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EXTRA-PANCREATIC EFFECTS OF *MOMORDICA CHARANTIA* IN RATS

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Summary

The extra pancreatic effects of the oral hypoglycaemic plant *Momordica charantia* have been investigated in rats. It was found that the fruit juice of this plant caused an increased glucose uptake by tissues in vitro without concomitant increase of tissue respiration. Oral treatment with the juice prior to a glucose load was found to increase the glycogen content of liver and muscle while it had no effect on the triglyceride content of adipose tissue. Pretreatment of fasted rats with *M. charantia* fruit juice had no significant effect on the gluconeogenic capacity of kidney slices. Similar results were obtained with kidney slices pre-incubated with *M. charantia* fruit juice.

Introduction

M. charantia of family Cucubitaceae is a common vegetable in Sri Lanka and other South East Asian countries. Traditional medical practitioners of these countries use the juice of the fruits of *M. charantia* to control diabetes (Bever and Zahnd, 1979). The results of recent in vivo studies (Karunanayake et al., 1984) in laboratory animals and experiments with diabetic patients (Welihinda et al., 1983) afforded evidence for the existence of oral hypoglycaemic activity in *M. charantia* that is traditionally cultivated and used in the treatment of diabetes in Sri Lanka (Jayaweera, 1981). Further, it was evident, from the results of experiments with isolated pancreatic islets of obese hyperglycaemic mice (Welihinda et al., 1982), that *M. charantia* was capable of stimulating insulin release in vitro.

The present studies were undertaken to investigate a possible extrapan-

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creatic action of *M. charantia* which may contribute towards its observed hypoglycaemic action. Thus the effects of the fruit juice of *M. charantia* on (a) the glucose uptake by tissue, (b) the tissue respiration, (c) the glycogen synthesis in liver and muscle tissue, (d) the triglyceride synthesis in adipose tissue and (e) gluconeogenesis were investigated.

MATERIALS AND METHODS

Experimental animals

In all experiments male Sprague—Dawley rats of body weight 200 ± 25 g, maintained on a standard laboratory diet, were used. The animals were fasted overnight before commencement of experiments in the instances where they were to be used for glycogen and triglyceride synthesis studies. For studies on gluconeogenesis, the animals were fasted for 48 h.

Preparation of M. charantia

Fresh fruits were taken and the seeds removed. The fleshy parts were cut into thin slices and mascerated in a mechanical grinder. The pulp was squeezed through a muslin cloth and the clear juice after centrifugation was taken for administration.

Dosages and administration

M. charantia juice (1 ml/100 g body wt) for the test animals, distilled water (1 ml/100 g body wt) for the control animals and glucose (1 ml/100 g body wt, 50% w/v) were separately administered via a stomach tube while the animals were under light diethyl ether anaesthesia.

Effect of M. charantia on tissue respiration

Animals were killed by decapitation and their diaphragm muscle was harvested for study. The respiratory activity of the tissue was estimated by manometry using the Warburg's Manometric apparatus (Umbreit et al., 1957; Beloff-Chain and Rookledge, 1968). The tissue (150 mg) was suspended in Krebs Henseleit Ringer phosphate buffer (2 ml, pH 7.4) (Long 1962), containing 20 mM glucose. After pre-incubation (40 min at 37°C and 100 strokes/min in a Warburg's apparatus) *M. charantia* (0.3 ml) supplemented with 20 mM glucose was tipped from the side arm into the main flask and incubated (40 min at 37°C and 100 strokes/min). In identical control experiments 20 mM glucose solution (0.3 ml) was tipped instead of *M. charantia*. The rate of oxygen uptake was calculated according to the method of Umbreit et al. (1957).

Effect of M. charantia on glucose uptake

For these studies animals were killed by decapitation and their diaphragm muscle tissue (150 mg) was pre-incubated in a Warburg apparatus (10 min at 37°C and 100 strokes/min) in Krebs-Henseleit Ringer phosphate buffer

(2 ml, pH 7.4) supplemented with 20 mM glucose. *M. charantia* juice (0.3 ml) supplemented with 20 mM glucose was then tipped from the side arm into the main Warburg flask. In identical control experiments, 20 mM glucose solution (0.3 ml) was tipped instead of *M. charantia*. After incubation (60 min at 37°C and 100 strokes/min) the tissues were removed for weighing and the glucose content in the incubation medium was estimated by the glucose oxidase method of Hugget and Nixon (1957).

Effect of M. charantia on glycogen and triglyceride synthesis

In these studies the animals were randomly divided into two groups. The first group (test) was given *M. charantia* juice (1 ml/100 g body wt) 30 min prior to receiving the oral glucose load (1 ml/100 g body wt; 50% w/v). The second group (control) received distilled water (1 ml/100 g body wt) instead of *M. charantia* juice. The animals were killed 90 min after glucose loading (i.e. 120 min after administration of *M. charantia*) by decapitation and their livers, muscle and adipose tissue were harvested. The decision to kill the animals 120 min after *M. charantia* administration was based on our previous observation (Karunanayake et al., 1984) that the maximum hypoglycaemic effect occurred approximately 120 min after administration of the plant juice. The glycogen content of the liver and muscle was estimated according to the micromethod of Kemp and Van Heijningen (1954) after elimination of free glucose in the tissue by extraction into methanol (80%, v/v). The triglyceride content was estimated according to the colorimetric method based on the Hantzsch reaction (Varley et al., 1980).

Effect of oral administration of M. charantia on gluconeogenesis

Two groups of rats were fasted for 48 h. One group (test) was given *M. charantia* juice (1 ml/100 g body wt.) and the second group (control) was given equivalent dose of distilled water. The animals were killed 120 min later and their kidneys were harvested for estimation of gluconeogenesis according to the method of Tutwiler and Brentzel (1980). The kidneys were rinsed in cold saline, sliced and cut into small pieces of 30—40 mg. After rinsing in well gassed (O_2 + CO_2, 95 : 5) cold Krebs Ringer bicarbonate medium (pH 7.4) the test and control kidney tissues were individually pre-incubated (in two groups, test and control) in the same medium (4 ml) for 25 min at 37°C with constant shaking. The tissues were then incubated (in two groups as before) in well aerated Krebs Ringer bicarbonate medium (4 ml, supplemented with 0.01 M pyruvate) for 60 min at 37°C with constant shaking. After incubation, the medium was sucked off for glucose estimation by the modified glucose oxidase method of Krebs et al. (1963). The tissues were dried (110°C) overnight and weighed.

Effect of pretreatment with M. charantia juice on the gluconeogenesis of kidney slices

Kidneys were harvested from 48 h fasted rats as described in the previous

experiment. Pieces of kidney slices were prepared as before and randomly divided into two groups. One group of kidney tissue (test) was individually pre-incubated (for 25 min at 37°C with constant shaking) in well aerated Krebs Ringer bicarbonate medium (3.4 ml) and *M. charantia* juice (0.6 ml). The other group (control) of kidney slices was similarly pre-incubated in Krebs Ringer bicarbonate medium (4.0 ml). Subsequently both test and control kidney slices were individually incubated in Krebs Ringer bicarbonate medium supplemented with pyruvate (0.01 M, 4 ml) for 60 min at 37°C with constant shaking. The medium was removed for glucose estimation and the tissues were dried and weighed as before.

Results and discussion

Table 1 shows the mean rates of respiration of diaphragm tissue measured in terms of oxygen uptake in μl/min per g tissue. The rate of respiration of the tissues (0.76 μl/min per g tissue) after the introduction of *M. charantia* juice was not significantly different from the rate of respiration of the tissue (0.78 μl/min per g tissue) before the introduction of *M. charantia* juice. The same pattern was observed in the control experiment (where only 20 mM glucose, 0.3 ml, was tipped from the side arm) with no significant alteration in the rate of respiration before and after tipping the contents of the side arm. Although the tissue respiration was not significantly altered with the introduction of *M. charantia*, there was a significant uptake of glucose in response to *M. charantia* (Table 2). This uptake of glucose in response to *M. charantia* was shown by the very significant ($0.01 > P > 0.001$) reduction of glucose content in the incubation medium of the test experiment (where

TABLE 1

EFFECT OF *M. CHARANTIA* ON TISSUE RESPIRATION

Diaphragm tissue of rats were pre-incubated in Krebs-Henseleit Ringer phosphate medium (supplemented with 20 mM glucose) in a Warburg's apparatus (40 min at 37°C and 100 strokes/min). *M. charantia* juice (supplemented with 20 mM glucose) was then tipped into the main flask and incubated under identical conditions for 40 min. In the control experiment 20 mM glucose was tipped instead of *M. charantia* juice. The rates of respiration are expressed as μl/min per g of gas absorbed ± S.E.M.

Rate of respiration μl/min per g ± S.E.M.			
Test (n = 12)		Control (n = 10)	
Initial	After *M. charantia* in 20 mM glucose	Initial	After 20 mM glucose
0.78 ± 0.044	0.76 ± 0.066	0.70 ± 0.040	0.64 ± 0.048

TABLE 2

GLUCOSE UPTAKE BY MUSCLE IN RESPONSE TO *M. CHARANTIA*

Diaphragm tissue was pre-incubated in Krebs Henseleit Ringer phosphate medium (supplemented with 20 mM glucose) in a Warburg apparatus (10 min 37°C and 100 strokes/min). *M. charantia* juice supplemented with 20 mM glucose was then tipped into the main flask and incubated for 60 min at 37°C and 100 strokes/min. The glucose concentration remaining in the respective incubation media are expressed as mg%.

Concentration of glucose mg% ± S.E.M.		
Stock medium without tissue ($n = 6$)	After incubation (60 min)	
	Test (with *M. charantia*) ($n = 7$)	Control ($n = 6$)
321.2 ± 3.14	261.92 ± 9.01	294.9 ± 2.92
	$P < 0.001$	$0.01 > P > 0.001$

M. charantia was introduced) when compared to that of the control. The reduction of glucose content in the control experiment compared to that of the stock medium represents the glucose uptake by the tissues for respiration.

Figure 1 illustrates the effect of *M. charantia* on the glycogen synthesis by liver and muscle. The glycogen content of the hepatic tissue of the treated animals were significantly higher ($0.01 > P > 0.001$) than that of the controls. Although the magnitude of the glycogen content in the muscle was lower than in the liver, yet a significantly higher ($P < 0.001$) glycogen accumulation was seen in the muscle tissue of the treated animals.

With regard to triglyceride synthesis in adipose tissue, there was no evidence of any significant stimulation in response to *M. charantia* administration (Table 3). The effect of the oral treatment with *M. charantia*, on the gluconeogenic capacity of the kidney slices obtained from 48 h fasted rats are illustrated in Fig. 2 (column A and B). The results indicate that there was no significant difference in the gluconeogenic capacity between the test and control experiments. Similar observations (Fig. 2, columns C and D) were made with the kidney slices pre-incubated with *M. charantia*. The overall data is suggestive of non-inhibition of gluconeogenesis by *M. charantia*.

The results of the present study while confirming the previous work (Karunanayake et al., 1984) may suggest several possibilities for the peripheral activity of *M. charantia* juice. The stimulation of glucose uptake by the diaphragm tissue in vitro, with no concomitant increase of tissue respiration clearly indicate the ability of the *M. charantia* juice to directly stimulate glucose uptake. In contrast, significant accumulation of glycogen in the liver

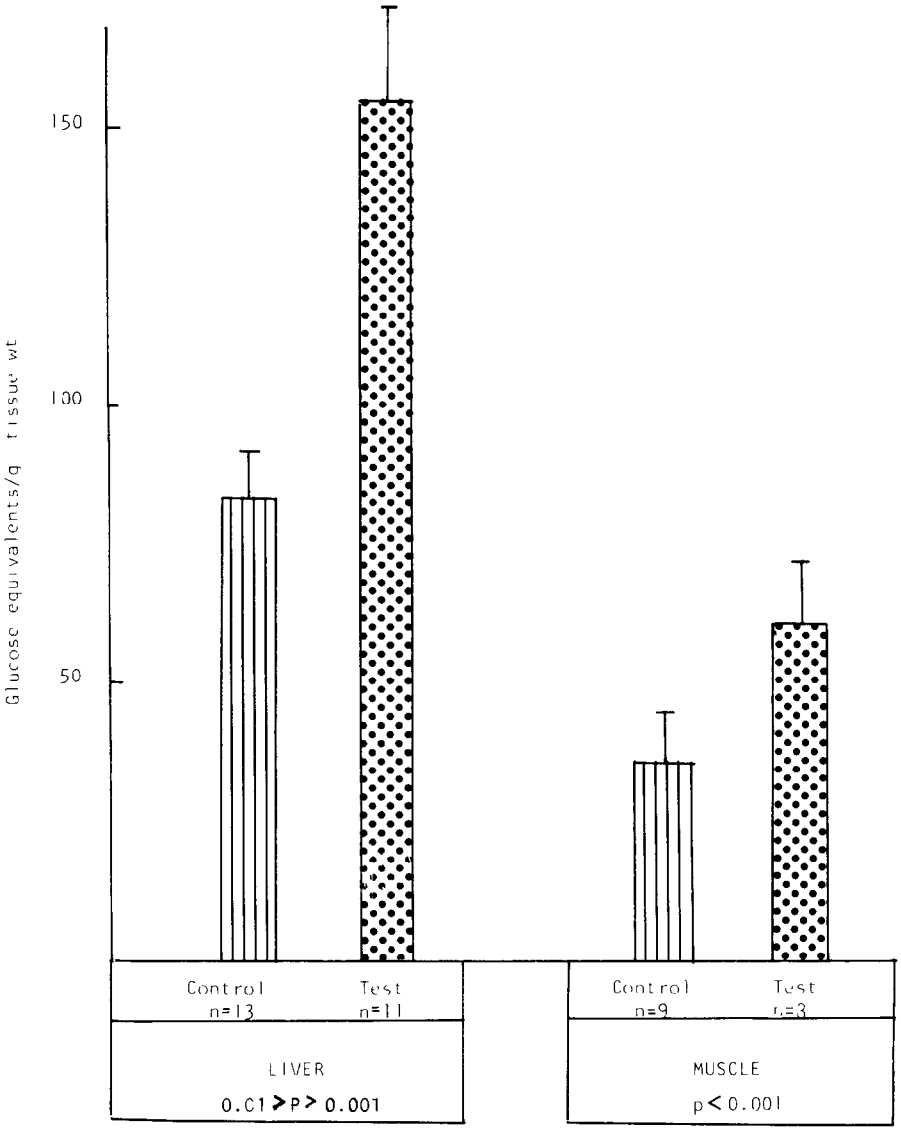

Fig. 1. Effect of *M. charantia* on glycogen synthesis. After overnight fasting rats were given *M. charantia* juice (1 ml/100 g body wt) 30 min prior to receiving a glucose load (50% w/v, 1 ml/100 g body wt). The control animals were given an equivalent dose of distilled water. The animals were killed 90 min after the glucose loading and their livers and muscle tissue were harvested for glycogen assay.

and muscle following prior oral administration of *M. charantia* could well have been due to increased insulin levels resulting from the stimulation of the

TABLE 3

EFFECT OF *M. CHARANTIA* ON TRIGLYCERIDE SYNTHESIS IN ADIPOSE TISSUE

After overnight fasting rats were given *M. charantia* juice (1 ml/100 g body wt) orally 30 min prior to receiving a glucose load (50% w/v, 1 ml/100 g body wt). The control animals received equivalent dose of distilled water. The animals were killed 90 min after receiving the glucose load and their adipose tissue samples were harvested for triglyceride assay.

Triglyceride in adipose tissue (mmol/l per g)	
Control (n = 8)	Test (n = 10)
1200.7 ± 112.2	1313.3 ± 97.8

B cells by the juice. However, the lack of simultaneous significant triglyceride accumulation in the adipose tissue of the treated animals contradicts this possibility. The interpretation of these results would have been very much facilitated if serum insulin levels were measured simultaneously. In the absence of such data the present results have to be interpreted with caution. However, in the light of the significant uptake of glucose by diaphragm tissue in vitro, the observed increased glycogen accumulation may have been due both to the stimulation of insulin release as well as due to a direct prepheral stimulation of glucose uptake by *M. charantia* juice. The present studies also showed that the pretreatment of fasted rats with *M. charantia* juice (1 ml/100 g body wt) and pre-incubation of kidney slices with *M. charantia* juice has no effect on the gluconeogenic potential of kidney slices. In contrast, hypoglycin, an oral hypoglycaemic agent from *Blighia sapida* has been shown (Patrick, 1966) to inhibit gluconeogenesis and thereby contribute towards its hypoglycaemic activity. This lack of inhibitory activity of *M. charantia* juice on gluconeogenesis may suggest that its mechanism of hypoglycaemic activity is similar to phenformin (a biguanide), where an absence of any effect on gluconeogenesis has been shown in non-diabetics (Kreisberg, 1968; Kreisberg et al., 1970).

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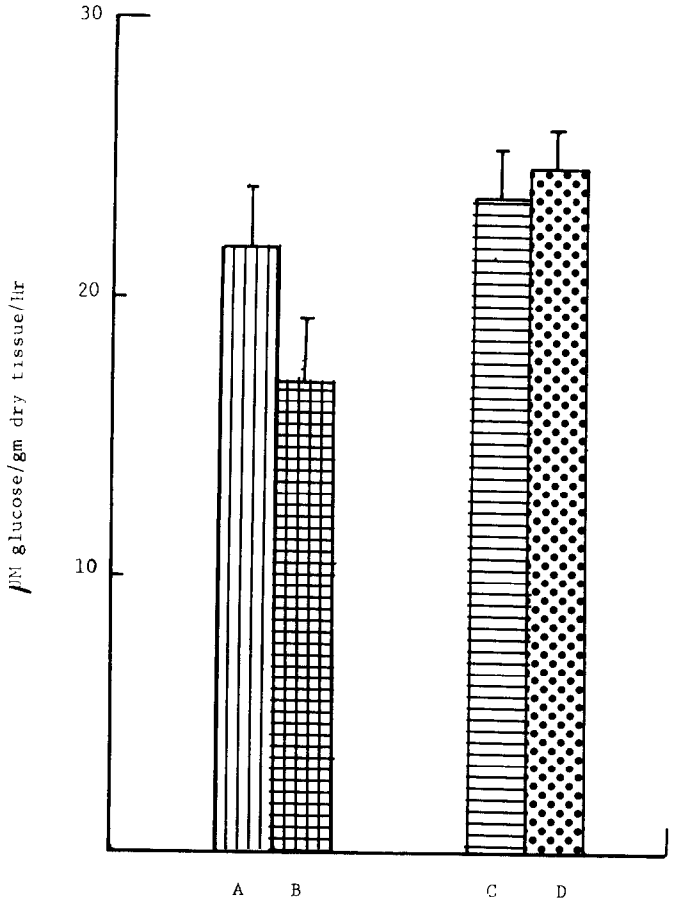

Fig. 2. Effect of *M. charantia* on gluconeogenesis. Columns A (n = 12) and B (n = 13) illustrate the effect of pretreatment of fasted rats with distilled water and *M. charantia*, respectively (1 ml/100 g body wt) on the gluconeogenic capacity of kidney slices. Columns C (n = 11) and D (n = 11) illustrate the effect of pre-incubation of kidney slices with buffer and *M. charantia*, respectively, on their gluconeogenic capacity. In either instance gluconeogenesis was estimated by incubating the kidney slices in Krebs Ringer bicarbonate medium supplemented with pyruvate substrate (0.01 M), for 60 min at 37°C. The results are given as μm glucose ± S.E.M. See text for experimental details.

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