Hypoglycaemic activity of *Coccinia indica* and *Momordica charantia* in diabetic rats: depression of the hepatic gluconeogenic enzymes glucose-6-phosphatase and fructose-1,6-bisphosphatase and elevation of both liver and red-cell shunt enzyme glucose-6-phosphate dehydrogenase

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Coccinia indica leaves were extracted with 60% ethanol, solvents were evaporated and the residue was suspended in water. This suspension was administered orally at a dose of 200 mg/kg body wt. after 18 h of fasting to normal fed and streptozotocin-induced male diabetic rats (180–250 g). After 90 min the rats were killed, and blood-glucose, hepatic glucose-6-phosphatase, fructose-1,6-bisphosphatase and glucose-6-phosphate dehydrogenase (G6PDH) and red-cell G6PDH were assayed. Blood sugar was depressed by 23% ($P < 0.01$) and 27% ($P < 0.001$) in the normal fed and streptozotocin-diabetic rats respectively compared with controls which were given distilled water. Hepatic glucose-6-phosphatase and fructose-1,6-bisphosphatase activities were depressed by 32% ($P < 0.001$) 30% ($P < 0.05$) respectively in the streptozotocin-diabetic rats, compared with 19% ($P < 0.02$) and 20% ($P < 0.01$) depression in the normal fed controls, whereas both the red-cell and hepatic G6PDH activities were found to be elevated by feeding the extract in the streptozotocin-diabetic and in the normal fed controls. Similar results were obtained with the 95%-ethanolic extract of *Momordica charantia*. Taken together, these results indicate that *Coccinia indica* and *Momordica charantia* extracts lowered blood glucose by depressing its synthesis, on the one hand through depression of the key gluconeogenic enzymes glucose-6-phosphatase and fructose-1,6-bisphosphatase and on the other by enhancing glucose oxidation by the shunt pathway through activation of its principal enzyme G6PDH.

INTRODUCTION

Coccinia indica, known in Bangladesh as 'tela kucha', has been shown to have hypoglycaemic activity in both laboratory animals (Chopra and Bose, 1925; Brahmachari and Augusti, 1963; Khuda et al., 1965; Mukherjee et al., 1972) and human subjects (Khan et al., 1980). *Momordica charantia*, known as 'karela', which is an all-year vegetable (fruit) in Bangladesh and parts of India, has also been shown to have a hypoglycaemic effect in animals (Pabrai and Sehra, 1962; Gupta and Variyer, 1963; Leatherdale et al., 1981) and human subjects (Pons et al., 1943; Aslam and Stockley, 1979; Leatherdale et al., 1981). However, the underlying biochemical mechanism of this hypoglycaemic activity remains unexplored. We have recently reported (Hossain et al., 1992) that *Coccinia* extract fed to 48 h-starved male rats depressed the activity of the key liver gluconeogenic enzyme glucose-6-phosphatase. Here we report for the first time that (i) an aqueous suspension of a 60%-ethanolic extract of *Coccinia* leaves administered orally after 18 h of fasting to normal fed and streptozotocin-induced-diabetic rats significantly elevated the activity of both hepatic and red-cell glucose-6-phosphate dehydrogenase (G6PDH) and depressed hepatic fructose-1,6-bisphosphatase, and (ii) a 95%-ethanolic extract of *Momordica charantia* gave identical results. Glucose-6-phosphatase and fructose-1,6-bisphosphatase activities were depressed, whereas the hepatic shunt enzyme G6PDH was activated.

MATERIALS AND METHODS

Reagents

The chemicals were purchased from Sigma (St. Louis, MO, U.S.A.).

Preparation of plant extracts

Coccinia indica

About 1 kg of fresh leaves of *Coccinia indica* was extracted with 3 litres of 60% ethanol with occasional shaking for 4 days. The suspension was filtered, and the filtrate was freeze-dried to give a green oily semi-solid material which weighed 18.60 g. When needed, this material was suspended in water and administered orally to rats at a dose of 200 mg/kg body wt.

Momordica charantia

Bought fresh from the local market, the vegetable was thoroughly washed in tap water, cut, and the seeds were removed manually. Then 1 kg of seedless vegetable was blended with 1500 ml of 95% ethanol in a blender, left at room temperature with occasional shaking for 48 h, and filtered. The residue was discarded, and the filtrate was evaporated in a Rotavapor (Büchi) at 40–50 °C to remove alcohol, followed by freeze-drying to remove water. A gummy yellowish residue resulted, which weighed 15 g. This material was stored in a freezer; when needed, a portion was suspended in water and was administered to the experimental animals at a dose of 200 mg/kg body wt.

Animal management

Male albino rats weighing between 180 and 250 g were used. Rats in one group were administered streptozotocin, whereas rats in the other were given saline, which was previously adjusted to pH 4.8 with 0.05 M citric acid. This latter group of rats was used as controls. *Coccinia indica* or *Momordica charantia* extract

Abbreviation used: G6DPH, glucose-6-phosphatase dehydrogenase.
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(approx. 1 ml volume) equivalent to a dose of 200 mg of extract/kg body wt. were administered to rats as described previously (Hossain et al., 1992). Intubation was accomplished by means of a narrow tube attached to a syringe, which was inserted down the oesophagus. The animals were starved for 18 h before administration of the extract. Before administration of extract or distilled water, blood was drawn from all rats for estimation of blood glucose as described by Somogyi (1945). Then 90 min after the administration of the extracts, the rats were killed, and blood samples and livers were taken for enzyme assays.

Blood collection

Blood was collected from rats by tail-puncture method. About 0.50 ml of blood was collected from each rat into a vial containing heparin (35 units/ml of blood) solution.

Dose and administration of streptozotocin

Streptozotocin (90 mg) was dissolved in saline (15 ml) previously adjusted to pH 4.3 with 0.05 M citric acid. This solution was administered intraperitoneally to 24 h-fasted rats (65 mg/kg body wt.). Then 3 weeks after the injection of streptozotocin, the herbal extracts were administered.

Enzyme assays

G6PDH (EC 1.1.1.49) was assayed by the method of Lohr and Waller (1974). The rate of formation of NADPH, which was a measure of enzyme activity, was measured spectrophotometrically. Blood (0.5 ml) was taken in a small tube containing 0.01 ml of sodium heparin (1%). To this was added 0.5 ml of sodium citrate solution (3.8%), followed by mixing and centrifugation at 1000 rev./min for 5 min. The supernatant was discarded, and the pallet (red cells) was washed twice, each time with 5 ml of physiological saline. To prepare a haemolysate, the final sediment was suspended in 1 ml of saline, to which were added 1 ml of water, 0.7 ml of Tris/HCl buffer (50 mM, pH 7.5) and 0.3 ml of digitonin (1%). After mixing and standing at room temperature for 15 min, the mixture was centrifuged at 1000 rev./min for 15 min to remove the insoluble material. The haemolysate thus prepared was used for G6PDH assay.

Hepatic G6PDH was assayed as follows. Liver (0.2 g) was chopped and homogenized in 5 ml of ice-cold EDTA/saline (66 mM EDTA in 0.85% saline), and centrifuged at 1000 rev./min at 2 °C for 30 min. The pellet was discarded and the supernatant was used as the source of hepatic G6PDH. Results were expressed as units of enzyme per mg of protein content of the supernatant fraction. One unit of the enzyme at 25 °C in a 3 ml assay mixture changes the A_{340} of NADPH by 0.001 in 1 min.

Glucose-6-phosphatase (EC 3.1.3.9) was assayed as described by Baginski et al. (1974), fructose-1,6-bisphosphatase (EC 3.1.3.11) as described by McGilvery (1955) and arginase (EC 3.5.3.1) by the method of Roman and Ruys (1964). For these determinations, 1 g of fresh/frozen liver was chopped and homogenized in ice-cold sucrose (15 ml, 250 mM) with a Potter–Elvehjem homogenizer for 2 min, centrifuged at 10000 rev./min for 30 min, and the pellet was discarded and the supernatant was used as the source of the above-mentioned enzymes. Protein content of the supernatant was determined by the method of Lowry et al. (1951).

RESULTS AND DISCUSSION

The results presented in Table 1 indicate that oral administration of an aqueous suspension of ethanolic extract of *Coccinia indica* leaves to 18 h-fasted rats lowered blood glucose level of both normal and streptozotocin-diabetic rats. This is consistent with our own results (Hossain et al., 1992) and results published by others on both laboratory animals and human subjects (Brahmachari and Augusti, 1963; Gupta and Variyer, 1963; Khuda et al., 1965; Mukherjee et al., 1972; Khan et al., 1980). In an attempt to gain an insight into the underlying biochemical mechanism of this hypoglycaemic effect, we assayed the key gluconeogenic enzymes glucose-6-phosphatase and fructose-1,6-bisphosphatase of liver, the master organ responsible for maintaining homoeostasis of blood glucose. The data presented in Table 1 clearly indicate that the *Coccinia* leaf extract significantly depressed glucose-6-phosphatase and fructose-1,6-bisphosphatase activities in both normal and streptozotocin-diabetic rats. Consistent with the depressing effect of the extract on these gluconeogenic enzymes are the data on the hepatic urea-cycle enzyme arginase, which is also depressed. The data on glucose-6-phosphatase and arginase are in total agreement with our recent data on these hepatic enzymes (Hossain et al., 1992). In that study, these enzymes were induced by starvation of the animals for 48 h, and the depressing effect of the *Coccinia* extract was observed only on the starvation-induced enzyme. The depressing effect of the *Coccinia* extract on fructose-1,6-bisphosphatase reported for the first time in the present study reinforces the preceding study (Hossain et al., 1992). Unlike the preceding experimental protocol, in the current investigation both the streptozotocin-diabetic and the normal fed animals were starved for 18 h before oral administration of *Coccinia* extract.

In addition to the depressing effect on these key hepatic enzymes, the extract had significantly elevated hepatic and red-cell shunt enzyme G6PDH in both normal and streptozotocin-diabetic rats. As would be expected, both hepatic and red-cell basal enzyme levels were decreased by streptozotocin.

Identical results were obtained with *Momordica charantia*, which is also used to obtain relief from diabetes mellitus (Pons et al., 1943; Aslam and Stockley, 1979; Leatherdale et al., 1981). Extracts of this plant material was tested only on streptozotocin-diabetic rats, as indicated in Table 1. As was observed with *Coccinia indica*, *M. charantia* extract also depressed hepatic glucose-6-phosphatase and fructose-1,6-bisphosphatase by 27% and 29% respectively, while stimulating the hepatic shunt enzyme G6PDH by 29%. Blood sugar was depressed by 22%.

Although the hypoglycaemic effect of *M. charantia* has been the subject of extensive investigations, to the best of our knowledge the enzymic effects observed in this study are the first reported.

Leatherdale et al. (1981) reported that *M. charantia* improved glucose tolerance in diabetes, and lowered blood glucose level among non-insulin-dependent diabetics as well as non-diabetic laboratory rats. These workers observed no rise in serum insulin, which was suggestive of the absence of any insulin secretelogue in the extract of *M. charantia*. They concluded that the blood-sugar-lowering effect could be ascribed to liver and peripheral glucose utilization, neglecting any effect that the herb could have on liver gluconeogenesis. Gupta and Seth (1962) showed that *M. charantia* extract did not decrease intestinal glucose absorption, which is consistent with our results. The hypoglycaemic effects reported here were observed on 18 h-starved animals, which would rule out any effect of the extracts on the intestinal absorption of sugars.

Table 1 Effect of oral administration of aqueous suspension of ethanolic extract of *Coccinia indica* and *Momordica charantia* on male rats made diabetic with streptozotocin

Each value is the mean ± S.E.M. for the number of observations shown in parentheses. Glucose-6-phosphatase is expressed in μmol of P_i produced/min per mg of protein. A unit of fructose-1,6-bisphosphatase is defined as the amount which liberates 1 μmol of P_i in 1 h under the experimental conditions. A unit of arginase is the amount of enzyme that produces 1 μmol of ornithine/min per mg of protein. A unit of G6PDH at 25 °C in a 3 ml assay mixture changes the A_{340} of NADPH by 0.001/min.

	Streptozotocin-diabetic rats					
	Given distilled water		Given *Coccinia* extract			
Parameters assayed	Before treatment	After treatment	Before treatment	After treatment	Effect of extract (%)	$P <$
Blood sugar (mg/100 ml)	177.13 ± 0.89 (8)	174.24 ± 0.77 (8)	174.58 ± 0.46 (9)	128.37 ± 0.63 (9)	−26	0.001
Liver glucose-6-phosphatase (units/mg of protein)	–	0.254 ± 0.001 (8)	–	0.174 ± 0.002 (9)	−30	0.001
Liver fructose-1,6-bisphosphase (units/mg of protein)	–	0.55 ± 0.01 (8)	–	0.41 ± 0.01 (9)	−25	0.001
Liver arginase (units/mg of protein)	–	4.26 ± 0.11 (4)	–	3.31 ± 0.04 (5)	−22	0.02
Liver G6PDH (units/mg of protein)	–	305.63 ± 3.01 (8)	–	421.44 ± 5.43 (6)	+39	0.001
Red-cell GDPH activity (units/l of haemolysate)	–	34750 ± 600 (4)	–	46420 ± 1000 (6)	+34	0.01
	Given distilled water ($n = 4$)		Given *M. charantia* extract ($n = 4$)			
Parameters assayed	Before treatment	After treatment	Before treatment	After treatment	Effect of extract (%)	$P <$
Blood sugar (mg/100 ml)	173.76 ± 0.36	173.26 ± 0.30	173.15 ± 0.61	135.75 ± 0.38	−22	0.001
Liver glucose-6-phosphatase (units/mg of protein)	–	0.247 ± 0.002	–	0.190 ± 0.001	−23	0.001
Liver fructose-1,6-bisphosphase (units/mg of protein)	–	0.54 ± 0.01	–	0.43 ± 0.01	−20	0.001
Liver arginase (units/mg of protein)	–	–	–	–	–	–
Liver G6PDH (units/mg of protein)	–	297 ± 3	–	386 ± 2.50	+29	0.001
Red-cell GDPH activity (units/l of haemolysate)	–	–	–	–	–	–
	Normal fed control rats					
	Given distilled water ($n = 5$)		Given *Coccinia* extract ($n = 5$)			
Parameters assayed	Before treatment	After treatment	Before treatment	After treatment	Effect of extract (%)	$P <$
Blood sugar (mg/100 ml)	71.64 ± 1.30	75.03 ± 0.70	75.45 ± 1.53	58.14 ± 0.90	−23	0.01
Liver glucose-6-phosphatase (units/mg of protein)	–	0.182 ± 0.003	–	0.147 ± 0.003	−19	0.02
Liver fructose-1,6-bisphosphase (units/mg of protein)	–	0.35 ± 0.01	–	0.28 ± 0.01	−20	0.01
Liver arginase (units/mg of protein)	–	3.67 ± 0.10	–	3.15 ± 0.03	−14	0.05
Liver G6PDH (units/mg of protein)	–	364 ± 5	–	434 ± 3	+19	0.001
Red-cell GDPH activity (units/l of haemolysate)	–	41380 ± 1200	–	63800 ± 900	+54	0.001

The data presented in Table 1 suggest that the hypoglycaemic effect of *Coccinia indica* and *Momordica charantia* is mediated through (*a*) suppression of the key hepatic gluconeogenic enzymes glucose-6-phosphatase and fructose-1,6-bisphosphatase, and (*b*) an accelerated rate of glucose metabolism through the pentose phosphate pathway.

How the extract might depress the activity of the hepatic gluconeogenic enzymes or elevate that of the shunt enzyme is not understood at present. The shunt enzyme G6PDH is regulated by the $NADP^+$/NADPH ratio, a high ratio favouring activation of the enzyme, whereas a low ratio is deactivating.

The depressing effects of the *C. indica* and *M. charantia* extracts on fructose-1,6-bisphosphatase and consequent depression of hepatic gluconeogenesis are consistent with the antihyperglycaemic effect of both the plants reported by Gupta and Variyar (1964). The hyperglycaemia in these cases was induced by somatotropin and corticotropin. It is now well established that the gluconeogenic effect of these pituitary hormones is mediated through an elevation of intracellular cyclic AMP. It is possible that the antihyperglycaemic effect of *Coccinia* and *M. charantia* extracts could be due to a lowering of intracellular cyclic AMP, which could be effected in a variety of ways. That activation of the enzyme fructose-1,6-bisphosphatase by the gluconeogenic pancreatic hormone glucagon is linked to an elevation of intracellular cyclic AMP is now well established (Van Schaftingen et al., 1980). Whether the depressing effect of *C. indica* and *M. charantia* extracts on fructose-1,6-bisphosphatase reported here is secondary to a lowering of intracellular cyclic AMP remains to be seen. This and other biochemical aspects of the hypoglycaemic effect of *C. indica* and *M. charantia* are currently under investigation in our laboratory.

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