Research Article

Evaluation of Calotropis Gigantea (l.) and S. Indicus on Glycemia and Lipidemia in Sterptozotocin induced Diabetic Rats

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ABSTRACT

In Ayurvedic system of medicine in India, not only extracts of one plant or the other but also a combination of plant extracts are used for the treatment of diabetes mellitus. The present paper reports the combined effect of Calotropis gigantea and S. indicus known to be useful for the treatment of diabetes in Ayurveda on the fasting blood sugar, glucose tolerance and lipid profile of Streptozotocin (STZ) induced albino rats. 300mg of water extract of the mixture of dried powdered leaves of Calotropis gigantea leaves and S. indicus in equal proportions was given once daily for 8 weeks. After 8 weeks of treatment of Streptozotocin (STZ) diabetic rats, the fasting blood sugar came down to almost normal value and improvement in glucose tolerance and serum lipid profile were also observed.

Keywords: Calotropis gigantea leaves, S. Indicus, Streptozotocin (STZ), type 2 diabetes.

INTRODUCTION

Diabetes, a metabolic disorder involving high blood sugar levels due to the non functioning of a key hormone called insulin, has been on the risk across the world. Diabetes mellitus (DM) is a metabolic disorder characterized by hyperglycemia resulting from defects in insulin secretion, insulin action or both.[1] The risk of diabetic complications, particularly cardiovascular diseases (CVD) peripheral vascular disease (PVD).[2] Complications such as coronary artery disease (CAD), stroke, neuropathy, renal failure, retinopathy amputations, and blindness etc are known to be associated with DM.[3] Insulin and various types of hypoglycemic agents such as biguanides and sulfonylureas including some of the recently developed ones are available for the treatment of diabetes. But none are ideal in treatment due to the toxic side effect and sometimes diminution in response after prolonged use.[3-4] The disadvantages of the presently available drugs are that they have to be given throughout the life and produce side effect.[4] A variety of plant preparations have been mentioned in Ayurveda and other indigenous systems of medicine, which are claimed to be useful in the treatment of diabetes mellitus.[5] World Health Organization (WHO) has suggested the evaluation of the potential of plants as effective therapeutic agents, especially in areas where we lack safe modern drugs.[6] In the ongoing search for more effective and safer drugs attention is being paid to new and safe drugs.[6-8] There are many studies on Calotropis gigantea which show that it exhibits anti-ulcer, anti-syphilis and anti-diabetic activities. Some of the plants useful for the treatment of diabetes mellitus including those from which some active constituents were isolated have been recently revived by Shukala et. Al.[9] Reported that Calotropis gigantea possesses not only anti-hyperglycemic effect but also hypolipidemic effect.[10-11] S. indicus is also known to be a drug useful for the treatment of diabetes mellitus.[12] In this paper the combined effect of water extract of a mixture Calotropis gigantea leaves and S. indicus in streptozotocin (STZ) induced diabetic in rats is reported.

MATERIALS AND METHODS

Calotropis gigantea was collected from the forest area of Ghaziabad, U.P., India and S. Indicus was obtained from the market of Ghaziabad, U.P and both plants were identified from the School of Pharmacy, Vishveshwariya Institute of Medical Science, Greater Noida, Gautam Budha, Nagar, U.P., India. They were assigned voucher specimen Ref. VIMS/CONSULT/2009/02/10 and Ref. VIMS/CONSULT/2009/02/11. The leaves of Calotropis gigantea leaves and S. indicus were air dried and powdered in a grinder and mixed in equal proportions.

Preparation of water extract

300 gm of powdered mixture of the two plant parts was extracted overnight with 360 ml of water with magnetic stirring in cold room (4°C). The water extract was separated and the residue was re-extracted with water. The combined water extract was concentrated in lyophilizer.

Animals

Wistar albino rats were obtained from R.V. Northland Institute, Greater Noida, Gautam Budha, Nagar, U.P., India, clearance is taken from animal ethics committee (IEC). Adult rats of either sex weighing between 150-200 gms were selected for the study. The animals were acclimatized to laboratory conditions and divided into various groups. Animals were housed and kept on the light and dark cycle throughout.

Induction of diabetes and associated neuropathy

Healthy adult albino wistar rats of both sex weighing between 150-200 gm were obtained from R.V. Northland Institute, Greater Noida, Gautam Budha, Nagar, U.P., India, and used in this study. The animals were fed on a pellet diet (Hindustan Lever, India) and water provided ad libitum. Diabetes was experimentally induced to produce diabetic neuropathy.[13] Sorbitol induced disfunction of inositol / metabolites leading to neuron-infraction by causing microangiopathy of vasa nervosum it deceases blood flow to nerves. Overnight fasting animals were injected with streptozotocin (STZ) 60 mg / kg dissolved in 3 mM citrate buffer (pH 4.5) intraperitoneally (i. p.). After 10 days only those rats which showed plasma glucose level > 300 mg / dl were classified as diabetic and were included in study as described earlier.[14] Animals were divided into three groups of five each. Group1 animals served as healthy controls, while those of the group 2 were untreated diabetic rats. Rats of group 3 were diabetic and treated for 8 weeks with 300 mg of water extract of Calotropis gigantea leaves plus S. indicus.[13-14] Blood samples were collected from overnight fasted rats at 0 and 8 weeks. Blood glucose serum total cholesterol, HDL and LDL cholesterol, triglyceride, and Glycosylated haemoglobin were determined using kits from Randox Mumbai. Total proteins albumin and creatinine in serum were determined by the method of Reinhold. Assay of plasma glucose and albumin and creatinine and total cholesterol, LDL – VLDL and HDL cholesterol and triglycerides were estimated as described earlier. Lipid peroxidation products were estimated as thiobarbituric acid reactive substance (TBARS) in plasma and tissues.[15-16]

Statistical analysis

All the data were statistically evaluated and the significance calculated using student's test. All the results were expressed as mean ± SD.

RESULTS AND DISCUSSION

The result obtained with untreated diabetic rats and diabetic rats treated with Calotropis gigantea plus S. indicus on fasting blood glucose and GTT are compared with normal healthy controls and shown in Table 1 and Table 1A. It is seen that treatment with water extract of Calotropis gigantea plus S. Indicus at a dose of 300 mg / kg body weight brought down fasting blood glucose (Table 1), from a higher value of 166.9 ± 25.4 mg / dl to a normal value of 85.4 ± 2.3 mg / dl while in the untreated group the FBG increased from the initial value of 172.2 ± 5.4 to 285.6 ± 42.6 mg / dl. There was considerable fall in FBG in diabetic rats treated with Caloptropis gigantea leaves alone or S. indicus alone. The effect was more with S. indicus. But the effect of the two plants in combination was more than that with either of the plants alone. Similar improvement to normal glucose tolerance was seen (Table 1A). In the diabetic untreated rats the blood sugar was 269.0 ± 92.2 mg / dl even after 2 hrs of glucose load in GTT. But in the Calotropis gigantean plus S. indicus treated rats the 2 hrs blood glucose value was in the normal range of 75.2 ± 1.0 mg /dl. The fasting (0hr) blood glucose values which were higher in the diabetic animals (160.5 ± 32.1) were brought down to 81.0 ± 3.5 mg / dl, when 300 mg of the extract of the mixture of the two plants was administered for 8 weeks. There was improvement in glucose tolerance in diabetic rats treated alone with either of the plants. The exact mechanism of action of the plant extracts either alone or in combination cannot be stated. However it is possible that these extracts increase blood insulin levels and also stimulate utilization of glucose by liver and extrahepatic tissues. The changes in the lipid profile have also been studied (Table 2). Before treatment the total cholesterol (TC), LDL cholesterol (LDL-C) and TG were higher than in normal animals. After 8 weeks, the TC, LDL-C, LDL-C/HDL-C and TG values were still higher in diabetic untreated animals then in control animals. There was no change in HDL-C in untreated diabetic animals. However in the case of diabetic treated animals, with Calotropis gigantea alone, total cholesterol, LDL cholesterol and LDL-C/HDL-C values returned close to normal values. There was very slight fall in TG values probably because these plants could not show much effect on triglycerols. But further improvement in increase in HDL-C value was seen. With Calotropis gigantea and S. indicus plus Caltropis gigantia treatments similar values were obtained. Further there was reduction in TG values also. This shows again that the water extract of the mixture of two plants, which contains less than 300 mg of each of the two plants is as effective as 300 mg of water extract of each of the two plants. Improvement in lipid profile is suggestive of the action of the two plants on enzymes and lipid metabolism. The total proteins, albumin and creatinine in serum, glycosylated hemoglobin in blood and total body weight and weight of kidneys (in the animals killed after the experiment) were also analyzed after 8 weeks of treatment and the values are shown in Table 3 and Table 4. The untreated diabetic animals showed signs of neuropathy e.g. tropic ulcer on tail, slight edema in the paws of the legs. The animals looked lethargic and sickly. All these symptoms disappeared after treatment for 8 weeks with water extract of the two plants Calotropis gigantea and S. indicus. The treatment with water extract was so effective that the above mentioned complications seen in untreated diabetic rats disappeared. The treated diabetic animals showed appearance almost like normal rats. The overall behavior of the rats was normal. In conclusion it can be stated that the two plants Calotropis gigantea and S. indicus, have synergistic effect when given together. They have a strong anti-hyperglycemic and anti-hyperlipidemic effect. In table, only values of with water extract of combination of the two plants are shown. An interesting observation is that glycosylated hemoglobin (HbA1c) decreased to 4.8 ± 0.1 and returned to normal values after 8 weeks of treatment. Glycosylated hemoglobin content rather than FBG is considered as a more reliable index of glycemic control in the management of diabetes mellitus. Return to normal of HbA1c (Table 3) after treatment is a clear indication that

the diabetic state was well regulated after the treatment of diabetic animals. Serum albumin, total body weight and kidney weight increases in diabetic untreated animals. There was increase in total proteins, serum creatinine and serum acid in diabetic animals. After treatment there was decrease except in total protein. But after treatment the values were closer to the normal value. There was an improvement in the hemoglobin content of the blood also (Table 3). Herbal treatment improves the weight of liver and kidney in diabetic rats fed high fat diet, although it was not completely reversible in liver (Tables 4 and Table 5). Recovery from this type of neuropathy is eluting, although months and even years may elapse before it happens. This form of neuropathy is often referred to as diabetic amyotrophy. When the clinical picture is dominated by deep sensory loss, ataxia and stone of the bladder, with only slight weakness of limbs, the similarity can be heightened by the presence of lancinating pains in the legs, unreactive pupils and neuropathic arthropathy. Loss of nerve fibers is a prominent pathologic finding in the distal symmetric form of neuropathy. Since myelin is formed from the cell membranes of Schwan cells, one may infer that the Schwan cell is a primary target of the pathologic process in this type of diabetic neuropathy.

Table 1: Effect of treatment for 8 weeks with water extract of (300 mg/kg bw) Calotropis gigantea and S. indicus and mixture of the two plants on fasting plasma glucose level in streptozotocin (STZ) diabetic rats.

Plasma glucose (mg/dl) men ± S.D.		
Group	**0 Weeks**	**8 Weeks**
Control	99.4 ± 19.5	89.8 ± 5.21
Diabetic untreated	172.2 ± 5.4	285.6 ± 42.6
Diabetic + Calotropis gigantea	164.6 ± 25.0	105.4 ± 26.6
Diabetic + S. indicus	158.6 ± 10.0a	98.2 ± 25.2a
Diabetic + Calotropis gigantea + S. Indicus	166.9 ± 25.4	85.4 ± 2.3

a = p < 0.001; Group of 5 animals were used for each set of experiments; All the data were statistically evaluated and the significance was calculated using student's't'- test. All the results were expressed as mean ± S.D.

Table 1A: Effect of water extract (300 mg/kg b.w.) of Calotropis gigantean and S. indicus and mixture of the two plants on plasma glucose tolerance in diabetic rats after 8 weeks

Blood glucose (mg/dl) men ± S.D.					
Group	**0 hr**	**0.5 hr**	**1 hr**	**1.5 hr**	**2 hr**
Control	94.3 ± 21.0	140.2 ±11.2	132.6 ±27.3	116.2 ±10.0	102.0 ±12.0
Diabetic untreated	160.5 ±32.1	245.0 ±68.6	273.4 ±89.3	290.6 ±82.6	269.0 ±92.2
Diabetic + Calotropis gigantea	87.9 ±25.6	112.2 ±20.6	114.3 ±15.0	102.0 ±10.3	95.6 ±24.0
Diabetic + S. indicus	82.0 ± 4.2	86.0 ± 4.3	81.0 ± 3.9	80.0 ± 3.2	84.0 ± 3.2
Diabetic + C. gigantea + S. indicus	81.0 ± 3.5	84.0 ± 4.2	86.0 ±3.3	81.0 ±3.6	75.2 ± 1.0

Table 2: Effect of treatment for 8 weeks with water extract of (300 mg/kg bw) Calotropis gigantea and S. indicus and both Calotropis gigantea + S indicus on plasma lipid in diabetic rats

Group	TC (mg/dl) men ± S.D.	LDLC (mg/dl) men ± S.D.	HDLC (mg/dl) men ± S.D.	LDLC/HDLC (mg/dl) men ± S.D.	TG (mg/dl) men ± S.D.
Control	170.3 ± 10.3	78.0 ± 12.2	44.9 ±13.3	1.7 ±0.3	115.6 ± 42.6
Diabetic untreated	250.0 ± 14.9	152.2± 12.6	45.0 ±12.0	3.3 ±0.4	181.8 ±18.8
Diabetic + Calotropis gigantea	175.0 ± 12.3	99.7 ±16.8	51.3 ±8.4	1.9 ±0.4	174.0 ±15.8
Diabetic + S. indicus	187.0 ± 14.9	95.6 ±15.7	50.2 ±8.5	1.6 ±0.3	135.0 ±13.2
Diabetic + C. gigantea + S. indius	174.0 ± 10.3	51.3 ±6.3	48.3 ±8.2	1.8 ± 0.5	131.0 ±11.2

TC, LDL-C, HDL-C, TG = Total Cholesterol, Low Density and High Density Lipoprotein Cholesterol respectively and Triglyceride. Number of animals is 5 in each group.

Table 3: Effect of water extract of Calotropis gigantea plus S. indicus on glycosylated haemoglobin and body weight, values shown are those at the end of 8 weeks treatment, haemoglobin in blood and serum albumin total proteins and creatinine

Parameter	Normal men ± S.D.	Diabetic men ± S.D.	Diabetic treated men ± S.D.
Glycosylated haemoglobin (HbA1c) %	3.04 ± 0.02	9.4 ± 2.4	4.8 ± 0.1
Albumin (g/l)	55.0 ± 1.4	45.0 ± 3.1	52.9 ± 3.0a
Total protein (g/l)	69.0 ± 3.0	73.2 ± 2.9	78.2 ± 3.8a
Creatinine ml/min	0.75 ± 0.04	1.30 ± 0.15	0.91 ± 0.05a
Body weight (g)	283.6 ± 25.8	225.5 ± 45.0	280.4 ± 9.0
Kidney weight (g)	1.40 ± 0.05	2.92 ± 0.08	1.06 ± 0.10
Haemoglobin	16.0 ± 4.2	____	ND
Urine sugar	10.5 ± 1.2	+++	++
Urine albumin	ND	++	ND

The values are men ± S.D; *a p < 0.05 when compared with diabetic untreated group.

Table 4: Effect of water extract of Calotropis gigantea plus S. indicus on the serum lipid profile in rats fed on high fat diet

	TC mg/dl	TG mg/dl	LDL	HDL-C
Normal	198.7 ± 44.9	94.9 ± 7.1	83.8 ± 16.2	42.3 ± 19.2
High-fat rats	622.3 ± 1.4	34.7 ± 0.9	3.58 ± 0.11	31.0 ± 0.10
High-fat diet plus extracts	182.5 ± 4.2	171.8 ± 0	148.8 ± 8.0	173.1 ± 23.4

Table 5: Effect of water extract on liver and kidney weight in rats fed on high fat diet

Tissues	Normal rats	High-fat diet untreated	High-fat diet plus extracts of two plants
Liver (gm)	7.82 ± 0.33	10.4 ± 0.41	9.8 ± 0.18[b]
Kidney (gm)	0.99 ± 0.05	1.08 ± 0.08	0.99 ± 0.06[b]

*b p<0.0005 *c p<0.01

CONCLUSION

In conclusion it appears that the water extract of combination of Calotropis gigantea plus S. indicus has got good hypoglycemic and hypolipidemic effect and also corrects complications associated with diabetes such as, retinopathy, nephropathy, neuropathy and musculopathy. Further research is needed to corroborate the same beneficial effects and to discuss the underlying mechanism involved in the above findings.

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