STUDIES ON THE ISOLATION AND EFFECT OF THREE ORALLY ACTIVE HYPOGLYCEMIC PRINCIPLES KAKARA Ib, IIIa AND IIIb, FROM BITTER GOURDS *(Momordica Charantia* LINN)

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Juice of unripe fruits of *Momordica Charantia* Linn) has been reported to produce fall in fasting blood glucose (FBG) and improve glucose tolerence in rabbits [1'2,3'4]. Dry powder of the unripe fruit was also shown to bring down FBG in rabbits[5]. Clinical trials in diabetic patients also have shown that the juice of fresh unripe fruits could bring down FBG and bring about satisfactory improvement [6]. Recently Leatherdale et al (1981) have observed improvement in glucose tolerance test in 9 patients of non insulin dependent diabetes mellitus with water extract[7]. A steroid glycoside charantin has been isolated as the active hypoglycemic principle[8]. Subsequent work by Pugazhenthi and Suryanarayana Murthy (1979) has indicated that charantin is actually a mixture and its biological activity is lost sometimes during the lengthy isolation procedure[6]. The systematic studies undertaken by us using a better animal model have resulted in the isolation of three active hypoglycemic principles.

Material and Methods

Alloxan recovered (AR) and severely diabetic {SD) rabbits : Rabbits were given alloxan 80 mg/kg intravenously and those which recovered in one month by showing normal and slightly elevated FBG but abnormal glucose tolerance pattern in glucose tolerance test (GTT) were called AR rabbits. The details of the development of AR rabbits were described earlier[10]. Those which had stabilized diabetes with FBG consistently above 250 mg/dL were called severely diabetic (SD) rabbits.

Assessment of hypoglycemic effect

a) *In S.D. rabbits:* Fall in FBG after oral administration of the drug was indicative of the extra pancreatic effect of the drug.

b) *In A.R.rabbits:* In overnight fasted rabbits basal glucose tolerance test (GTT) pattern was established using water or control solvent in place of the drug. One week later to the same group of animals, the test drug was given orally and 90 minutes later glucose (2g/kg) was administered orally and GTT performed. Thus the The same animal served as its own control. A separate untreated group of AR rabbits was also used as a control group. Improvement in glucose tolerance as indicated by decrease in peak blood glucose, (usually at 90 min) decrease in blood glucose at 2½ hrs and overall suppression of GTT curve were used as a measure of hypoglycemic effect (both pancreatic and extra pancreatic put together).[10]

Blood glucose estimation: Blood glucose was estimated by the glucose oxidase method of Huggett and Nixon 1957.[11]

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Isolation of hypoglycemic principles

Dry powder from fresh fruits was extracted exhaustively with benzene. The benzene extract was purified by silicic acid column chromatography with petroleum ether (diethylether) as the solvent. Four major fractions I, II, III and IV were obtained out of which I and II showed hypoglycemic activity. Rechromatography of fractions I and fraction III yielded three fractions called Kakara Ib, Kakara IIIa and Kakara IIIb. They were homogenous by the criteria of thin layer as well as high performance liquid chromatography. Since they are not steroids by Liebermann and Burchard reaction they are different from charantin isolated earlier by Lotlikar and Rao (1966).

Results

Preliminary studies with Charantin and conventional SD rabbits gave uncertain results. So the new model, AR rabbits, were used here.

Hypoglycemic activity in AR rabbits with tolbutamide

In order to make sure that the AR model of rabbits would be useful for the assessment of hypoglycemic activity of unknown drugs, the effect was tried with tolbutamide which is known to produce its effect mostly through pancreas. The results in table I show that in AR rabbits, the peak blood glucose values at 90 min reached 286 ± 15 mg/dL when compared to 134 ± 10 mg/dL in healthy controls. However, in telbutamide treated group of rabbits the peak value was only 209 ± 12 mg/dL i.e. 27% less.

However, the same dose of tolbutamide did not produce any significant fall in severely diabetic rabbits with FBG values of about 400 mg/dL. These results indicate that SD rabbits are unsuitable for a arylsulphonylurea type of drug like tolbutamide whereas AR rabbits are useful.

Effect of single dose of Kakara Ib, IIIa and IIIb on fasting blood glucose levels

In AR rabbits and SD rabbits the three principles Kakara Ib (400 mg/kg) Kakara IIIa (100 mg/kg) and Kakara IIIb (300 mg/kg) did not produce any significant fall in FBG values. However, with kakara IIIb, there was some transient but not substained fall in FBG (20%) which however increased by 2 hours (only 13% fall).

Table 1
Glucose tolerance test in normal, AR and tolbutamide treated AR rabbits.

Type of	Blood glucose mg/dL Mean±S.D.					
Rabbits*	Fasting	Durring GTT minutes after glucose				
(6 in each group)		30	60	90	120	150
Control	93±8	132±12	160±11	134 + 10'	98± 9	96± 8
ARuntreated	95±9	191±13	252±12	286±15	241 ±14	209±14
AR tolbutamide treated (300mg/kg)	88±7	183±12	208±13	223±13	201±11	189±12

Effect of single dose of Kakara Ib, IIIa, and IIIb on glucose tolerance in AR rabbits:

All the three principles showed improvement in glucose tolerance as judged by all the three criteria mentioned above namely reduction in the peak blood glucose level at 2½ hours and overall suppression of the GTT curve. Kakara Ib (400 mg/kg) produced 20% fall (less active than tolbutamide) Kakara IIIa, (100 mg/kg) 30% fall (more active than tolbutamide) and Kakara IIIb (300 mg/kg) 22% fall (as active as tolbutamide). GTT could not be performed in SD rabbits as they died when glucose was given orally.

Mode of action of active principles with multiple doses:

In order to understand the mechanism of action Kakara Ib was given orally daily once at a dose of 300 mg/kg for one week, Kakara IIIa, 100 mg/kg daily once for 2 weeks and kakara IIIb, 300 mg/kg daily once for 2 weeks. At the end of the experiment serum lipid profile was assessed and GTT performed. During GTT serum insulin levels were also determined to see whether the principles would act by increasing serum insulin levels an indication of pancreatic effect. With Kakara Ib, there was 54% decrease in serum free fatty acids (FFA) level ($P<0.001$), improvement in glucose tolerance (21%) but there was no change in other serum lipid parameters like cholesterol, triglycerides (TG) and phospholipids and even FBG. There was no increase in serum insulin. This indicates that this principle acts by decreasing circulating serum FFA levels which in turn have a sparing effect on serum insulin without actually increasing serum insulin. Its effect persisted for another two weeks even after withdrawal of the drug.

Kakara IIIa, improved glucose tolerance (30%), decreased the serum FFA levels without affecting other serum lipids TG, cholesterol and PL on FBG. There was no increase in serum insulin levels during GTT. Therefore, the principle also had no effect on pancreas as far as insulin release is concerned but improved the utilization of administered glucose by suppressing circulating FFA.

Kakara IIIb on the other hand could not only improve glucose tolerance (29%) but also increase serum insulin levels and even produce fall in FBG. This indicates that it has pancreatic effect also.

Discussion

Three orally active hypoglycemic principles called Kakara Ib, IIIa and IIIb were isolated from the unripe fruits of *M. Charantia* Linn. Improvement in glucose tolerance in alloxan recovered (AR) rabbits with nearly normal or slightly elevated fasting blood glucose (FBG) levels but with impaired glucose tolerance was used as the criterion for assessing hypoglycemic activity.

Kakara Ib (400 mg/kg) and IIIa (100 mg/kg) had no effect on FBG but improved glucose tolerance 20% and 30% respectively in single dose in AR rabbits. In multiple doses (Kakara Ib for one week and IIIa for two weeks) seemed to act by suppressing circulating free fatty acids without affecting other serum lipid parameters (cholesterol, triglycerides and phospholipids). Both of them could not increase glucose induced serum insulin levels during GTT. Their effect seems to be extra pancreatic. Kakara Ib is less

effective than tolbutamide on weight basis. Kakara IIIb in a single dose (300 mg/kg) produced only a transient but not sustained fall in FBG and improved glucose tolerance. In multiple doses for 1 week, there was improvement in glucose tolerance and increase in serum insulin during GTT indicating pancreatic effect. There was fall in FBG also. Thus M. *charantia* contains at least three non steroidal orally active hypoglycemic principles having pancreatic (IIIb) as well as extrapancreatic (Ib and IIIa) mechanism of action.

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