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SHORT COMMUNICATION

Effect of Bitter Melon (*Momordica Charantia*) Fruit Juice on the Hepatic Cytochrome P450-Dependent Monooxygenases and Glutathione S-Transferases in Streptozotocin-Induced Diabetic Rats*

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ABSTRACT. Bitter melon (*Momordica charantia*), commonly known as karela, has been reported to have hypoglycemic, antiviral, antidiabetic, and antitumor activities. In the present study, we have investigated the effects of oral feeding of karela fruit juice on the hepatic cytochrome P450 (CYP) and glutathione S-transferase (GST) drug-metabolizing enzymes in the streptozotocin (STZ)-induced diabetic rat. Hepatic CYP contents, ethoxycoumarin-O-deethylase (ECOD), ethoxyresorufin-O-deethylase (EROD), aniline hydroxylase (AH), and aminopyrene N-demethylase (APD) activities were measured in control, diabetic, and karela juice fed animals. Diabetic rats exhibited a 50–100% increase in AH and EROD activities that was reversed by karela juice feeding. In addition, a decrease (17–20%) in the activities of APD and ECOD was observed in diabetic rat liver. Feeding of karela juice to the diabetic animals brought the level of APD close to that of control animals, while ECOD was further reduced to 60% of the control value. The cytosolic glutathione concentration was decreased in diabetic rats, and karela juice feeding normalized the effect. However, an increase (of 20–30%) in the GST activity was observed in both diabetic and karela juice fed rats. Western immunoblot analysis of CYP and GST isozymes exhibited a differential response during diabetes. The expression of CYP1A1, 2B1, 2E1, 3A4, and 4A2 was apparently increased in the diabetic rat liver. The expression of GST alpha and pi appeared to be increased in diabetes, while a decrease in GST mu was observed. Our results suggest that the changes in hepatic phase I and phase II drug-metabolizing enzyme activities in the STZ-induced diabetic animals may be associated with the altered expression of different CYP and GST isozymes. In addition, we have also observed that karela does not always reverse the effects on drug-metabolizing enzymes in STZ-induced diabetes. BIOCHEM PHARMACOL 52;10:1639–1642, 1996. *Copyright © 1996 Elsevier Science Inc.*

KEY WORDS. streptozotocin-diabetes; karela juice; microsomes; cytosol; CYP; GST; GSH; and immunoblotting

Momordica charantia, commonly known as karela or bitter melon, is used as a vegetable in South Asia, South America, and Oriental countries. Most often, karela has been used as a hypoglycemic and antidiabetic agent [1–4] and many components have been identified in extracts from the karela fruits and plant which possess antidiabetic properties [5, 6]. Karela fruit juice also has been shown to stimulate glycogen storage by liver and insulin secretion by isolated islets of Langerhans [6, 7]. The hypoglycemic activity of karela fruit has been found in both normal and diabetic (type 1 and type 2) humans, suggesting that karela fruit is mimicking insulin action in humans [2].

Hepatic microsomal CYP‡ enzymes, which are involved in the metabolism of various drugs, xenobiotics, and physiological substrates, may be induced or suppressed in many pathophysiological conditions, such as diabetes, hypertension, and cancer [8, 9]. Alterations in the hepatic CYPs, GSTs, and GSH-dependent metabolism are being used to monitor the toxicity of various xenobiotics and toxicants [10, 11]. Spontaneous or chemically induced diabetes in rats produces changes in the expression and activities of CYP isozymes in hepatic and renal microsomes [12, 13].

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‡ *Abbreviations:* AH, aniline hydroxylase; APD, aminopyrene N-demethylase; CDNB, 1-chloro-2,4-dinitrobenzene; CYP, cytochrome P450; ECOD, 7-ethoxycoumarin-O-deethylase; EROD, 7-ethoxyresorufin-O-deethylase; GSH, glutathione; GST, glutathione S-transferase; and STZ, streptozotocin.

Treatment of STZ-induced diabetic rats with insulin was found to reverse the changes and has a normalizing effect on CYP isozyme expression [12–14]. However, there is insufficient information on the effect of STZ-diabetes on GSH-dependent detoxication mechanisms, and therefore in the present study we examined changes in activity and expression of CYP and GST isozymes in the liver of STZ-induced diabetic rats. In addition, we also studied the effect of oral feeding of antidiabetic karela fruit juice on CYP and GST activities in diabetic and control animals.

MATERIALS AND METHODS

Aminopyrene, NADPH, 7-ethoxycoumarin, 7-ethoxyresorufin, reduced GSH, STZ, and dithio-bis(nitrobenzoic acid) were purchased from the Sigma Chemical Co. (St. Louis, MO, U.S.A.). CDNB, aniline-HCl, and 4-aminophenol were obtained from the Aldrich Chemical Co. (Milwaukee, WI, U.S.A.). Chemicals and reagents for electrophoresis and western blotting were purchased from Bio-Rad Laboratories (Richmond, CA, U.S.A.). Monoclonal antibodies to CYP 2B1 and CYP 1A1 were gifts from Dr. S. S. Park, NCI-Frederick Cancer Research and Development Center, Frederick, MD, U.S.A. Polyclonal antibodies against CYP 4A2 were obtained from Dr. R. T. Okita, Washington State University, Pullman, WA, U.S.A. Antibodies for CYP 2E1 and 3A4 were purchased from Amersham Int. plc (Buckinghamshire, England). Polyclonal antibodies to purified GST alpha, mu, and pi isozymes were gifts from Professor Y. C. Awasthi, University of Texas Medical Branch, Galveston, TX, U.S.A.

Treatment of Animals and Enzyme Assays

Male Wistar rats (200–250 g body wt) were obtained from the animal house facility of the UAE University. Animals were treated, to induce diabetes, by a single intraperitoneal injection of STZ (60 mg/kg body wt in 0.05 M sodium citrate, pH 4.5). Animals were divided into four groups with five animals in each group: STZ-induced diabetic group, diabetic-karela juice fed (10 mL/kg body wt daily for 10 weeks) group, karela juice fed control group, and a control group subjected to the oral intubation of water alone (10 mL/kg body wt). Animals were considered diabetic if blood glucose values were more than 300 mg/100 mL. Oral feeding of karela juice was performed from the freshly prepared karela fruit pulp juice as described by Sharma *et al.* [15]. At the end of week 10, animals were killed, and liver microsomes and cytosols were prepared by ultracentrifugation [16]. Protein concentration was measured by the method of Bradford [17], using bovine serum albumin as a standard.

CYP content and CYP-dependent ECOD, EROD, APD, and AH activities were measured as described previously [16, 18].

The GSH concentration in all four group of animals was measured in the cytosolic fraction as protein free sulfhydryl content using Ellman's reagent as described previously [18, 19]. Cytosolic GST was measured using CDNB as a substrate according to the method of Habig *et al.* [20].

SDS–PAGE and Western Blot Analysis

Microsomal and cytosolic proteins from control and experimental rat liver were separated by SDS–PAGE according to the method of Laemmli [21]. Western immunoblotting was performed according to the method of Towbin *et al.* [22] as described previously [16, 18].

RESULTS AND DISCUSSION

The administration of a single dose of STZ to rats raised the blood glucose concentration (200–400% above normal value), which remained high throughout the study period of 10 weeks (data not shown). The treatment of diabetic and control rats with karela juice produced a 30–50% reduction in the blood glucose concentration of diabetic rats after 10 weeks of daily feeding (results of the hypoglycemic effect of karela juice on STZ-diabetic rats is published elsewhere [15]).

Microsomal CYP Monooxygenases and Cytosolic GSTs

The total amount of CYP in hepatic microsomes of diabetic rats was not significantly different from that of control rats (Table 1). Although not significant, feeding of karela juice to diabetic rats caused a slight (12–15%) decrease in CYP content when compared with that of control rats. Diabetic rats, however, exhibited more than 100% increase in AH activity which was reduced to a 33% higher level after karela juice feeding when compared with that of control rats, while karela juice feeding to control rats caused a 24% increase in AH activity. Similarly EROD activity was also increased (59%) in diabetic rats. Karela juice feeding brought the enzyme activity down to the level of control rats. An inhibitory effect of karela juice feeding was also seen in control rats. In contrast, a decrease in APD and ECOD activities was observed in diabetic rat liver. However, karela juice reversed the inhibition of APD activity in diabetic rats, while ECOD activity was inhibited further after karela juice feeding. Feeding of karela juice to control rats also inhibited ECOD activity.

Table 1 also shows the results of the alterations in the concentration of cytosolic GSH and GST activity in the control and diabetic rat liver. A 29% decrease in GSH concentration was observed in diabetic rats, and karela juice feeding reversed the inhibition. However, karela juice feeding to control rats has no significant effect on GSH concentration. GST activity in diabetic rat liver, using CDNB as a substrate, was found to be increased (20% above control). This activity was further enhanced after feeding karela juice to diabetic rats. An increase in GST was also observed in control rat liver after karela juice feeding.

TABLE 1. CYP and GSH contents, CYP-monooxygenase, and GST activities in control and diabetic rat liver

	Control	Control + karela	Diabetic	Diabetic + karela
CYP contents (nmol/mg protein)	0.81 ± 0.04	0.82 ± 0.05	0.82 ± 0.02	0.72 ± 0.10
AH (nmol/min/mg protein)	0.55 ± 0.01	0.68 ± 0.05	1.13 ± 0.03*	0.73 ± 0.03*
APD (nmol/min/mg protein)	2.62 ± 0.10	2.42 ± 0.10	2.15 ± 0.14*	2.42 ± 0.15
ECOD (pmol/min/mg protein)	333.92 ± 46.90	204.15 ± 37.61*	277.62 ± 15.50*	191.87 ± 22.01*
EROD (pmol/min/mg protein)	723.16 ± 88.01	551.56 ± 40.01*	1148.75 ± 91.31*	703.65 ± 70.06
GSH (mmol/g tissue)	2.42 ± 0.40	2.58 ± 0.7	1.73 ± 0.06*	2.83 ± 0.25
GST (nmol CDNB conjugated/min/mg protein)	997.72 ± 85.88	1190.70 ± 148.4	1200.56 ± 00.00*	1321.45 ± 107.54*

CYP content and monooxygenase activities in microsomal preparations from all four groups of animals were determined as described in Materials and Methods. Cytosolic GSH concentration and GST activity were measured in post-microsomal supernatant from control and treated animals as described in Materials and Methods. Values are means ± SEM, N = 4 (number of animals). Two determinations were done on each animal.

* Significantly different ($P \leq 0.05$) from control group.

Western blot analysis using isozyme-specific CYP antibodies showed a pattern of expression of CYP isozymes in diabetic rat liver microsomes (Fig. 1) which differed from that of control animals. Although no quantitation was attempted, an increase in the levels of isozyme CYP 1A1, 2B1, 2E1, 3A4, and 4A2 was apparent in diabetic rat liver microsomes. Feeding karela juice to diabetic rats resulted in an apparent reduction in the expression of CYP with the exception of the expression of CYP 3A4 and 4A2, which remained enhanced after karela juice feeding (Fig. 1).

The results of experiments to determine the expression of cytosolic GST in diabetic rat liver are shown in Fig. 2. There was an abundant expression of GST alpha and mu in control and diabetic rat liver, but very little expression of GST pi was seen. An apparent increase in the expression of GST alpha and a decrease in the expression of GST mu were observed in STZ-induced diabetic rat liver. A slight increase in the expression of GST pi was also seen in diabetic rat liver. Karela juice feeding appeared to have normalized the altered expression of GST isozymes.

There are reports that indicate that diabetes induces changes in hepatic levels of several CYP isozymes in rats [12–14]. A CYP isozyme 2E1, which is induced by acetone, ethanol, or fasting, has been shown to be induced markedly in microsomes from the livers of chemically induced or spontaneously diabetic rats [14]. An increased level of ketone bodies and an increase in the metabolism of fatty acids during diabetes seem to be responsible for the induction of CYP 2E1 in these rats [12–14]. Our results in the present study also showed an increased expression of CYP 2E1 and CYP 4A2 and an increase in CYP-dependent aniline hydroxylation activity. In addition, we observed an increase in the expression of 1A1 with concomitant increase in EROD activity. However, the APD and ECOD activities were found to be inhibited in microsomes from diabetic rat liver. These findings suggest that CYP isozymes are differently affected during diabetes.

We also investigated the effects of oral feeding of karela on the drug-metabolizing enzyme activity and expression in control and diabetic rat liver. Karela has been reported to have antidiabetic, antiviral, and antitumor activities [1–6]. The antidiabetic effect of karela is presumably associated with the stimulation of insulin secretion by the pancreas. Insulin has been reported to reverse the effects of diabetes on drug-metabolizing enzyme activities in the liver and kidney [12–14]. Effects of karela juice feeding in the present study also exhibited a variation in affecting the CYP isozymes in diabetic and control rat liver. The most profound effect seen was an apparent reversal of alterations in CYP 1A1, 2B1, and 2E1, which is similar to the effect of insulin as reported by Favreau and Schenkman [13]. However, some of the CYP isozyme selectivity of the karela juice is comparable as we did not see any reversal of CYP 3A4 and 4A2 expressions. The possible mechanisms of action of

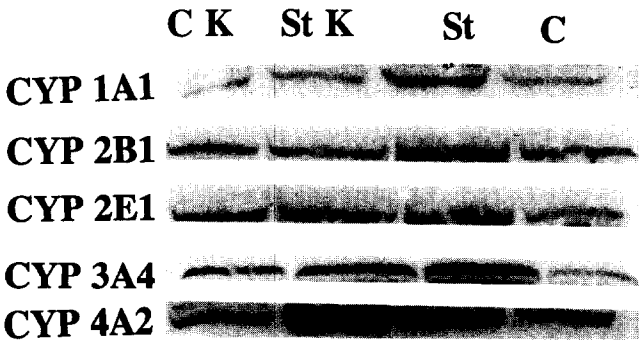

FIG. 1. Western blot analysis of liver microsomes from control and diabetic rats. Hepatic microsomal preparations (40 μg protein) from control, STZ-induced diabetic, and karela juice fed rats were subjected to 12% SDS–PAGE. Western blotting and immunoreactivity of proteins with CYP isozyme specific antibodies were determined as described in Materials and Methods.

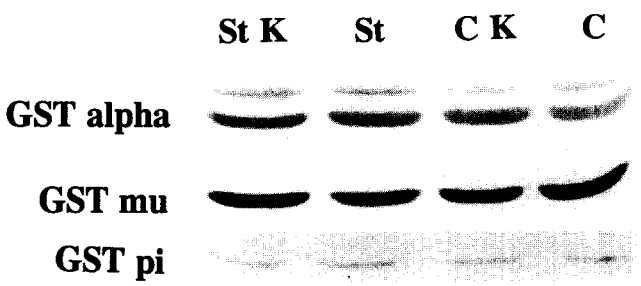

FIG. 2. Western blot analysis of liver cytosol from control and diabetic rats. Immunoreactivities with cytosolic proteins from control and experimental groups of animals were determined, using antibodies to GST alpha, mu, and pi, as described in Materials and Methods.

karela juice feeding to diabetic rats may be associated with alterations in the ketone bodies or fatty acid metabolism, a mechanism described for insulin action [3, 14]. The karela juice may also be altering insulin and/or the secretion of growth hormones, thereby affecting the drug-metabolizing enzymes.

A decrease in hepatic GSH concentration and an increase in GST activity were observed in diabetic rats. Karela juice feeding reversed these effects, which may suggest a protection mechanism against the deleterious effects during diabetes. An increase in GST catalytic activity in diabetic rats was in agreement with an observation of apparent increased expression of GST alpha and pi by western blot analysis. The karela juice feeding apparently increased the expression of GST mu, which was found to be depressed in diabetic rats. These results suggest that the alterations in the hepatic drug-metabolizing activities in diabetes may be related to the differential expression of various CYP and GST isozymes. The karela juice feeding, however, appears to have selectively normalized the alterations in some of the drug-metabolizing enzymes during diabetes, presumably because of multiple reagents present in karela juice.

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