbH, Germany) at 10,000 rpm for 1 minute. All sera were stored at - 20°C and the serum glucose levels were determined within 48 hours by the glucose hexokinase/UV procedure (16) using a commercially available kit (DMA, Arlington, Texas, USA). Briefly, 5µl each of serum and the glucose standard (200 mg/dl) were added separately to 1 ml of Glucose Hexokinase reagent and the samples were incubated at 37°C for 3 minutes. The absorbance of each sample was measured at 340nm using an UV spectrophotometer (Cecil CE2040, Cecil Instrument Limited Cambridge), to determine the glucose concentration of serum samples.

Results are given as means ± SEM and area under the curves. Statistical comparisons were made by the Student's t-test using statistical software package MINITAB. Area under the curves was calculated by normal trapezium method.

3. Results

Post administration serum glucose levels are presented in Table 1. The blood glucose levels obtained post administration of control solutions of each experiment were not significantly different ($P<0.01$) form each other. Therefore these values were pooled. The effect on MH, DMS, BK and TB on fasting blood glucose levels of rats, its shown in Figure1. The effect of the drug formulations in depicted as a mean

percentage deviation in the blood glucose level from that of the fasting blood glucose level (%) at zero time (prior to dose administration).

Table 1. Blood glucose levels (mean ± SEM) of fasting rats, prior (0 h) and post administration (1-4h) of control solution (water of 0.05 M NaOH, n=42), MH (2.00ml, n=12),=DMS (0.81ml, n=10), BK ().11 g in 3.00 ml of 0.05 M NaOH, n=10) and TB (0.06 g in 3.00 ml of 0.05 M NaOH, n=10).

	Blood glucose concentration (mg/dl)				
	Fasting	Post administration			
	0 h	1 h	2 h	3 h	4 h
Control	88.7 ± 2.9	95.2 ± 1.9	91.9 ± 2.7	99.8 ± 2.2	97.8 ± 2.7
MH	88.8 ± 4.0	68.8 ± 4.7**	70.1 ± 5.2**	88.7 ± 6.2*	96.2 ± 5.6
DMS	96.4 ± 8.8	92.8 ± 5.2	73.7 ± 10.0*	84.5 ± 5.00**	85.3 ± 7.6*
BK	93.1 ± 5.7	98.7 ± 3.2	105.4 ± 6.2	107.2 ± 5.6	106.7 ± 5.4
TB	87.6 ± 3.8	69.0 ± 4.3**	70.9 ± 4.8**	70.5 ± 3.4**	69.6 ± 5.3**

As compared with controls : *P<0.05, **P<0.01 (Student's t-test, Statistical software package Minitab).

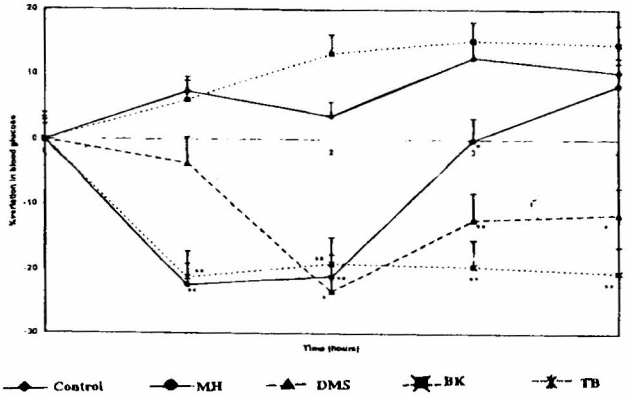

Figure. 1. Mean Percentage variation of blood glucose level following administration of the Control solution (water or 0.05 M NaOH, n=42), MH (2.00ml, n=12), DMS.

(0.80ml, n=10), BK (0.11)g in 3.00ml of 0.05 M NaOH, n=10) and TB (0.06 g in 3.00 ml of 0.05M NaOH, n=10)

In contrast, BK did not exhibit an oral hypoglycaemic activity under our experimental conditions. The oral administration of TB resulted in a sustained

reduction (approximately 20%, $P<0.01$) of the fasting blood glucose levels of rats throughout the 4h assay.

The oral hypohlycaemic activity of each Ayurvedic drug formulations was also evaluated with respect to the reference drug TB. This was performed by calculating the area under the curves (AUC) depicted if Figfure 1, and comparing with the values obtained for TB (Figure2). The AUC The oral administration of MH, compared to the control, showed a significant reduction in the fasting blood glucose levels during the 1h ($P<0.01$), 2 h ($P<0.001$) and the 3 h ($P<0.05$) of treatment. This hypoglycaemic effect was most prominent in the first two hours (21.4% and 19.7 reduction, respectively) of the 4 h assay.

DMS also exhibited a significant hypoglycaemic effect. This effec was observed during the 2 h ($P<0.05$), 3 h ($P<0.01$) and 4 h ($P<0.05$) of the assay. The maximum reduction of blood glucose level (26.2%) was observed during the second hour post administration.

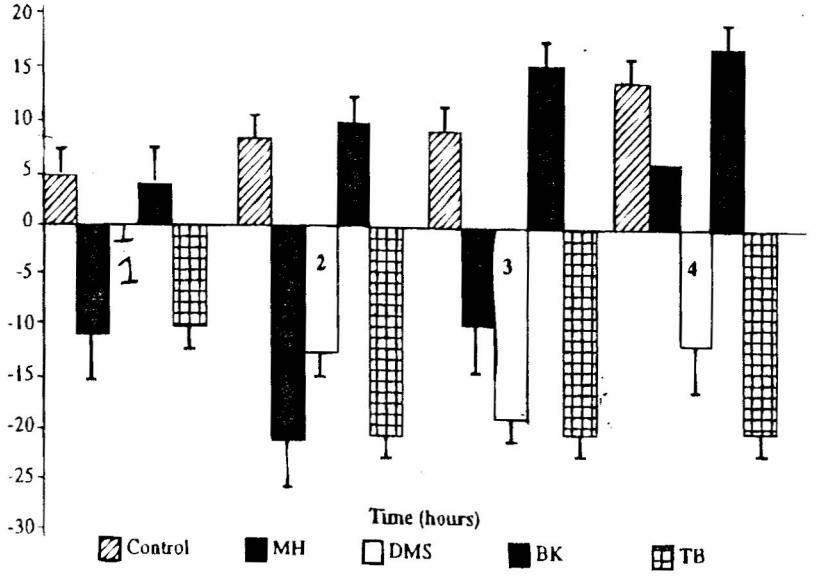

Figure 2. Area under the percentage variation curve for the Control Solution (water or 0.05 M Na oH, n=42), MH (2.00ml, n=12), DMS (0.80 ml, n=10), BK (0.11g in 3.00ml of 0.05 M NaOH, n=10) and TB (0.06 g in 3.00 ml of 0.05 M NaOH, n=10).

obtained in this study following the treatment with for the 4 h assay period is-69 .1. The AUC obtained for MH and DMS were -34.5 and -39.9 respectively. Hence, MH and DMS showed 49.9% and 57.6 of oral hypoglycaemic activity demonstrated by TB in the 4 h period tested. However, the efficacy of MH during the first 2 h of the assay was 5% higher than that of TB.

4. Discusion

Of the three Ayuredic drug formulations marketed in Sri Lanka (Madumeha Harani, Dilini Madumeha Suwaya and B-Kapu) for the treatment of diabetes mellitus only MH and DMS syrups possessed oral hypoglyceamic activity when tested on normoglycaemic fasted rats when the recommended dosages were administered. In our experimental setting the efficacy of these two herbal preparation was about 50% less than that of TB over the 4 h period tested. TB is sulphonylurea drug which is widely used clinically in the treatment of NIDDM (type 2 diabetes mellitus). The onset of the hypoglycaemic effect of MH (1h) and DMS (2h) was rapid and short-lived (MH up to 1st 3 h of 4 h study and DMS in the last 3 h of 4 h study). This is a desirable feature expected of a good drug used in the treatment of NIDDM as this would prevent the development of potentially serious cllinical hypoglycaemia (17). On the other hand, the hypoglycaemic action of the two herbal preparations was associated with peaks (maximum nadir at 1h with MH and 2 h with DMS) whilst TB had a rather uniform action through out the 4 h assay period with no hypoglycaemic peaks.

Extracts of some Sri Lankan plants such as *Salacia reticulata, Aegle mameleos, Momordica charantia* (7) and *Ficus bengalensis* (9) have shown greater hypoglycaemic action compared to MH and DMS syrups on normoglucaemic fasting rats over a 4 h study period. However, the efficacy of these plant extracts cannot be directly compared with that of the Ayurvedic drug formulations as neither the principal constituents nor their quantities are known. It is important to note that previous studies (7,9) have shown that the hypoglycaemic activity of plant extracts has decreased significantly within 72 h of storage at room temperature (29-30^{0}C). However, the two Ayurvedic syrups used in our study were stored at room temperature during the 3 months study period indicating that the oral hypoglycaemic activity is retained in both MH and DMS over a long priod.

Most of the previous work on hypoglycaemic effect have performed using glucose oxidase method to determine the blood glucose level (3,7,9,13,14). In contrast, in our study, DMA glucose Hexokinase UV procedure was used, which is based on a modification of the coupled enzyme method developed by Slein (16). This method has a higher specificity for glucose and to our knowledge we were the first to use DMA glucose Hexokinase UV method.

The hypoglycaemic mechanisms of MH and DMS are likely to be mediated through potentiation of insulin release and/or insulinotropic action: since the hyoglycaemic action had a rapid onset and a short-lived action in fasting rats more or less similar to TB. However, it does not preclude other potential extra pancreatic mechanisms.

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The Ayurvedic formulation in tablet form, BK tested in this study, did not reduce the fasting blood glucose level of rats under our experimental condition. However, as shown for *Momordica dioica* (13) and *Gynema sylvestre* (18), it is possible for a formulation not to reduce the pasting blood glucose level, but to possess a significant oral hypoglycaemci activity by improving the glucose utilisation in glucose tolerance test. Hence, a further investigation based on the glucose tolerance test is required to evaluate the hypoglycaemic activity of BK. The results of our study merit further investigation on MH and DMS to determine their mode of action and HPLC separation to find out the active constituents.

In conclusion, for the first time this study demonstrates potent oral hypoglycaemic activity on two syrups marketed for the treatment of diabetes mellitus in Sri Lanka.

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