ANTIDIABETIC EFFECT OF DIASANSAR IN STREPTOZOTOCIN AND FRUCTOSE INDUCED TYPE-2 DIABETES IN RATS

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Summary

Diasansar, a polyherbal formulation, was tested for its anti-diabetic activity against neonatal Streptozotocin (STZ) and fructose induced type 2 diabetes in Wistar rats. Diasansar was tested at two dose levels of 400 and 800 mg/kg, p.o. Glibenclamide (500 µg/kg, p.o.) was used as the standard. Serum glucose, lipid profile, creatinine, liver glycogen and liver weight were measured. Diasansar shows a significant antidiabetic effect at both the dose levels employed in both streptozotocin and fructose induced type 2 diabetes models. It significantly reduces the serum glucose, cholesterol, triglyceride and creatinine and significantly increases the serum HDL-cholesterol, liver glycogen and absolute liver weight when compared to the untreated diabetic control ($p<0.01$). The results are comparable to the standard glibenclimide. In normal rats, diasansar significantly prevents the glucose load induced elevation of serum glucose levels in oral glucose tolerance test ($p<0.01$). The results of the present study indicate that diasansar has significant beneficial effects against streptozotocin and fructose induced hyperglycemia and hyperlipidemia in diabetic rats and on glucose tolerance in normal animals.

Key words: Type 2 diabetes, glucose tolerance, diasansar, streptozotocin, fructose

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Introduction

Type 2 diabetes is primarily a lifestyle disorder, with huge adverse impact on the global health and mortality. It accounts for around 90% of the diabetes cases. In 1995 it was estimated that around 135 million people were affected from this condition and it was expected to affect 300 million by the year 2025 (1). The current therapeutic agents available for the treatment of type 2 diabetes are associated with several side effects. Biguanides are found to cause lactic acidosis and GI disturbances. Sulphonylureas are associated with hepatotoxicity, hypoglycemia, weight gain and GI disturbances. Meglitinides cause hypoglycemia at high doses. Thiazolidinediones, the most widely used antidiabetic drugs, have been reported to cause hepatotoxicity, weight gain, fluid retention and congestive heart failure (2).

Plants have always been a very good source for drugs and many of the currently available drugs have been derived directly or indirectly from plants. Many of the plant derived products have been reported to possess antidiabetic activity and are widely prescribed because of their effectiveness, less side effects and relatively low cost (3-5). Investigations on such plant derived products are, therefore, an important area of current research interest. In the present study, diasansar, a polyherbal formulation marketed by Pradhan Herbals, Bangalore, was screened for its antidiabetic activity in streptozotocin and fructose induced diabetes in Wistar rats.

Material and Methods

Animals:

Wistar rats weighing between 170-190 g were procured from IISc, Bangalore. The animals were housed under standard conditions of temperature (22±3° C) and relative humidity (30-70%) with a 12:12 light:dark cycle. The animals were fed with standard pellet diet (Amrit feeds Ltd. Bangalore.) and water *ad libitum*. The Institutional Animal Ethics Committee (IAEC) of Visveswarapura Institute of Pharmaceutical Sciences approved the proposal.

Chemicals:

Streptozotocin and glibenclamide were obtained from Sigma co., St. Louis, MO, USA. Triglyceride, cholesterol, creatinine, HDL-cholesterol and glucose estimation kits were from Ecoline, Merck Ltd. Mumbai, India. All the other reagents and chemicals used in the study were of analytical grade.

Dose selection:

Based on the clinical dose (5-10g twice a day) the polyherbal formulation, diasansar, was tested at two dose levels of 400 and 800 mg/kg, b.wt. The standard, glibenclamide, was tested at 500 µg/kg, b.wt. All the drugs were suspended in 0.5% w/v sodium carboxymethyl cellulose (CMC) and administered at a dose volume of 10 ml/kg body weight.

Oral glucose tolerance test in normal rats:

Twenty four rats were divided into four groups of six each, Group 1 received 0.5 % carboxymethylcellulose (CMC) at a dose of 10 ml/kg, p.o. and served as control. Group 2 and 3 received 400 and 800 mg/kg, p.o. diasansar, respectively. Group 4 received glibenclamide, 500 µg/kg, p.o. All the animals were fasted for 16 h before the test and received assigned treatment 1 h before the glucose load. A glucose load of 10 g/kg, b.wt. was administered orally to all the animals and blood was collected from retro orbital plexus at 0, 30, 60 and 120 min. Serum was separated for the estimation of glucose using Ecoline glucose estimation kit.

Streptozotocin induced type-2 diabetes:

Type-2 diabetes was induced by injecting to the 2 day old wistar rat pups (either sex) with 90 mg/kg, b.wt. of streptozotocin, intraperitoneally. At 12 Weeks of age, all the animals were screened for oral glucose tolerance and for basal glucose levels. Animals showing glucose intolerance and basal hyperglycemia were selected for the study.
Twenty four animals (either sex) showing basal hyperglycemia and glucose intolerance were divided into four groups of six each. Group 1 received 0.5 % CMC (10 ml/kg, p.o.) and served as control. Group 2 and 3 received 400 and 800 mg/kg, p.o. diasansar, respectively. Group 4 received glibenclamide 500 µg/kg, p.o. All the animals received their assigned treatment twice daily for a period of 30 days. At the end of the treatment period blood was collected under light ether anesthesia for the estimation of serum glucose, cholesterol, triglyceride, creatinine and HDL-cholesterol. After blood collection animals were culled using deep ether anesthesia and the liver was excised, weighed and subjected to tissue glycogen estimation.

Fructose induced insulin resistance:

Thirty male Wistar rats were divided into five groups of six each. Group 1 and 2 received 0.5 % CMC (10 ml/kg, p.o.) and served as normal and fructose control, respectively. Group 3 and 4 received 400 and 800 mg/kg, p.o. diasansar, respectively. Group 5 received glibenclamide 500 µg/kg, p.o. Animals of group 1 were fed with standard laboratory chow (Amrit feeds Ltd., Bangalore) and animals of groups 2 to 5 were fed with fructose-rich diet. This diet contained 66% fructose, 20% casein, 2% soyabean oil, 0.02% cholesterol, 0.7% necessary amino acids, 1.28% vitamins and minerals. All the animals received their assigned treatment twice daily for a period of 30 days. At the end of the treatment period, blood was collected under light ether anesthesia for the estimation of serum glucose, cholesterol, triglyceride, creatinine and HDL-cholesterol. After blood collection animals were culled using deep ether anesthesia and the liver was excised, weighed and subjected to tissue glycogen estimation.

Results

Oral glucose tolerance test in normal rats:

The results of the study are given in Table 1. The results reveal that diasansar, at both the dose levels (400 and 800 mg/kg, b.wt.), shows a significant decrease in the serum glucose levels at 30, 60 and 120 min when compared to the control (P<0.01). The standard, glibenclamide, also significantly reduces the serum glucose at 30, 60 and 120 min when compared to the control (P<0.01).

Table 1: Effect of diasansar on glucose tolerance test in normal rats

GROUP	Treatment	Serum glucose levels (mg/dl)			
		0 min	**30 min**	**60 min**	**120 min**
Group 1	Control, 0.5% CMC, 10ml/kg, p.o.	71.7± 0.6	97.9± 0.7 (36.5± 2.1)	123.8± 0.9 (72.7 ± 1.9)	100.9±0.7 (40.8± 2.1)
Group 2	Diasansar 400 mg/kg, p.o.	81.8± 0.4	90.0± 0.3 (10.0± 0.4)*	78.1± 0.2 (-4.5± 0.8)*	88.6± 0.3 (8.3± 0.8)*
Group 3	Diasansar 800 mg/kg, p.o.	77.8± 0.7	86.66±0.3 (11.4± 1.3)*	76.2± 0.2 (-1.8± 0.8)*	81.3± 0.3 (4.6± 1.3)*
Group 4	Glibenclamide 500 µg/kg, p.o.	76.5± 0.6	108.2± 0.5 (41.3± 1.4)*	88.3± 0.4 (17.9± 2.8)*	100.6± 0.1 (31.3±1.1)*

Values are in mean ± SEM, n = 6.
Values in the parentheses represent percentage change in serum glucose,
* P < 0.01 when compared to the control group.

Streptozotocin induced type-2 diabetes:

The results of the study are given in Table 2. The results reveal that diasansar at dose levels 400 and 800 mg/kg, b.wt., and glibenclamide at 500 µg/kg, b.wt. significantly reduce the serum glucose, cholesterol, triglyceride and creatinine, and significantly increase the serum HDL-cholesterol, liver glycogen and absolute liver weight when compared to the control (P<0.01).

Table 2: Effect of diasansar on STZ induced diabetes in rats.

	GP-1 (Control)	**GP-2 (Diasansar 400 mg/kg, p.o.)**	**GP-3 (Diasansar 800 mg/kg, p.o.)**	**GP-4 (Glibenclamide 500 µg/kg, p.o.)**
Glucose (mg/dl)	65.3 ± 2.0	42.3 ± 2.8*	45.4 ± 2.4*	39.2 ± 3.1*
Cholesterol (mg/dl)	98.1 ± 2.1	57.3 ± 2.2*	63.9 ± 1.3*	49.4 ± 1.1*
Triglyceride (mg/dl)	74.6 ± 2.2	50.3 ± 2.1*	52.4 ± 1.3*	47.2 ± 1.1*
HDL-cholesterol (mg/dl)	39.6 ± 1.4	58.8 ± 1.3*	55.3 ± 0.6*	64.3 ± 1.8*
Creatinine (µmol/l)	67.9 ± 0.9	48.0 ± 0.8*	51.3 ± 0.9*	40.1 ± 0.6*
Liver glycogen (mg/100g)	524.3 ± 2.0	743.5 ± 3.4*	687.9 ± 5.4*	912.8 ± 3.7*
Liver weight (g)	5.4 ± 0.3	6.4 ± 0.2*	6.2 ± 0.1*	6.8 ± 0.1*

Values are mean ± SEM, n = 6

* $P < 0.01$ when compared to the control group

Fructose induced insulin resistance:

The results of the study are given in Table 3. The results reveal that diasansar, at dose levels of 400 and 800 mg/kg, b.wt, significantly reduce the serum glucose, cholesterol, triglyceride and creatinine, and significantly increase the serum HDL-cholesterol, liver glycogen and absolute liver weight when compared to the untreated fructose control ($P<0.01$) (Table-3). The standard, glibenclamide (500 µg/kg, b.wt.), also significantly reduce the serum glucose, cholesterol, triglyceride and creatinine, and significantly increase the serum HDL-cholesterol, liver glycogen and absolute liver weight when compared to the fructose control ($P<0.01$).

Table 3: Effect of diasansar on fructose induced insulin resistance in rats.

	GP-1 (Normal control)	**GP-2: (Fructose control)**	**GP-3 (Diasansar 400 mg/kg, p.o.)**	**GP-4 (Diasansar 800 mg/kg, p.o.)**	**GP-5 (Glibenclamide 500 µg/kg, p.o.)**
Glucose (mg/dl)	78.8 ± 1.8	106.6 ± 2.7*	90.2 ± 2.2*	82.4 ± 3.3*	74.7 ± 1.7*
Cholesterol (mg/dl)	81.5 ± 2.0	117.4 ± 3.3*	93.4 ± 2.1*	82.6 ± 2.0*	71.2 ± 1.4*
Triglyceride (mg/dl)	72.9 ± 0.8	290.4 ± 1.4*	213.4 ± 2.7*	191.4 ± 3.4*	147.6 ± 2.8*
HDL- Cholesterol (mg/dl)	45.2 ± 1.4	35.7 ± 1.0*	47.6 ± 1.4	58.8 ± 1.3*	65.4 ± 2.1*
Creatinine (µmol/l)	69.3 ± 1.2	82.8 ± 1.2*	57.8 ± 1.7*	50.7 ± 1.1*	46.3 ± 1.5*
Liver glycogen (mg/100g)	524.9 ± 4.2	490.8 ± 5.5*	628.5 ± 2.7*	662.7 ± 4.3*	702.8 ± 5.2*
Liver weight (g)	5.0 ± 0.1	6.6 ± 0.1*	7.1 ± 1.1*	7.7 ± 0.1*	6.25 ± 0.1*

Values are mean ± SEM, n = 6

* $P < 0.01$ when compared to the control group

Discussion

Diasansar, a polyherbal formulation manufactured by Pradhan herbal company, Bangalore, contains *gymnema sylvestris*, *withania somnifera*, *emblica officinalis*, *curcuma longa, azadirachta indica, eugenia jambolana, trigonella foenum graceum* and *momordica charantia*. These plants have been individually shown to possess antihyperglycemic activity through varied mechanisms of action (3). *Gymnema* contains gymnemic acid and gymnemosides, which have been reported to show antihyperglycemic activity in different models of diabetes (3). *Azadirachta indica* has been reported to inhibit the action of epinephrine on glucose metabolism, *eugenia jambolana* and *trigonella foenum graceum* have been reported to stimulate insulin secretion(6,7). The soluble and insoluble fibers present in *curcuma longa* has been reported to retard the absorption of carbohydrates from the gastrointestinal tract (7). *Momordica charantia* has been shown to increase peripheral utilization of glucose and also reported to posses insulin like molecules (8-10).

Streptozotocin induces diabetes in rats by β cell destruction, through the generation of free radicals, causing alkylation of DNA and ultimately inducing hyperglycemia. Rats fed with high fructose diet develop hyperlipidemia, insulin resistance, hyperinsulinemia and mild hypertension, which are features associated with the obesity-related hypertension (11-13).

In the present study, diasansar, shows a significant beneficial effect on the glucose tolerance in normal rats, against streptozotocin and fructose induced changes in the serum glucose, lipid, and creatinine levels, in addition to the liver weight and liver glycogen levels. Both low and high doses of diasansar show almost equal degree of antidiabetic activity comparable to standard, glibenclamide. Diasansar thus seems to improve insulin resistance through enhanced insulin sensitivity in peripheral tissues, as is evident from the decreased serum glucose and increased liver glycogen levels in diasansar treated animals. Diasansar also decreases the diabetes induced changes in the serum lipid and creatinine profile, thus indicating an added benefit in the management of diabetes mellitus.

In conclusion, the results of the present study indicate that the polyherbal formulation, diasansar, has significant beneficial effects against streptozotocin and fructose induced hyperglycemia and hyperlipidemia in diabetic rats. The results are comparable to the standard, glibenclamide.

References

1. King H, Aubert RE, Herman WH. Global burden of diabetes 1995–2025: prevalence, numerical estimates, and projection. Diabetes Care 1998; 21: 1414–31.
2. Silvio EI. Oral antihyperglycemic therapy for type 2 diabetes. JAMA 2002; 287: 360-72.
3. Gover JK, Yadav S, Vats V. Medicinal plants of India with anti-diabetic potential. J Ethnopharmacol 2002; 81: 81-100.
4. Mukherjee PK, Maiti K, Mukherjee K, Houghton PJ. Leads from Indian medicinal plants with hypoglycemic potentials. J Ethnopharmacol 2006; 106: 1-28.
5. Mukherjee PK, Wahile A. Integrated approaches towards drug development from Ayurveda and other Indian system of medicines. J Ethnopharmacol 2006; 103: 25-35.
6. Sauvaire Y, Petit P, Broca C, Mantegheti M, Baissac Y, Fernandez ALVJ, et al. 4-Hydroxy iso-leucine, a novel amino acid potentiator of insulin secretion. Diabetes 1998; 47: 206-10.
7. Suresh Kumar G, Shetty AK, Sambaiah K, Salimath PV. Antidiabetic properties of fenugreek seed mucilage and spent turmeric in streptozotocin-induced diabetic rats. Nutr Res 2005; 25: 1021-8.
8. Ng TB, Wong CM, Li WW, Yeung HW. Insulin like molecules in Momordica Charantia Seeds. J Ethnopharmacol 1986; 15: 107-17.
9. Raza H, Ahmed I, John A. Tissue specific expression and immunohistochemical localization of glutathione S-transferase in streptozotocin induced diabetic rats: Modulation by Momordica chirantia (karela) extract. Life sci 2004; 74: 1503-11.
10. Gover JK, Yadav SP. Pharmacological actions and potential uses of Momordica chirantia: a review. J Ethnopharmacol 2004; 93: 123-32.

11. Szkudelski T. The Mechanism of Alloxan and Streptozotocin Action in B Cells of the Rat Pancreas. Physiol Res 2001; 50: 536-46.
12. Arulmozhi DK, Veeranjaneyulu A, Bodhankar SL. Neonatal streptozotocin-induced rat model of type 2 diabetes mellitus: A glance. Indian J Pharmacol 2004; 36: 217-21.
13. Storlien LH, Higgis JA, Thomas TC, Brown MA, Wang HQ, Huang XF. Diet composition and insulin resistance action in animal models. B J Nut 2000; 83 Suppl 1: 85-90.