

ORIGINAL RESEARCH ARTICLE

A Comparative Study of the Hypoglycaemic Potentiation of Metformin, by Different Preparations of *Momordica charantia* (Bitter Gourd), in Hyperglycaemic Lab RatsBhuvaneshwari S^{1*}, Rohanti Ravikulan², Bhuvaneshwari K³¹Professor, Department of Pharmacology, ²MBBS Student, ³Professor and HOD, Department of Pharmacology, PSG IMS & R, Peelamedu, Coimbatore, 641004, Tamilnadu, India

Received 24 Jun 2013; Revised 07 Oct 2013; Accepted 17 Oct 2013

ABSTRACT

This study was aimed to determine variation and effectiveness in the effect on blood glucose by different preparations of *M. charantia* in combination with Metformin (single dose), in diabetic rats; and variation in feeding habits with different preparations of *M. charantia*. Each preparation [raw juice (Prep A), decoction (Prep B) and raw juice with buttermilk (Prep C); strength of *M. charantia*: 100g/100ml], with Metformin (M), was administered to three groups of rats, at doses of 0.5, 1 and 2ml/100g body weight and Metformin alone to another group. The blood glucose was checked before and at intervals after administration. The food supply was weighed before and at 24 hours. At 4 hours, Preparation B + M and Preparation C + M lowered blood glucose significantly more than Metformin given alone. [Mean of % change in blood glucose: Control= 29.1; Prep B + M = 39.2(1ml), 42.5 (2mL); Prep C + M = 52.8 (0.5mL), 56.5 (1mL), 43.3 (2mL)]. At 24 hours, Prep B + and Prep C + had significantly lower blood glucose values than at 0 hours, compared to Metformin which showed an increase (Mean of % increase in blood glucose= 1.3). There was no variation in the food intake among the groups. *M. charantia* with Metformin showed greater reduction in blood glucose than with Metformin alone at 4-hours and 24 hours. Preparations C + M had the highest reduction. There was no change in feeding habits when *M. charantia* was added to Metformin.

Key words: *Momordica charantia*, preparation, Metformin, Rats.**INTRODUCTION**

The prevalence of Type II diabetes is increasing worldwide. Although several forms of treatment are available, patients are generally more inclined towards the use of natural foods in order to maintain their blood glucose levels [1]. Of these, the most popular source of herbal treatment used is *Momordica charantia*, also known as *bitter gourd*, *bitter melon*, *balsam pear* or *karela* [2].

For several years, the fruit has been consumed as a hypoglycaemic agent and referred to as the “vegetable insulin” in parts of Asia, Africa and South America [3]. Today, diabetic patients generally consume *M. charantia* in addition to prescribed oral hypoglycaemic drugs such as glibenclamide, tolbutamide, etc., of which the most common is Metformin [2]. Previous studies have proven that *M. charantia* acts synergistically and potentiates the effect of oral hypoglycaemic drugs such as Metformin, and causes a greater

reduction in blood glucose level when taken in combination, than when Metformin is taken alone [3]. The components in *M. charantia* believed to attribute to this action are charantin, polypeptide P and vicine [4]. *M. charantia* increases glucose utilisation in liver and cellular uptake [5].

However, not all diabetic patients consume the fruit in one form. The fruit has been known to be consumed as several preparations such as the fresh fruit, powdered forms, extracts, in combination with other foods, etc [6]. Among the different forms in which it may be taken, we narrowed the options down to the three most common preparations: raw fruit juice, cooked form and raw fruit juice mixed with buttermilk. Furthermore, there was no standard dose at which the fruit was consumed, and the amount of bitter gourd consumed varied from person to person without any relation to their body weight and other such

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parameters. This study was therefore required in order to determine the optimum preparation and in order to receive the maximum therapeutic effects of *Momordica charantia* in diabetic patients.

The results of this study may be used as a guide for clinical trials, in order to extend the benefits to humans.

MATERIALS AND METHODS

Nature of study:

The study was a comparative interventional study with single dose of *M. charantia* and Metformin. The study was conducted in the laboratory, the Department of Pharmacology, PSG IMS&R, Coimbatore.

Preparation technique of the three preparations of *M. charantia*:

Preparation A (Prep A) was prepared by cutting raw *M. charantia* into pieces and grinding them into a paste, which was then mixed with water at a dilution of 1kg *M. charantia*: 1L water. Preparation B (Prep B) was prepared by boiling pieces of raw *M. charantia* in water for 30 minutes and then grinding the pieces to a paste with water at a dilution of 1kg *M. charantia*: 1L water. Preparation C (Prep C) was prepared by grinding pieces of raw *M. charantia* to a paste, mixed with water and standard buttermilk, at a dilution of 1kg *M. charantia*: 500ml of buttermilk: 500ml of water. The standard buttermilk used for Preparation C was "Amul Masti" available at the local supermarkets.

Induction of Non-insulin Dependent Diabetes Mellitus:

Hyperglycaemia was induced by a single intraperitoneal (i.p) injection of streptozocin at a dose of 25mg/kg of body weight, in a citrate buffer (pH 4.5). The 0.1M citrate buffer was prepared by adding 50mL of distilled water to a volumetric flask and dissolving 1.4705g of sodium citrate. The correct pH was then achieved by required addition of citric acid. The buffer was refrigerated till the time of injection.

Animals:

A total of 24 male, Wistar albino rats were used in this study. All rats were more than 6 months of age and had a body mass between 150 and 350g.

Administration of doses:

The 24 rats were divided into four groups of 6 rats each. One group was allocated as the control group, which received a standard dose of Metformin (M) at 120mg/kg of body weight. A

500mg tablet of Metformin was dissolved in 10ml of water and the appropriate volume was administered orally to each rat using a syringe. The remaining three groups of rats each received one of the three preparations of *M. charantia* as well as the standard dose of Metformin received by the control group. The *M. charantia* preparation was orally administered at doses of 0.5ml, 1ml and 2ml/100g of body weight, with an interval of 3 days between each dose.

Monitoring blood glucose level:

The blood glucose levels were checked at 4 hours, 8 hours and 24 hours after the administration of the dose. A superficial incision was made on the tail of each rat and the drops of blood produced were tested using a digital glucometer, and the blood glucose level recorded.

Feeding habits:

The food supply was weighed before and 24 hours after the administration of each dose and recorded.

Ethics:

Study was conducted after Institutional Animal Ethics committee approval and have adhered to the guidelines of our institution regarding the care and use of laboratory animals.

Statistical Methods:

The data was expressed as % change in blood glucose levels from the time of administration of each dose. Analyses were carried out using SPSS for Windows, Version 19.0.1. To determine the variation in values between the groups that received preparations of *M. charantia* combined with Metformin, and the group that received Metformin alone, independent samples T-testing was used. The variation between the preparations of *M. charantia* at the same dose and the variation within the same group receiving one preparation of *M. charantia* at different doses were determined using one-way ANOVA. Statistical significance was accepted at $P < 0.05$.

RESULTS AND DISCUSSION

Change in blood glucose at 4 hours:

- All doses of all preparations of *M. charantia* show higher mean change in blood glucose values except for the 0.5ml doses of Preparation A + M and Preparation B + M. Preparation C + M at 1ml/100g shows the highest mean change in blood glucose (**Table 1**).
- There is a significant variation in the change in blood glucose at 2mL of preparation A + M, at 1mL of Preparation

B + M and at all three doses of preparation C + M compared to Metformin group (Table 2).

- There is a significant variation in the blood glucose values of the different preparations at doses of 0.5ml and 1ml (Table 3).
- Within the group, there is significant variation in the values among the different doses of Preparation B. There is no significant variation of values among the other two groups (Table 4).
- This proves the hypothesis that a minimum dose is required in order for the effect of *M. charantia* to exceed the blood glucose lowering capacity of Metformin.

Change in blood glucose at 8 hours:

- All doses of all preparation of *M. charantia* show higher change mean blood glucose values (Table 1).
- There is a significant variation in the blood glucose values of the three preparations of *M. charantia* at all doses when compared to that of the control group, except that of Preparation A + M at 0.5ml/100g (Table 5).
- There is a significant variation in the blood glucose values of the different preparations only at 1ml/100g (Table 3).
- Within the group, there is significant variation in the values among the different doses of Preparation B + M. (Table 4).
- This proves the theory that the combination of *M. charantia* with Metformin potentiates its action, thus there is sustained hypoglycaemia [3]

Change in blood glucose at 24 hour:

- All doses of all preparations of *M. charantia* show higher change mean blood glucose values except those of Preparation A + M at 1ml/100g and Preparation C + M at 1ml/100g (Table 1).
- There is no significant variation in the mean change in blood glucose values of Preparation A + M when compared to the control group. However, there is a significant variation at all three doses of Preparation B and 0.5ml and 2ml doses of Preparation C + M (Table 6).
- A statistical significance is seen in the values of change in blood glucose, between the three preparations of *M. charantia* at 0.5ml and 1ml/100g doses

(Table 3).

- There is significant variation seen among the mean change in blood glucose values of the three doses of Preparation B + M and C + M (Table 4).

.Food intake:

- The mean change in food intake values of all *M. charantia* preparations at all doses are equal to higher than that of the control group (Mean: 6g) (Table 1).
- There was no significant variation in the values of food intake of the three preparations of *M. charantia* in comparison to that of the control group.
- If the effect on food intake could only be observed after prolonged administration of *M. charantia* preparation, this effect could not have been observed in this study. Thus, further study is required in order to conclude whether an effect on food intake is present.

The results support the hypothesis that *M. charantia* combined with Metformin is more effective at lowering blood glucose levels than when Metformin is administered alone, which is consistent with the results from previous studies [1].

The highest mean reduction of blood glucose is shown by Preparation C + M at the 1ml dose, although according to the other values, the expected dose was 2 ml. This inconsistency in the values may be due to factors such as the inter-individual variations of the particular group.

The results of this study are fairly consistent with a few exceptions. The deviation from the expected values in certain cases may be due to the small sample size, which results in an exaggerated change in the average even if there are very few random errors. Thus this study may be used as a guide and may be conducted on a much larger sample size, which is likely to produce more consistent values.

In conclusion, there is a significant variation in the blood glucose lowering capacity of different preparations of *M. charantia* in combination with Metformin. Of these, the most effective preparation is Preparation C + M, which consists of raw *M. charantia* juice mixed with buttermilk and water, with the dose of 1ml/100g body weight being the most effective. At 4 hours, this preparation, at doses 0.5ml, 1ml and 2ml/100g body weight is more effective at lowering blood

glucose than the other two preparations of *M. charantia* at the same doses as well as Metformin administered alone. Preparation B + M is the next most effective and is more potent than Preparation A + M. At 4 hours, preparation A + M is only significantly more effective than Metformin at 2ml/100g. Significant sustained hypoglycaemic effect at 24 hours is seen only with Preparations B + M and C + M. There is no significant variation in the food intake values with the preparation of *M. charantia* with Metformin when compared to the control group. Further studies, involving

prolonged administration of the *M. charantia* preparations may determine whether a chronic effect exists on the feeding habit. This study may be conducted on a larger sample size in order to derive more consistent values.

The study may also be used as a model and extended to humans, by administering the most effective preparations of *M. charantia* at the different doses to patients on oral hypoglycaemics and compared to a control group of patients that are on oral hypoglycaemics alone.

Table 1: Mean % decrease in blood Glucose levels and change in food 24 hrs from the time to administration of each dose

	Metformin	Prep A + M			Prep B + M			Prep C + M		
	120 mg/kg	0.5 ml / 100 mg	1 ml / 100 mg	2 ml / 100mg	0.5 ml / 100 mg	1 ml / 100 mg	2 ml / 100mg	0.5 ml / 100 mg	1 ml / 100 mg	2ml / 100mg
Average weight(kgs)	0.31	0.32			0.32			0.30		
% Change										
4 Hrs	29	29	33	40	20	39	42	53	57	43
8 Hrs	-2	14	17	24	7	36	9	33	48	23
24 Hrs	-1	1	-3	3	3	31	7	29	-3	13
Change in food(g)	6	9	8	8	7	7	17	11	6	9

Table 2: Statistical significance of the blood glucose values (% change), at 4 hours, of each preparation of *M. charantia* when compared with those of the Metformin group

Dose	p-value		
	Prep A + M	Prep B + M	Prep C + M
0.5ml/100g	0.98	0.06	0.001
1ml/100g	0.6	0.03	.001
2ml/100g	0.03	0.13	0.005

Table 3: Statistical significance of the blood glucose values (% change), at 4 hours, of all three preparations of *M. charantia* at the same dose, compared to one another

Dose	p-value		
	4 hrs	8hrs	24hrs
0.5ml/100g	0.002	0.24	0.001
1ml/100g	0.001	0.001	0.002
2ml/100g	0.86	0.1	0.09

Table 4: Statistical significance of the blood glucose values (% change), at 4 hours, at three different doses of the same preparation of *M. charantia*

Dose	p-value		
	4 hrs	8hrs	24hrs
Prep A	0.31	0.54	0.816
Prep B	0.005	0.001	0.001
Prep C	0.12	0.095	0.001

Table 5: Statistical significance of the blood glucose values (% change), at 8 hours, of each preparation of *M. charantia* when compared with those of the Metformin group

Dose	p-value		
	Prep A + M	Prep B + M	Prep C + M
0.5ml/100g	0.1	0.001	0.017
1ml/100g	0.009	0.001	0.001
2ml/100g	0.001	0.001	0.001

Table 6: Statistical significance of the blood glucose values (% change), at 24 hours, of each preparation of *M. charantia* when compared with those of the Metformin group

Dose	p-value		
	Prep A + M	Prep B + M	Prep C + M
0.5ml/100g	0.55	0.004	0.001
1ml/100g	0.9	0.001	0.153
2ml/100g	0.11	0.004	0.007

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