Indian Journal of Experimental Biology
Vol. 47, July 2009, pp. 564-570

Antihyperglycemic, antihyperlipidemic and antioxidant effects of Dihar, a polyherbal ayurvedic formulation in streptozotocin induced diabetic rats

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Received 22 July 2008; revised 9 March 2009

Present investigation was undertaken to evaluate antihyperglycemic, antihyperlipidemic and antioxidant activities of Dihar, a polyherbal formulation containing drugs from eight different herbs viz., *Syzygium cumini, Momordica charantia, Emblica officinalis, Gymnema sylvestre, Enicostemma littorale, Azadirachta indica, Tinospora cordifolia* and *Curcuma longa* in streptozotocin (STZ, 45mg/kg iv single dose) induced type 1 diabetic rats. STZ produced a significant increase in serum glucose, cholesterol, triglyceride, very low density lipoprotein, low density lipoprotein, creatinine, and urea levels in diabetic rat. Treatment with Dihar (100 mg/kg) for 6 weeks produced decrease in STZ induced serum glucose and lipids levels and increased insulin levels as compared to control. Dihar produced significant decrease in serum creatinine and urea levels in diabetic rats. There was a significant decrease in reduced glutathione, superoxide dismutase, catalase levels and increase in thiobarbituric acid reactive species levels in the liver of STZ-induced diabetic rats. Administration of Dihar to diabetic rats significantly reduced the levels of lipid paroxidation and increased the activities of antioxidant enzymes. The results suggest Dihar to be beneficial for the treatment of type 1 diabetes

Keywords: Antidiabetic, Antioxidant, Polyherbal formulation, Lipid profile, Oxidative stress, Streptozotocin

The number of patients with diabetes mellitus is markedly increasing worldwide. Diabetes mellitus is associated with impaired glucose metabolism that leads to an increase in free radical production and increase in triglyceride and lipoprotein levels[1]. Oxygen free radical can initiate peroxidation of lipids, which in turn stimulates glycation of protein, inactivation of antioxidant enzymes and play a role in the long-term complications of diabetes[2]. Therefore, among the various therapeutic strategies, combination of antihyperglycemic, antihyperlipidemic and antioxidant activity can be beneficial in the prevention of diabetes mellitus and its complications. In spite of the presence of known antidiabetic medicines in the pharmaceutical market, remedies from medicinal plants are used with success to treat this disease possibly because they are considered to be less toxic and free from side-effects compared to synthetic one[3]. Poly herbal formulation rather than individual herbal formulation are commonly used and many herbal formulations such as D-400[4], Trasina[5], Hyponidd[6], Cogent db[7], tincture of punchparna[8], Diasulin[9] and Diamed[10] have been shown for their antidiabetic, antihyperlipidemic, antioxidant or all the effects.

Dihar is one such polyherbal formulation composed of 10 medicinal plants (Table 1). These plants are known to possess antidiabetic, antihyperlipidemic and antioxidant activity (Table 2) and have been used in indigenous system of medicine to treat diabetes mellitus. According to the traditional system of Indian medicine, a combination of substances is used to enhance the desired activity and eliminate unwanted side effects. In view of above information the present study has been undertaken to evaluate the antihyperglycaemic, antihyperlipidemic and antioxidant activity of Dihar and its effect on serum urea, serum creatinine in streptozotocin-induced type 1 diabetic rats.

Materials and Methods

Materials—All the plants used in formulation were authenticated by Department of Pharmacognosy, L.M.College of Pharmacy, Gujarat University, Ahmedabad, India. The antidiabetic plants were mixed and formulation was prepared by Rajsha

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Pharmaceuticals (Ahmedabad, India) named as Dihar (Table 1) on the basis of an ayurvedic antidiabetic formulation proposed by Pandey[11]. Streptozotocin (STZ) was purchased from Sigma Chemicals (St. Louis, USA). Glucose, triglyceride, total cholesterol, cholesterol-HDL, creatinine, urea kits were purchased from Span Diagnostics (Vadodara, India). Radioimmunoassay kit for rat insulin was obtained from Bhabha Atomic Research Centre, Mumbai. Other chemicals used were of analytical grade.

Experimental animals—Male Wistar rats weighing 200-250 g were obtained from the animal facility of Zydus Research Centre, Ahmedabad, India. They were maintained under standard environmental conditions (12h light/dark cycle at 20°-25°C and controlled humidity) and provided with feed and purified water *ad libitum.* All experiments and protocols described in present study were approved by Institutions Animal Ethics Committee (IAEC) and are in accordance with guidelines as per "Guide for the care and use of laboratory animal" and with permission from Committee for the Purpose of Control and Suppression of Experiments on Animals (CPCSEA).

Experimental protocol—Diabetes was induced by single injection of STZ (45 mg/kg, iv) dissolved in normal saline. The control animals were injected with equal volume of vehicle. After 48 hr of STZ injection, animals showing glucosuria (>2%) were considered as diabetic. Animals (24) were divided into following 4 groups of 6 animals each: non-diabetic control, non diabetic treated, diabetic control and diabetic treated. Treatment with Dihar prepared in 0.5% CMC (100 mg/kg/po/day) was started after 3 days of STZ

Table 1—Composition of Dihar

Common name (Hindi name)	Botanical name	Part used	Family	Composition (%)
Black berry(Jamun)	*Syzygium cumini*	Seed	Myrtaceae	10
Bitter gourd (Karela)	*Momordica charantia*	Fruit	Cucurbitaceae	10
Indian gooseberry (Amla)	*Embelica officinalis*	Fruit	Euphorbiaceae	20
Ram's horn (Mesha Shringi)	*Gymnema sylvestre*	Leaves	Asclepiadaceae	10
Nagajivha (Chota chirayata)	*Enicostemma littorale*	Entire plant	Gentianaceae	10
Neem	*Azadirachta indica*	Leaves	Meliaceae	10
Gulancha tinospora (Guduchi)	*Tinospora cordifolia*	Root	Menispermaceae	10
Turmeric (Haridra/Haldi)	*Curcuma longa*	Rhizome	Zingiberaceae	18

Table 2—Phytochemical present in plants contained in Dihar and their pharmacological effects

Plant	Phytochemicals	Pharmacological effect	Reference
Syzygium cumini	Gallic acid, oxalic acid, citric acid, glycolic acids, ellagic acid, betulinic acid, friedelin, friedelan-3-α-ol β-sitosterol, anthocyanins, petunidin-3-gentiobioside.	Antidiabetic, Antihyperlipidemic, antioxidant	Prince *et al*.,[13].
Momordica charantia	Charantin, momordicosides A & B, acylglucosyl sterols, P-insulin,V-insulin, stigmasterol	Antidiabetic, Antihyperlipidemic, antioxidant	Raman & Lau[14]
Emblica officinalis	Gallic acid, ellagic acid, phyllantin, phyllantidine, emblicanin A & B, flavonoids	Antidiabetic, Antihyperlipidemic, antioxidant	Bhattacharya *et al*.[15].
Gymnema sylvestre	Gymnemic acid, saponins, stigmasterol, quercitol, betaine, choline, trimethylamine	Antidiabetic, Antihyperlipidemic, antioxidant	Bhaskaran *et al*.[16]. Wang *et al*.,[17].
Enicostemma littorale	Swetiamerin, vanillic acid, ferulic acid, p-coumaric acid, apigenin, genkwanin, isovitexin, swertisin, seponarin, gentiocrucine, enicoflavine	Antidiabetic, Antihyperlipidemic, antioxidant	Murali *et al*.[18].
Azadirachta indica	Isonimolicinolide, nimolicinoic acid, apœuphane, azadirachtin, nimbocinol, azadiradione and salannin	Antidiabetic, Antihyperlipidemic, antioxidant	Sonia & Srinivasan[19]
Tinospora cordifolia	Tinosporin, isocolumbin, palmatine, tinocordiside, Cordioside, ß-sitostrol	Antidiabetic, Antihyperlipidemic, antioxidant	Gupta *et al*.[20].
Curcuma longa	Curcumin, desmithoxy curcumin, bisdesmithoxy Curcumin, dihydrocurcumin, α & ß turmerome, eugenon, campestrol, stigmastrol,	Antidiabetic, Antihyperlipidemic, antioxidant	Halim & Ali[21].

injection and it was given daily for 6 weeks. Weekly food water intake and body weight gain were measured.

Blood sampling and biochemical analysis—At the end of 6 week treatment, the animals were kept for an over night fasting and the blood samples were collected and allowed to clot for 30 min at room temperature. The blood samples were centrifuged at 5000 rpm for 20 min and serum was separated and stored at -20º C until analysis was done. The same animals were subjected to Oral Glucose Tolerance Test (OGTT)[12]. To perform OGTT the animals were orally administered with 1.5 g/kg glucose and blood samples were collected from the tail vein under light ether anaesthesia before i.e. 0 min and 30, 60 and 120 min after oral glucose administration. Samples were analyzed for glucose and insulin. Plotting the glucose concentration versus time gives a curve showing rise and fall in glucose and insulin levels with time and expressed as integrated area under the curve for glucose and insulin ($AUC_{glucose,}$ AUCinsulin). This was calculated by applying trapezoid rule [$AUC = (C_1 + C_2)/2 \times (t_2 - t_1)$] and changes in glucose and insulin concentrations over 120 min during OGTT were expressed as $AUC_{glucose}$ (mg/dl.120min) and $AUC_{insulin}$ (μU/ml.120min) respectively.

Serum samples were analyzed spectrophotometrically for serum glucose, triglyceride, total cholesterol, HDL-cholesterol using their respective kits using UV-Visible spectrophotometer (Shimadzu UV-1601, Japan). Serum insulin was estimated by radioimmunoassay technique using gamma counter (Packard). Very low density lipoprotein (VLDL) and low density lipoprotein (LDL) were calculated as per Friedevald's equation:

$$VLDL = \frac{\text{total serum glycerides}}{5}$$

$$LDL = \text{Total cholesterol} - \frac{\text{Total triglycerides}}{5} - \text{HDL}$$

Assessment of oxidative stress related markers—Two days after the OGTT the animals were sacrificed and liver was isolated and weighed. Tissue were finely sliced and homogenized in chilled tris buffer. The homogenate were centrifuged and clear supernatant was used for estimation of various antioxidant parameters. Superoxide dismutase (SOD)[22], catalase[23] and reduced glutathione (GSH)[24] were determined. TBARS formation was determined as per Slater and Sawyer[25]. Result of antioxidant activity in liver was expressed in terms of protein content which was measured as per Lowry *et al*[26].

Statistical analysis—The results were analyzed using one-way factorial analysis of variance ANOVA followed by Tukey's multiple comparison test. The value of *P* less than 5% ($P<0.05$) was considered as significant.

Results

General features of experimental animals—Intravenous injection of STZ produced cardinal signs of type 1 diabetes i.e., loss of body weight, polyphagia, and polydipsia in rats. Chronic treatment with Dihar significantly ($P<0.05$) prevented loss of body weight, polydipsia, polyphagia in STZ-diabetic rats. There was no significant effect on the food and water intake of non-diabetic rats (Table 3).

Serum glucose, insulin—STZ induced diabetic rats exhibited significant hyperglycemia with a corresponding hypoinsulinaemia as compared to control rats. Treatment with Dihar produced significant ($P<0.05$) decrease in elevated serum glucose levels and serum insulin levels was increased significantly by the treatment with Dihar. It did not produce any significant effect on the serum glucose and insulin levels in non-diabetic rats (Table 4).

Oral glucose tolerance test—Results of oral glucose tolerance test revealed that $AUC_{glucose}$ significantly increased in diabetic control as compared to non-diabetic control. Treatment with

Table 3—Effect of treatment with Dihar on general features in STZ-induced diabetic rats
[Values are mean ± SE from 6 animals in each group]

Parameter	Non-diabetic control	Non-diabetic treated with Dihar	Diabetic control	Diabetic treated with Dihar
Body weight after treatment(g)	216 ± 0.37	207 ± 0.36	155 ± 1.75*	159 ± 1.32*#
Food intake (g/animal/day)	23 ± 1.59	20 ± 0.67	43 ± 1.59*	38 ± 1.03*#
Water intake (ml/animal/day)	27 ± 1.08	30 ± 0.96	70 ± 2.31*	60 ± 3.66*#

P values: < 0.05; significantly different from *control, #diabetic control

Dihar significantly decreased elevated $AUC_{glucose}$ of diabetic animals. $AUC_{insulin}$ of diabetic control was significantly decreased as compared to non-diabetic control group. Treatment with Dihar produced significant increase in $AUC_{insulin}$ of diabetic rats as compared to that of diabetic control (Table 4).

Serum lipid profile—Serum triglyceride, total cholesterol, LDL-cholesterol and VLDL-cholesterol levels were found to be increased significantly (P<0.05) in STZ induced diabetic rats as compared to non diabetic control. HDL-cholesterol was found to be significantly decreased in diabetic rats. Treatment with Dihar produced a significant reduction in elevated serum triglyceride, total cholesterol, LDL-cholesterol and VLDL-cholesterol levels in diabetic rats. There was an increase in HDL-cholesterol however, it was not statistically significant (P<0.05). Treatment of non-diabetic rats with Dihar did not produce any significant effects on lipid profile (Fig. 1).

Serum creatinine and urea—STZ induced diabetic rats were found to have significantly (P<0.05) elevated serum creatinine and urea levels as compared to non diabetic control rats. Treatment with Dihar produced a reduction in elevated serum creatinine and urea levels in diabetic or non diabetic rats (Table 5).

Effect antioxidant parameters in liver—STZ induced diabetic rats were found to have decreased SOD, GSH and catalase enzyme levels in liver as compared to control. Treatment with Dihar produced significant increase in these enzyme levels. Treatment of non-diabetic rats with Dihar did not produce any effect on the SOD, catalase, and GSH levels. STZ-diabetic rats were found to exhibit significant increase in TBARS levels in liver as compared to control rats. Treatment with Dihar produced significant decrease in TBARS level. Treatment of non-diabetic rats with Dihar did not produce any significant effect on the TBARS levels (Fig. 2).

Table 4—Effect of treatment with Dihar on glucose and insulin in STZ-induced diabetic rats
[Values are as mean ± SE from 6 animals in each group]

Parameter	Non-diabetic control	Non-diabetic treated with Dihar	Diabetic control	Diabetic treated with Dihar
Serum glucose (mg/dl)	98.4 ± 5.25	102.4± 3.22	426.6 ± 29.87*	314.3 ± 25.11*#
Serum insulin (μU/ml)	18.44 ± 0.79	19.00 ± 0.89	12.67 ± 1.20*	17.67 ± 0.60#
AUCglucose (mg/dl.min)×10^3	13.51 ± 0.79	13.93± 0.67	44.66 ± 5.65*	21.25 ± 2.35*#
AUCinsulin (μU/ml.min)×10^3	3.49 ± 0.20	3.54 ± 0.19	1.65 ± 0.17*	2.96 ± 0.05*#

P values: < 0.05; significantly different from *control, #diabetic control

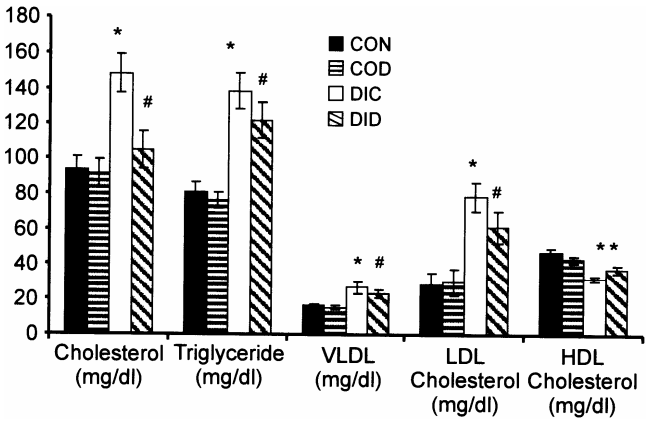

Fig. 1—Effect of chronic treatment with Dihar on serum cholesterol, triglyceride, VLDL-cholesterol, LDL-cholesterol and serum HDL-cholesterol in control and diabetic rats. Each bar represents mean ± SE of 6 animals. [CON-nondiabetic control. COD-control animals treated with Dihar, DIC-diabetic animals. DID-diabetic animals treated with Dihar. *P* values: < 0.05; significantly different from *control, #diabetic control]

Discussion

STZ induced diabetic rats exhibited decreased body weight, polyphagia and polydipsia associated with decrease in endogenous insulin and hyperglycemia. Chronic treatment with Dihar to diabetic rats not only decreased food and water consumption and improved loss of body weight, but also caused decrease in blood glucose and increase in insulin level in STZ diabetic rats. These effects may be attributed to either inhibition of increase in insulin output, inhibition of the intestinal absorption of glucose and increase in glucose metabolism because Dihar contains eight medicinal plants each having different mechanisms of action for antidiabetic activity. *Momordica*

Table 5—Effect of treatment with Dihar on serum creatinine and urea in STZ-induced diabetic rats
[Values are mean ± SE from 6 animals in each group]

Parameter	Non-diabetic control	Non-diabetic treated with Dihar	Diabetic control	Diabetic treated with Dihar
Serum creatinine (mg/dl)	1.31 ± 0.13	1.34 ± 0.09	1.82 ± 0.12*	1.78 ± 0.12*#
Serum urea (mg/dl)	38.49 ± 3.87	43.45 ± 1.90	64.02 ± 3.68*	55.29 ± 1.85*#

P values: < 0.05; significantly different from *control, #diabetic control

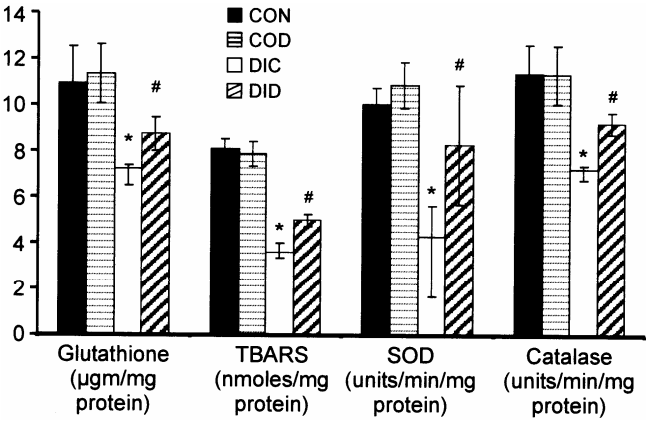

Fig. 2—Effect of chronic treatment with Dihar in control and diabetic rats on SOD, Catalase, TBARS and GSH Each bar represents mean ± SE of 6 animals. [CON- nondiabetic control animals. COD- control animals treated with Dihar, DIC-diabetic animals, DID-diabetic animals treated with Dihar. *P* values: < 0.05; significantly different from *control, #diabetic control]

charantia[27], *Azadirachta indica*, *Tinospora cordifolia*[28] and *Gymnema sylvestre*[29] have been reported to produce antihyperglycemic effect by increase in insulin secretion. While, *Enicostemma littorale*[18], *Emblica officinalis*[19] and *Syzigium cumini*[13] have been reported to produce antidiabetic activity by increasing insulin sensitivity. Many of plants have been reported to contain substances like glycosides, alkaloids, terpenoids, flavonoids, tannoids etc. which have been proved to be antidiabetic by different mechanism of action. Dihar showed significant decrease in $AUC_{glucose}$ and increase in $AUC_{insulin}$ on orally administered glucose load in oral glucose tolerance test in diabetic rats. These increased glucose disappearance rate may be due to increased insulin release. In the present investigation STZ induced diabetic rats found to produce increase in triglyceride, total cholesterol, LDL cholesterol, VLDL cholesterol levels which correlates with earlier findings that there is an increase in lipid levels is observed not only in diabetic animals but also in diabetic patients[31]. Chronic treatment of Dihar produced significant decrease in hypercholesterolemia and hypertriglyceridemia in diabetic rats. Thus apart from the regulation of carbohydrate metabolism, Dihar also played an important role in the metabolism of lipids. The present findings coincide with those of earlier studies, which reported that, plants present in Dihar like *Syzigium cumini* and *Momordica charantia* reported to produced antihyperlipidemic activity in high fat fed rat[13,14]. *Emblica officinalis* is reported to produce hypolipidemic effect in cholesterol fed rabbit, *Gymnema sylvestre* is reported to produce hypolipidemic effect by inhibition of intestinal absorption of fatty acid in rats[17], *Enicostemma littorale, Azadirachta indica, Tinospora cordifolia* and *Curcuma longa* are also reported to produce antihyperlipidemic activity in various animal experiments[18,19,21]. The possible mechanism for decreased lipid levels could be either insulin releasing effect of Dihar or insulin sensitizing activity, because insulin has been proved to inhibit the activity of the hormone sensitive lipases in adipose tissue and suppresses the release of lipids[32].

The HDL-cholesterol is involved in transport of cholesterol from peripheral tissues to liver and thereby it acts as a protective factor. In the present study also level of HDL-cholesterol was found to be decreased in diabetic rats. The level of HDL-cholesterol was increased in STZ induced diabetic rats when treated with Dihar. This indicates that Dihar may help to increase transport of peripheral tissue cholesterol to liver and thereby decrease blood cholesterol level.

STZ diabetic animals showed a significant increase in serum creatinine and urea levels as compared to control animals. The increase in serum creatinine and urea levels may be due to hyperglycemia that causes osmotic diuresis and depletion of extracellular fluid volume; several studies also have shown an increased correlation between serum creatinine and urea in diabetic patients[33]. Treatment with Dihar found to decrease serum creatinine and urea levels. This may be correlated with decrease in glucose levels by Dihar and thereby decrease in osmotic diuresis and depletion of extracellular fluid volume.

Numerous experimental and clinical observations have indicated that hyperglycemia may directly or indirectly contribute to an increased formation of free radicals and consequently to the onset of oxidative stress which has been implicated in diabetes associated complications[34]. Oxidative stress is a condition of reduction in antioxidative enzymes like SOD, GSH, and catalase levels[35]. Antioxidants thus play an important role to protect the human body against damage by reactive oxygen species. In the present investigation, a decrease in SOD, GSH, catalase levels was observed in the liver of diabetic rats. Chronic administration of Dihar produced antioxidant effect by increase in SOD, GSH and catalase levels. STZ diabetic rat also showed

increased level of TBARS, a marker of fatty chain paroxidation because high concentration of lipid was found to be present in liver of diabetic rat which results in the activation of NADPH dependent microsomal lipid paroxidation in liver[36]. The treatment with Dihar decreased TBARS level significantly in diabetic rats indicating protection against lipid paroxidation. The results of the present study indicate that the antioxidant effects of Dihar may be due to inhibition of lipid paroxidation and increase in antioxidant enzymes by medicinal plants present in Dihar, because all plants of Dihar contains substantial amounts of antioxidants like flavonoids and tannins which are reported to produce antioxidant action. Hence, in addition to antidiabetic and antihyperlipidemic effect, Dihar also possess antioxidant potential that may be beneficial for correcting the hyperglycemia and preventing diabetic complications due to lipid peroxidation and free radicals. On the basis of these results, it could be concluded that Dihar, a combination of eight herbal plants exerts a significant antidiabetic, antihyperlipidemic and antioxidant effect. This could be due to different types of active principles from various plants, which may have different mechanisms of action. Therefore, combination may be beneficial. However, it cannot be concluded that combination of eight plants may have synergistic or additive effect. Although, further studies remains to be conducted to investigate this hypothesis. The herbal formulation considered as safe supplementary therapy for a long-term and effective management of diabetic patients.

Acknowledgement

This study was supported by National Facilities in Engineering and Technology with Industrial Collaboration (NAFETIC) scheme of All India Council on Technical Education, New Delhi

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