Effects of a Mixture Of *Salacia reticulata* W. and *Catharanthus roseus* L. Extracts in Streptozotocin-Induced Juvenile Diabetic Rats

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Abstract

Background: Currently, the focus in management of diabetes is to look for balanced modulation of several targets that can provide a superior therapeutic effect and a decrease in side effect profile. Most of the studies have looked for the effects of a single plant extract in adult rats. Hence, in the present study, the hypoglycemic and hypolipidemic effects of a mixture of two herbal plant extracts, *Salacia reticulata* W. (*S. reticulata*) and *Catharanthus roseus* L. (*C. roseus*) have been evaluated in streptozotocin-induced juvenile diabetic rats.

Methods: The combined extract of *S. reticulata* and *C. roseus* (100 mg/kg body wt. each) was administered intragastrically to streptozotocin-induced (50 mg/ kg body wt.) juvenile diabetic Wistar rats for 30 days. The body weight, blood glucose, serum cholesterol and serum triglycerides were measured and compared with control, diabetic control and insulin treated groups of rats (n = 6 per group).

Results: Treatment with combined extract of *S. reticulata* and *C. roseus* (100 mg/kg body wt. each) to diabetic rats for 30 days significantly reduced blood glucose, serum cholesterol, and serum triglycerides to near control levels.

Conclusion: The present results show that the combined extracts of *S. reticulata* and *C. roseus* have hypoglycemic and hypolipidemic effects in streptozotocin-induced juvenile diabetic rats.

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Keywords: *Salacia reticulata, Catharanthus roseus, diabetes mellitus, blood glucose, serum lipids*

Diabetes mellitus (DM) is a metabolic disease characterized by hyperglycemia due to absolute or relative deficiency of insulin. The term Type 1 DM (T1DM) is used to denote childhood diabetes associated with autoimmunity and absolute insulin deficiency that result from an autoimmune destruction of insulin producing cells in the pancreas.[1] Over the last few years, the incidence of DM has increased in an epidemic-like fashion all over the world. T1DM is the commonest form of DM in children (1.7 of 1000 children) and the second most common chronic disease during childhood around the globe.[2] The study undertaken by Madras IDDM Registry group suggest that the incidence of T1DM in south India is approximately 10.5 cases / 100,000 per year.[3] In T1DM, there are many metabolic disturbances, which, if not treated, progress and lead to central nervous system depression, coma and death.[2] Management of DM without any side effects is a challenge to the medical system. The only available treatment for T1DM is insulin, which has undesirable side effects and is not cost effective as well. This has lead to an increased demand by patients to use natural products with antidiabetic activity.[4,5]

Ayurvedic medicine is an ancient system of medicine that is native to the Indian subcontinent. Many medicinal herbs are presently being used either as medicine or food supplement by millions of people in India, Nepal and Srilanka.[6,7] *Salacia reticulata* W. belonging to the Hippocrateaceae family, is one such plant that has been used traditionally in Indian Ayurvedic system of medicine and is said to be effective for the prevention and treatment of diabetes.[6,8] Since several years people use extract of its roots as a herbal therapy for diabetes mellitus. Recent studies report that an extract of *S. reticulata* contains α-glucosidase inhibitors and that its polyphenolic constituents have lipase-inhibitory activity.[9-12]

Another plant that is supposed to have antidiabetic activity is *Catharanthus roseus* L. (Apocyanaceae). This plant is also known as Madagascar periwinkle/ *Vinca rosea/ Lochnera rosea*.[13] It is cultivated mainly for its alkaloids, which have anticancer properties.[14] The leaves are known to contain pharmacologically active compounds including nearly 150 different alkaloids. Significant antihyperglycemic and hypotensive activity of the leaf extracts have been reported in laboratory animals.[15] Fresh leaf juice of *C. roseus* has been reported to have hypoglycemic activity in normal and alloxan diabetic rabbits.[13] Even in streptozotocin-induced diabetic rats, the leaves and twigs of *C. roseus* have been reported to have hypoglycemic activity.[16,17]

However, most of the studies are done on adult animal models and for varying time duration using a single plant extract. Hence in the present study, streptozotocin-induced juvenile diabetic young rats are used, and the hypoglycemic and hypolipidemic effects of the combined plant extract treatment have been evaluated after prolonged treatment for 30 days.

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Materials and Methods

Streptozotocin was purchased from Sigma Aldrich Chemicals, Pvt., Ltd., Bangalore, India. All other chemicals and reagents used were of analytical grade.

Preparation of plant extracts

The roots of *S. reticulata* were collected from the forests of Western Ghats of India, with the help of local ayurvedic practitioners. The roots were thoroughly washed, shade dried and fine powdered. The root powder was then subjected to defatting with petroleum ether (60-80 %) and extracted with ethanol (95 % v/v) using soxhlet apparatus. The ethanol was removed from the extract by distillation under reduced pressure to obtain a dried residue. The dried residue thus obtained was stored in a refrigerator at 2-8^{o}C for subsequent experiments.

The leaves of *C. roseus* were collected from the local surrounding area and shade dried and powdered. The leaf powder was also subject to similar procedure to obtain a dried residue, which was also stored in a refrigerator for subsequent experiments.

Experimental animals and induction of diabetes

Highly inbred Wistar strain albino rats (150-160 g body weight) of both sexes were used. The animals were housed in groups of six in standard laboratory conditions of temperature (23 ± 5^{o}C), relative humidity (55 ± 5 %), lighting (08:00-20:00 h) with food and water *ad libitum*. The protocol was approved by the Institutional Animal Ethical Committee.

The animals were fasted overnight and T1DM was induced by a single intra-peritoneal (i.p.) injection of freshly prepared STZ (50 mg/kg body weight) in 0.1 M cold citrate buffer (pH 4.5).[18] The animals were allowed to drink 5% glucose solution overnight to overcome the drug-induced hypoglycemia. The animals were considered as diabetic, if their fasting blood glucose values were above 126 mg/dl on the third day after STZ injection. The treatment was started on the fourth day after STZ injection and this was considered as the first day of treatment. The treatment was continued for 30 days.

The rats were divided into four groups comprising of six animals in each group (n = 6) as follows:

Group 1: Normal control rats

Group 2: Diabetic control rats

Group 3: (Insulin-treated) Diabetic rats treated with plain insulin injection subcutaneously (2-4 units /kg body weight/day)

Group 4: (Formula-treated) Diabetic rats treated orally with a mixture of *S. reticulata*[19] and *C. roseus*[20] (each 100 mg/kg body weight/day) for 30 days.

The body weight was recorded in the different groups both at the beginning of study and at the end of 30 days. The data recorded at the end of 30 days treatment was used for statistical comparison. At the end of 30 days, fasting blood sample was collected from the retro orbital vein of all the rats in plain tubes and serum was separated immediately and used for various biochemical estimations.

Fasting blood glucose levels were estimated enzymatically by glucose oxidase-peroxidase method using commercially available kits.[21] The serum was used for the estimation of total cholesterol[22] and triglycerides[23] enzymatically.

Statistical analysis

The laboratory results were statistically analyzed using one-way analysis of variance (ANOVA) followed by Bonferroni multiple comparison test and Kruskal-Wallis test as required. P values < 0.05 were considered significant. All values are expressed as mean ± standard deviation.

Results

The results are given in Table 1. The body weight of diabetic control rats had decreased when compared to normal control group. Insulin treatment significantly increased ($P < 0.01$) the body weight when compared with the diabetic control group. The mean weight of the formula-treated rats had also increased when compared with the diabetic controls; however, the increase was not statistically significant.

A significant increase in fasting blood glucose level was observed in diabetic rats when compared with normal controls. Treatment with insulin significantly ($P < 0.001$) decreased the blood glucose levels. Administration of formula mixture significantly ($P < 0.001$) decreased the fasting blood glucose levels when compared to both

Table 1 Blood sugar and lipid profiles in the different groups of rats

Groups	Body weight (gm)	Fasting blood glucose (mg/dl)	Serum total cholesterol (mg/dl)	Serum total triglycerides (mg/dl)
Normal controls	150 ± 16.73	110.5 ± 10.41	60.5 ± 7.03	82 ± 27.66
Diabetic controls	113 ± 24.22	267.1 ± 16.84*	66 ± 15.78	117.16 ± 11.07
Diabetic + Insulin-treated	167.5 ± 24.39*	224.1 ± 18.94*[a]	54.16 ± 11.19	79.3 ± 23.17[a]
Diabetic + Formula-treated	154.16 ± 23.5	159.2 ± 25.61*[bc]	53.5 ± 6.12	78.33 ± 17.30[b]

Values are given as mean ± SD for groups of six animals each. Values are statistically significant at $P < 0.05$:
*Diabetic, Insulin-treated, Formula-treated rats were compared with control rats;
[a] Insulin-treated diabetic rats were compared with diabetic controls;
[b] Formula-treated diabetic rats were compared with diabetic controls;
[c] Formula-treated diabetic rats were compared with Insulin-treated rats.

diabetic controls and insulin treated diabetic rats.

Serum total cholesterol levels were increased in the diabetic control group when compared with normal controls, and this was decreased in insulin-treated group. In the formula-treated group the serum cholesterol levels were lowered, but to lesser extent than the insulin treated group. However, the decrease was not statistically significant.

The total triglyceride levels were higher in the diabetic control group when compared to normal controls, and this was decreased significantly by insulin treatment. Formula-treated rats also showed a significant decrease in total triglyceride concentration that is comparable to insulin treatment.

Discussion

In the present study, streptozotocin (STZ) has been used for the induction of diabetes in rats. STZ is an antibiotic derived from *Streptomyces achromogenes* and structurally is a glucosamine derivative of nitrosourea. STZ causes deficiency of insulin mainly by its selective, cytotoxic action on the pancreatic beta cells,[24,25] which in turn leads to hyperglycemia due to poor glucose utilization by tissues.[26]

In the present study, based on previous standardized studies, we have used an optimal dose of plant extracts.[19-20] The lethal dose for *S. reticulata* and *C. roseus* is 1 g/kg bodyweight and 0.5 g/kg body weight respectively.[27, 28] The toxicity of combined extract is yet to be studied. The results of our study show that the combined formula of *S. reticulata* and *C. roseus* possesses significant hypoglycemic and hypolipidemic effects.

Previous pharmacological studies in both humans and rodents have shown that the root extracts of *Salacia*, act as hypoglycemic and hypolipidemic agent. The possible mechanisms include inhibition of different enzyme activity like alpha-glucosidase, aldose reductase and pancreatic lipase. It may even act at the level of peroxisome proliferator-activated receptor-alpha mediated lipogenic gene transcription and angiotensin II/angiotensin II type 1 receptor.[29]

Many studies have proposed that antidiabetic action of *C. roseus* could be attributed to its active principles like catharanthine, vindoline and vindolinine.[30,31] These active principles possess multiple sites of action and the prolonged antidiabetic activity of *C. roseus* leaf extracts results from either an increase in glucose utilization by peripheral tissues[16] or by enhanced secretion of insulin from the beta cells of Langerhans.[13]

The hypolipidemic effect of the formula was comparable to that of insulin. The improvement in body weight of the formula treated animals further supports the antidiabetic effect of the formula, as diabetic condition is associated with loss of body weight.

Many traditional schools of medicine believe that, a balanced modulation of several targets can provide a superior therapeutic effect and decrease in side effect profile compared to a single action from a single selective ligand, especially in the treatment of chronic and complex disease like T1DM.[25] Many herbal medicines, as single agents or in different combined formulations have been recommended for diabetes mellitus, as they are less toxic than oral hypoglycemic agents and insulin. Antidiabetic herbal therapy is less expensive and have oral route of administration, when compared to insulin preparations.[32,33] In many developing countries, insulin availability is intermittent, and even may not be included in national formularies of essential drugs.[34] In such countries alternative medicine, especially preparations of locally available herbal plants may be much more feasible. In the present study, a combination of two different herbs have been used, each of which have been individually used as hypoglycemic agents in traditional medicine, which are available abundantly. The added advantage is that the combination may act at multiple levels to bring about the therapeutic effects.

Conclusion

The present investigation reveals that the treatment of the combined extracts of *S. reticulata* roots and *C. roseus* leaves has significant hypoglycemic effects in streptozotocin-induced juvenile diabetic rats. The hypoglycemic effect is more significant when compared with insulin treatment. The hypolipidemic effects are comparable to that of insulin treatment.

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Conflict of Interest

None to declare.

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