Vol 3 | Issue 1| 2012 | 1-5.

e - ISSN 2249-7544
Print ISSN 2229-7464

INTERNATIONAL JOURNAL
OF
PHYTOPHARMACY RESEARCH
www.phytopharmacyresearch.com

STUDIES ON ANTI DIABETIC POTENTIAL OF *SYMPLOCOS RACEMOSA* ROXB. BARK EXTRACT IN STREPTOZOTOCIN INDUCED DIABETIC RATS

Venkidesh R*, Dilipkumar Pal[o], Ashok Kumar C.K.[b], Saravanakumar A.[b], Subhash C. Mandal[a]

*Faculty of Pharmacy, AIMST University, Bedong, Kedah, Malaysia.
[b]Sree Vidyanikethan College of Pharmacy, Tirupati, A.P., India.
[o]Institute of Foreign Trade & Management (IFTM), Moradabad, India.
[a]Dept. of Pharmaceutical Technology, Jadavpur University, Culcutta, India.

ABSTRACT

In modern medicine sulphonylureas, biguanides and thiazolidinediones, are used for controlling diabetes but there is a renewed interest in plant medicine due to their toxicity. *Symplocos racemosa* barks are used for various ailments and ethnopharmacologically the drug is valued for its treatment against diabetes. The aim of the present study was to evaluate the anti-diabetic effects of *Symplocos racemosa* Roxb. methanol extracts (MESR) in streptozotocin (STZ) induced diabetic rats. The bark of plant was extracted with methanol. Albino Wistar rats were used for the study. Diabetes was induced using STZ. The treatment was continued for 14 days. The rats were divided into groups. Blood glucose level, biochemical parameters and histopathological observation were done. MESR, administered at doses of 250 & 500 mg/kg to STZ-treated diabetic rats caused significant ($p < 0.01$) reduction of blood glucose levels. MESR showed a dose related significant ($p < 0.01$) reduction in triglycerides compared to pretreatment levels. The reduced glucose levels suggested that MESR might exert insulin-like effect on peripheral tissues by either promoting glucose uptake metabolism by inhibiting hepatic gluconeogenesis. The altered serum lipid profile was reversed towards normal after treatment with the MESR. MESR exhibited hypocholesterolemic and hypotriglyceridemic effects, while increased the levels of HDL in STZ - induced diabetic rats.

Key words: *Symplocos racemosa* Roxb., STZ-induced diabetic rats, anti-diabetic, LDL, VLDL, TG, Total cholesterol.

INTRODUCTION

Diabetes mellitus (DM) comprises a group of common metabolic disorders characterized by hyperglycemia. Many distinct types of DM exist and the etiology being a complex interaction of genetics, environmental factors, and life-style choices. Depending on the cause for the DM, factors contributing to hyperglycemia may include reduced insulin secretion, decreased glucose utilization, and increased glucose production. The metabolic dysregulation associated with DM causes secondary pathophysiologic changes in multiple organ systems that impose a tremendous burden on the individual with diabetes and on the health care system [1].

The crude drugs used to treat diabetes are of higher significance to ethnobotanical community as they are recognized to contain valuable medicinal properties in different parts of the plant. The characterization and standardization of such crude drugs involve the identification of the various composition of secondary metabolites present, which are present in the form of whole plant, parts of the plants(s)like root, seed, and on.

Rarely does drug activity in a plant depend upon single component. It is believed that the result of synergistic or mixed activity of several active components as well as the inert substances the crude drug exerts its activity. Though the involvement of the inert principles in Pathology of disease process or in the biochemical process is nil or limited, it is reasonable to use such components, which might influence bioavailability, stability of the active principle and excretion of active component. The rate of side effects is minimized. If there are different active

Corresponding Author: **Venkidesh R.** Email:- venkidesh2001@yahoo.com

components present in a plant drug, they might have synergistic or potentiation effect [2-5].

Governing and controlling diabetes is a general problem as there is no satisfactory effective therapy available till now. In modern medicine insulin, sulphonylureas, biguanides and thiazolidinediones, are used but there is a renewed interest in plant medicine due to their toxicity [6,7] for the treatment against different diseases as herbal drugs are generally out of toxic effect [8,9] reported from research work conducted on experimental model animal.

Symplocos racemosa is a tree in Sanskrit called as Lodhra or Srimata meaning "Propitious" and "Tilaka" because it was used in making the Tilaka mark on the forehead. A decoction of the bark was used for gargling when the gums were spongy and bleeding. It is remarked that the bark was popular among the dyes of red in Calcutta and seemed to be used as a mordant only. In Europe it was formerly looked upon as a *cinchona* bark and had been known at various times as "Ecorce de Lautour", "*China nova"* ,"*China Calafornica*", "*China Brasilarsis*" and "*China paraquatan*".

In the previous study hypoglycemic and hypolipidemic activity of *Symplocos racemosa*. in alloxan induced diabetes in albino wistar rats was reported. Therefore the present study is to conform the anti-diabetic activity of *Symplocos racemosa* in STZ induced diabetic rat model.

MATERIALS AND METHODS

Plant collection

The Plant materials of *Symplocos racemosa* Roxb. bark *were collected from the market in Chennai*. The bark of the plant was authenticated by Dr. K. Madhava Chetty, Department of botany, S.V.University, Tirupathi.

Preparation of extracts

The bark of plant were dried in shade, separated and made to dry powder. It was then passed through the 40 # sieve. A weighed quantity (200g) of the powder was subjected to continuous hot extraction in Soxhlet Apparatus. The extract was evaporated under reduced pressure using rotary evaporator until all the solvent has been removed to give an extract sample. Percentage yield of MESR (methanolic extract of *Symplocos racemosa*) was found to be 16.5% w/w.

Animals Used

Albino Wistar rats, weighing 150–200 g were used. The selected animals were housed in acrylic cages in standard environmental conditions (20–25ºC), fed with standard rodent diet and water *ad libitum*. The experiments on animals were conducted in accordance with the internationally accepted principles for laboratory animal use and the experimental protocols duly approved by the Institutional Ethical Committee.

Experimental induction of diabetes

The animals were not fed overnight and made to fast diabetes was induced by a single intra-peritoneal injection of a freshly prepared solution of streptozotocin (STZ) (55 mg/kg body weight) in 0.1 M cold citrate buffer (pH 4.5). The animals were fed with 1% glucose solution overnight to overcome the drug induced hypoglycemia. Control rats were injected with citrate buffer. The animals were considered as diabetic, if their blood glucose values were above 250 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 first day of treatment. The treatment was continued for 14 days [10-12].

Experimental design

The rats were divided into four groups comprising of six animals in each group as follows Group I. Control rats receiving 0.1 M citrate buffer (pH 4.5).Group II. Diabetic control and rats received only vehicle (2 ml/kg p.o) 2% v/v Tween 80.Group III. Diabetic rats received methanol extract of *Symplocos racemosa* Roxb. (250 mg/kg/day p.o) suspended in 2% v/v Tween 80.Group IV. Diabetic rats received methanol extract of *Symplocos racemosa* Roxb. (500 mg/kg/day p.o) suspended in 2% v/v Tween 80.Group V. Diabetic rats treated with glibenclamide (600 µg/kg b.w/day) suspended in 2% v/v Tween 80 orally for 14 days.

Testing of fasting blood glucose level

Fasting blood glucose levels were measured on 0, 3, 7, and 14 days of treatment of methanol extract of *Symplocos racemosa* Roxb. bark *supplement* from the animals of all these groups. Blood was collected from tip of the tail vein and fasting blood glucose level was measured using single touch glucometer .The results were expressed in terms of milligram per deciliter (dL) of blood. At the end of the experimental period, all the animals were sacrificed under light ether anesthesia. The guide line of our institutional ethical committee for this purpose was followed strictly. The rats were sacrificed by decapitation and blood was collected with anti-coagulant and the serum was used for the estimation of total cholesterol and triglycerides.

Histopathological study of pancreas

Pancreas were isolated and preserved in 10% formalin. Histopathological observation of the tissue was carried out at the Sri Venkateswara University, Pathology Laboratory, Tirupati, Andhra Pradesh -517 502.

Statistical Analysis

The data were expressed as mean ± standard error mean (S.E.M). The Significance of differences among the group was assessed using one way and multiple way analysis of variance (ANOVA). The test followed by Dunnet's test *p* values less than 0.05 were considered as significance.

RESULTS

Effect of MESR on fasting blood glucose in STZ induced diabetic rats

The effect of repeated oral administration of MESR (methanolic extract of *Symplocos racemosa*) on blood glucose levels in STZ-diabetic rats is presented in Table 1. MESR, administered at doses of 250 & 500 mg/kg to STZ-treated diabetic rats caused significant ($p < 0.01$) reduction of blood glucose levels which was related to dose and duration of treatment. Maximum reduction was

observed on day 14. MESR 500 mg/kg exhibited maximum glucose lowering effect in diabetic rats. Glibenclamide exhibited significant reduction in blood glucose levels at the end of the study when compared to diabetic control.

Effect of MESR on serum lipids in STZ-induced diabetic rats

MESR showed a dose related significant ($p < 0.01$) reduction in triglycerides compared to pretreatment levels (Table 2). MESR at the doses of 250 and 500 mg/kg was dose dependently reduced the Total cholesterol, LDL, VLDL, TG levels than diabetic control rats.

Table 1. Effect of methanolic extract of *Symplocos racemosa* bark on the blood glucose regulation of diabetic rats

Group (n=6)	Fasting Blood Glucose Levels			
	0 th Day	5 th Day	10 th Day	15 th Day
Group I (Normal Control)	95±1.15	95.66±1.14**	95.16±0.79**	95.±1.15
Group II (Diabetic Control)	279.50±2.47	272.33±2.19	282.33±0.04	293.66±2.96
Group III (MESR-250 mg/Kg)	259.47±2.18	230.06±1.12	200.63±1.52	178.93±0.09
Group IV (MESR-500 mg/Kg)	254.76±1.40	201.64±2.65	183.16±0.04	154.03±1.29
Group V (Glibenclamide 0. 6 mg/Kg)	281.5±3.05	206.33±2.24	138.16±3.56	100±0.66

*Values are given as mean ± SEM for groups of six animals in each group. Values are statistically significant at *p<0.05 and **p<0.01 and ns-non significant. Significance compared within the groups as follows:* ***a****. diabetic + MESR - 250 & 500 treated rats vs. diabetic control rats.* ***b****. diabetic + glibenclamide treated rats vs. diabetic control rats.*

Table 2. Effect of *Symplocos racemosa* Roxb bark on the total lipid profile of STZ- induced diabetic rats

Groups(n=6)	Biochemical Parameters				
	TG(mg/dL)	TC(mg/dL)	HDL(mg/dL)	LDL(mg/dL)	VLDL (mg/dL)
Group-I (Normal Control)	80.72±0.43**	90.86±0.17**	34.39±0.50**	33.14±0.63**	15.01±0.90**
Group-II (Diabetic Control)	137.57±1.10	152.72±5.89	15.87±4.19	75.52 ± 4.19	48.2±5.12
Group-III (MESR-250mg/Kg)	120.14±1.59*	101±1.51**	26.01±0.76**	37.16±0.49**	31.81±0.50**
Group-IV (MESR-500mg/Kg)	100.07±2.49**	92.35±1.49**	29.72±0.95**	37.75±0.64**	27.59±1.82**
Group-V (Glibenclamide-0.6mg/Kg)	84.14±0.61**	82.00±0.49**	28.71±0.34**	32.17±0.25**	20.52±0.58**

*Values are given as mean ± SEM for groups of six animals in each group. Values are statistically significant at *p<0.05 and **p<0.01 and ns-non significant. Significance compared within the groups as follows: a. diabetic + MESR - 250 & 500 treated rats vs. diabetic control rats. b. diabetic + glibenclamide treated rats vs. diabetic control rats.*

DISCUSSION AND CONCLUSION

The currently available drug regimens for management of diabetes mellitus have certain drawbacks and therefore there is a need to find safer and more effective antidiabetic drugs. The aim of the present study was to evaluate the anti-diabetic effects of *Symplocos racemosa* Roxb. in STZ-induced diabetic rats. The experimental diabetic model used in this study was type 2 since low dose of STZ (55 mg/Kg body weight) destroyed some population of pancreatic beta cells. There were residual beta cells which secreted insufficient insulin causing type 2 diabetic model [13]. The mechanism by which streptozotocin STZ, a highly cytotoxic agent of pancreatic ß-cells induces diabetes by damaging the cells that causes reduction in insulin. The increased levels of plasma glucose in STZ-induced diabetic rats were lowered by the administration of *Symplocos racemosa* Roxb. The reduced glucose levels suggested that *Symplocos racemosa* Roxb. Might exert insulin-like effect on peripheral tissues by either promoting glucose uptake metabolism by inhibiting hepatic gluconeogenesis, [13,14] or by absorption of glucose into the muscle and adipose tissues, [15] through the stimulation of a regeneration process and revitalization of the remaining beta cells [16,-18].

The possible mechanism by which MESR mediated its antidiabetic effect could be by potentiating of pancreatic secretion of insulin from existing ß-cells of islets, as was evident by the significant increase in the level of insulin in the extract treated animals. In this context, a number of other plants have been reported to have antihyperglycemic activity with a stimulatory effect on insulin release [19,20]. Since the extract produced highly significant antihyperglycemic effect even in streptozotocin-induced diabetic rats in which most of the ß- cells are damaged, it is likely that MESR might have extra pancreatic mechanism of action. The other effects such as increase in the levels of total cholesterol, triglycerides and LDL-cholesterol and decrease in HDL cholesterol in STZ-treated animals could be secondary to a partially restored beta-cell function with increased insulin levels. From the results of the present study, it may be suggested that the mechanism of action of MESR may be similar to glibenclamide action. In diabetes, hyperglycemia is accompanied with dyslipidemia, [21,22], i.e.

characterized by increase in TC, LDL, VLDL, TG and fall in HDL. Hypercholesteremia and hypertriglyceridemia are primary factors involved in the development of atherosclerosis and coronary heart diseases which are the secondary complications of diabetes. This altered serum lipid profile was reversed towards normal after treatment with the MESR. MESR exhibited hypocholesterolemic and hypotriglyceridemic effects, while increased the levels of HDL in streptozotocin - induced diabetic rats. However, MESR was found to be more effective in reducing the levels of TG and LDL as compared to its effect on TC. The elevated atherogenic index, i.e. TC/ HDL ratio, which is a useful determinant of cardiovascular risk, [23], was also shifted towards normal after MESR treatment. Thus, it is reasonable to conclude that MESR could modulate blood lipid abnormalities. Summarizing, it could be proofed that the traditional use of *Symplocos racemosa* Roxb. as a hypoglycemic agent is justified and that extracts from this plant show a dose dependent activity which is comparable to the standard hypoglycemic drug glibenclamide.

REFERENCES

1. Fauci, Braunwald, Hauser, Longo, Jameson, Loscalzo, *Harrison's Principles of Internal Medicine,* 16th Edition, McGraw-Hill, 2005, 235-279.
2. Anonymous, *The International Pharmacopeia*, Vol. 4: Tests Methods, and General Requirements, 3rd edn. World Health Organization, Geneva, 1988.
3. Anonymous, WHO. *Guidelines for the Assessment of Herbal Medicines*. WHO Technical Report Series, No 863. World Health Organization, Geneva, 1996.
4. Anonymous, UNESCO, FIT/504-RAF-48.Terminal Report: *Promotion of Ethnobotany and the Sustainable Use of Plant resources in Africa*. Paris. 60, 1998.
5. Anonymous, WHO. *General Guidelines for Methodologies on Research and Evaluation of Traditional Medicine.* World Health Organization, Geneva, 2002.
6. Prince PS, Menon VP, Pari L. Hypoglycemic activity of Syzigium cuminii seeds: effect on lipid peroxidation in alloxan diabetes rats. *Journal of Ethnopharmacology*, 61, 1998, 1–7.
7. Ladeji O, Omekarah I, Solomon M. Hypoglycemic properties of aqueous bark extract of Ceiba pentandra in streptozotocin induced diabetic rats. *Journal of Ethnopharmacology*, 84, 2003, 139–142.
8. Geetha BS, Biju CM, Augusti KT. Hypoglycemic effects of leucodelphinidin derivative isolated from Ficus bengalensis (Linn.). *Indian Journal of Pharmacology*, 38, 1994, 220–222.
9. Rao BK, Sudarshan PR, Rajasekhar MD, Nagaraju N, Rao CA. Antidiabetic activity of *Terminalia pallida* fruit in alloxan induced diabetic rats. *Journal of Ethnopharmacology*, 85, 2003, 169–172.
10. Rakieten N, Rakieten ML, Nadkarni MV. Studies on the diabetogenic action of streptozotocin (NSC-37917). *Cancer Chemo Therapy Reports*, 29, 1963, 91–98.
11. Pari L, Uma Maheswari J. Antihyperglycemic activity of Musa sapientum flowers: effect on lipid peroxidation in alloxan diabetic rats. *Journal of Ethnopharmacology*, 14, 2000, 136–138.
12. Gomes A, Vedasiromoni JR, Das M, Sharma RM, Ganguly DK. Antihyperglycemic effect of black tea (*Camellia sinensis*) in rat. *Journal of. Ethnopharmacology*, 27, 2001, 243–275.
13. Ali L, Azad Khan AK, Mamun MIR, Mosihuzzaman M, Nahar N, Alan MNE, Rokeya B. Studies on the hypoglycemic effects of fruits pulp, seed and whole plant of Momordica charantia on normaland diabetic model rats. *Planta Med*., 59, 1993, 408–412.
14. Gray AM, Abdel-Wahab YHA, Flatt PR. The traditional plant treatment, Sabucus nigra (Elder), exhibits insulin-like and insulin releasing actions *in vitro*. *Journal of. Nutrition* 130, 2000, 15–20.
15. Kamanyi A, Dajmen D, Nkeh B. Hypoglycemic properties of the aqueous root extract of Morinda lucida (Rubiacea) study in the mouse. *Phytother Research*, 8, 1994, 369–371.
16. Bolkent S, Yamardag R, Tabakogluoguz A, Sacaon OO. Effects of chord extract on pancreatic β- cells in Streotozotocin-diabetic rats: a morphologic and biochemical study. *Journal of Ethnopharmacology*, 73, 2000, 251–259.
17. Rokeya B, Nahar N, Ali L, Hassan Z, Alam MNE, Choudhury SN, Azad Khan AK, Mossihuz zaman M. Effects of five medicinal plants on blood glucose levels in non-diabetic and diabetic model rats. *Diabetes Research*, 34, 1999, 219– 228.
18. Shanmugasundaram ERB, Gopinath KL, Shanmugasundaram KR, Rajendran VM. Possible regeneration of the islets of Langerhans in streptozotocindiabetes rats given Gymnema sylvetre leaf extracts. *Journal of Ethnopharmacology*, 30, 1990, 265– 279.
19. Esmaeili MA, Yazdanparas R. Hypoglycemic effect of Teucrium polium: studies with rat pancreatic islets. *Journal of Ethnopharmacology*, 95, 2004, 27–30.
20. Sharma SB, Nasir A, Prabhu KM, Murthy PS. Antihyperglycemic effect of the fruit-pulp of Eugenia jambolana in experimental diabetes mellitus. *Journal of Ethnopharmacology*, 104, 2006, 367–373.
21. Bierman EL, Amaral JAP, Belknap BH. Hyperlipidemia and diabetes mellitus. *Diabetes*, 15, 1966, 675–679.
22. Garber AJ. Attenuating CV risk factors in patients with diabetes: clinical evidence to clinical practice. Diabetes, *Obesity and Metabolism*. 4, 2002, S5–S12.

23. Grover JK, Vats V, Rathi SS. Antihyperglycemic effect of Eugenia jambolana and *Tinospora cordifolia* in experimental diabetes and their effects on key metabolic enzymes involved in carbohydrate metabolism. *Journal of Ethnopharmacology*, 73, 2000, 461–470.