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Research Article

ANTI HYPERGLYCEMIC ACTIVITY OF PETROLEUM ETHER EXTRACT & ETHYL ACETATE EXTRACT OF *SARCOCOCCA SALIGNA.*

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ABSTRACT

Sarcococca saligna is an evergreen shrub. It has many ethno botanical uses, the leaves and shoots are used to relief pain and as a laxative. Medicinally used in traditional, Ayurvedic system. It has a long history of uses by indigenous and tribal people and in Ayurvedic or Natural Herbal Medicine. The effect of Pet. Ether extract & Ethyl acetate extract of the leaf of *Sarcococca saligna* on blood glucose level in 18h fasted Rats & in High Fatty Diet Fed, STZ treated Rats was determined. 250 mg/kg of the leaf extracts were administered separately to a set of overnight fasted rats. Blood glucose concentration was evaluated at 0h and 3h after treatment in 18h fasted rat model & Overnight Fasted in High Fatty Diet Fed, STZ treated Rats respectively. Both extracts showed significant reduction in blood Glucose level in 18h fasted rat model compared to 0h blood glucose level & in High Fatty Diet Fed, STZ treated Rats compared to HFD, STZ Control.

Keywords: Sarcococca saligna, Streptozotocin, Antihyperglycemic, Fenofibrate, High Fatty Diet.

INTRODUCTION

Diabetes is any disorder characterized by excessive urine excretion. The most common form of Diabetes is Diabetes mellitus, a chronic, progressive, systemic condition of impaired Carbohydrate metabolism[1]. Diabetes mellitus is thus defined as a state in which homeostasis of carbohydrates and lipid metabolism is improperly regulated In this metabolic disorder, there is either defective, deficient insulin secretory response for normal function of many cells of the body resulting in persistent hyperglycemia or inadequate utilization of insulin at receptors level Under the condition when insulin is inadequate in the body, there are disorders of all kinds of metabolism, commonly with an increase in blood sugar accompanied by glycosuria, polyphagia, polyurea and polydipsia[2]. Insulin unavailability may be due to degenerative changes in β-cells in the pancreatic islets, reduced effectiveness of the hormone owing to the formation of anti-insulin antibodies or inactive complexes, immune-mediated islet Cytotoxicity or inappropriate secretion of hormones by neoplasm in other endocrine organs[3].

In this disease, glucose accumulates rapidly in the body fluids and as the blood glucose concentration increases beyond a certain point, it is excreted by the kidneys. Glycosuria causes a continual waste of this essential nutrient and due to reduced ability to use glucose, produces a depression of the functions of brain, muscles and many other tissues and follows with other serious metabolic consequences[4]. However, when normal amounts of insulin are inadequate to produce a normal insulin response from fat, muscle and liver cells, evolves a state of diabetes, known as insulin resistance. Insulin resistance (IR) in fat cells reduces the effects of insulin and results in elevated hydrolysis of stored triglycerides in the absence of measures which either increase insulin sensitivity or which provide additional insulin. Increased mobilization of stored lipids in these cells elevates free fatty acids in the blood plasma[5]. Insulin resistance (IR) in muscle cells reduces glucose uptake whereas insulin resistance in liver cells reduces storage of glycogen, making it unavailable for release into the blood when blood insulin levels fall (normally only when blood glucose levels are low). Both cause elevated blood glucose levels. High plasma levels of insulin and glucose due to insulin resistance often lead to metabolic syndrome and type II diabetes, including its complications[6].

Sarcococca saligna is an evergreen shrub, belonging to family *Buxaceae*. It has many ethno botanical uses, the leaves and shoots are used to relief pain and as a laxative. Medicinally used in traditional, Ayurvedic system. It has a long history of uses by indigenous and tribal people and in Ayurvedic or Natural Herbal Medicine, widely distributed throughout the northern areas of Pakistan and Kashmir at 5000–9000 ft altitudes. It is also widely distributed in W. Himalayas from Afghanistan to west of Nepal between 1-3000 m.[7]

The plant has been used in Pakistan for the hyperactive states of the gastrointestinal tract, liver diseases, syphilis, infections, fever, pain, inflammation and rheumatism. The ethanol extract of S. saligna showed antifungal activity[8].

MATERIALS AND METHODS

Experimental animals

Adult male/female Wistar rats (8 weeks), weighing 160-180 g bred in the Animal House, Indian Institute of Integrative Medicine (IIIM), Formerly Regional Research Laboratory (CSIR), Jammu, were used. All animal experiments were approved by the Institutional Animal Ethic Committee (IAEC), Indian Institute of Integrative Medicine, IIIM (Formerly Regional Research Laboratory) (CSIR), Jammu. The animals were housed in polycarbonate cages in a room with a 12h day-night cycle, temperature of 22 ± 2°C, humidity of 45–64%. During the whole experimental period, animals were fed with a balanced commercial pellet diet (Ashirwad Industries, Chandigarh, India) and water *ad libitum.*

Plant material

Sarcococca saligna In the present study, the plant material was collected in winter 2007 i.e. 12-01-2007 from the forest area of Patnitop, (Jammu) having altitude 7000 ft. The plant was authenticated by botanist Dr. S.N. Sharma Department of Taxonomy, I.I.I.M, Jammu, India and a voucher specimen was deposited in the Herbarium of Department of Botany, IIIM Jammu. (#IIIM 19186). After authentification, plant material was dried at room temperature until it was free from the moisture and subjected to physical evaluation with different parameters. The parameters which were used for evaluation are nature, odour, colour, taste, size, shape, width, length. Finally aerial parts were subjected to size reduction to get coarse powder. Then the uniform powder was subjected to standardization with different parameters Plant extracts received from NPC division IIIM, Jammu encoded as RJM-P01-A001 &RJM-KP-A002 for pet.ether extract & ethyl acetate extract of *sarcococca saligna* respectively.

METHODS

Effects of pet.ether extract & ethyl acetate extract of *sarcococca saligna* on Blood Glucose Levels in 18h Fasted rats model:

Wistar rats (male/female), 6 animals in each group were fasted overnight. The animals were divided into Normal Vehicle Control,

test (test extract/fraction) treated and reference (Glibenclamide) treated group. Blood glucose determination was done at 0h (prior to any treatment), 3h (Post-drug administration).

Effect of pet.ether extract & ethyl acetate extract of *sarcococca saligna* on blood glucose in high fatty diet fed, STZ treated Rats

Adult Wistar rats, 5 animals/Cage were housed at 22-26°C, 12h-12h light/dark cycle, fed with normal feed with a composition of Crude protein- 22.05%, Crude Oil- 4.55%,Crude fibre-3.2%, Ash- 6.45%, Sand silica- 1.25%. As calculated from 9 KCal/g of fat, 4 KCal/g of protein and 4 KCal/g of carbohydrates, 100 g of Normal Fat Diet (NFD) gives 142 KCal energy of which only 29% is derived from 4.5 g of Oil/fat present in the feed.

The High Fat Diet (HFD) should contain enough fat to denote 60% of KCal energy. Therefore, adding of extra fat 912.5 g of lard, to 100 g NFD, makes diet of composition per 100g feed- Protein : 22g, Fat : 4.5 g, lard : 17g and Carbohydrate : 3.2g.

Normal fat diet: Normal chaw fed As per capacity of the animals (M/S Ashirwad)

High fat diet: Normal diet as per capacity of the animal + (15g/kg Amul Butter +1g/kg cholesterol once daily p.o.)

Diet schedule: 0 - 42 days

STZ Treatment: 40mg/kg, i. p. on Day 14 (overnight fasted, feed and water 1 hr after STZ)

Sample collection: 0, 14th, 28th, and 42nd day

Blood Parameters: Blood Glucose.

Estimation of blood glucose concentration

Blood glucose was estimated by glucose oxidase/peroxidase (GOD/ POD) method, followed procedure as per manufacturer of enzymatic kit, using commercially available from Siemens Medical Solutions Diagnostics Ltd., Baroda, Gujarat, India. (formarly known, Bayers Daignostics India Limited, Baroda.).

Table 1: Grouping of animals

Groups	Treatments
Group 1:	NFD (Normal Control)
Group 2:	NFD (STZ Control)
Group 3:	NFD (STZ+ Fenofibrate)
Group 4:	NFD (STZ +A001)
Group 5:	NFD (STZ+A002)
Group 6:	HFD (Normal Control)
Group 7:	HFD (STZ Control)
Group 8:	HFD (STZ +Fenofibrate)
Group 9:	HFD (STZ +A001)
Group 10:	HFD (STZ +A002)

Statistical analysis

Results obtained were expressed as Mean ± Standard error of the mean. Analysis of variance test were used to compare the means. Values of $p<0.001$ were regarded as being significant.

RESULTS

Effect on blood glucose level in 18h fasted rats

A total Six Samples were evaluated for their Hypoglycemic effect on 18h fasted Rats in the single dose treatment at 250 mg/kg p.o., Two Samples of them RJM-P01-A001 & RJM-KP-A002 showed hypoglycemic activity in 18h fasted Rat model when which indicates that these extracts may have insulin secreting or insulin like activity. The data on blood glucose level in 18h fasted Rats has been shown in the table 2 and graphically represented in the fig. 1.

Table 2: Effect of *different herbal plant* extracts on blood glucose level in 18h fasted rats

S.NO	Treatment group (mg/kg p.o.)	Blood Glucose level (Mg/dl) Mean ± S.E	
		0 h	3 h
1.	Vehicle Control	82±2.99	83±4.06
2.	Glibenclamide Control (0.5)	92±5.0	64±1.81***
3.	RJM-P01-A001 (250)	80±3.32	57±2.36**
4.	RJM-P01-A002 (250)	87±4.54	83±3.62
5.	RJM-KP-A002 (250)	81±5.12	58±1.04**
6.	RJM-KP1-A003 (250)	76±6.57	74±1.85
7.	PIM2 (250)	92±1.35	87±5.15
8.	BUB(DE) (250)	89±2.9	83±3.5

n (Number of animals) : 6

*** $p < 0.001$; ** $p < 0.01$ compared to 0h blood glucose level

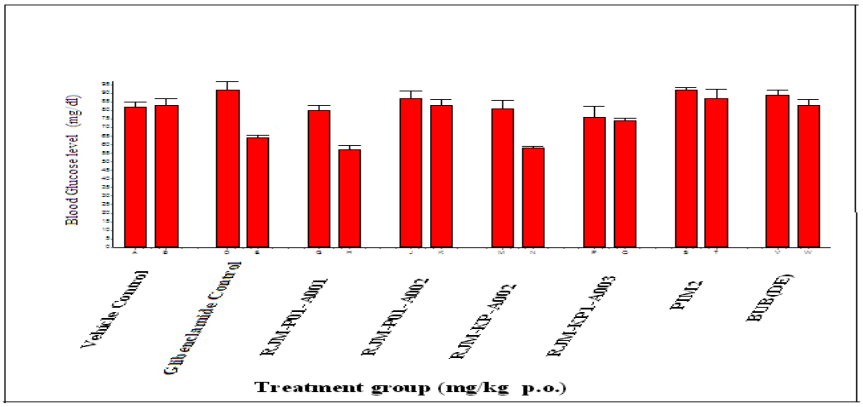

Fig. 1: Effect of *different herbal plant* extracts on blood glucose level in 18h fasted rats

Effect of pet. ether extract & ethyl acetate extract on blood glucose in High Fatty Diet Fed, STZ treated rats

These two extracts were evaluated on blood glucose in High Fatty Diet Fed, STZ treated Rats in the single dose treatment at 250 mg/kg p.o., these two sample showed significant decreased in blood glucose Level as compare to HFD: STZ Control. The data on blood glucose in High Fatty Diet Fed, STZ treated Rats has been shown in the table 3 and graphically represented in the Fig.2.

Table 3: Effect of RJM-P01-A001& RJM-KP-A002 on blood glucose in high fatty diet fed, STZ treated rats

S.no	Treatment group (mg/kg p.o.)	Blood glucose level (mg/dl) (Mean ±S.E.)			
		Day 0	Day 14th	Day 28th	Day 42th
1.	NFD: Normal Control	70±4.15	79±6.77	73±6.55	75±6.38
2.	NFD: STZ Control	69±5.16	77±7.02	260±7.86	243±11.23
3.	NFD: STZ+Fenofibrate(300)	80±2.98	85±9.51	160±24.7***	124±21.09***
4.	NFD: STZ+A001(250)	75±5.51	76±6.20	211±13.95***	197±13.37***
5.	NFD: STZ+A002(250)	88±6.35	90±6.71	267±9.78	231±13.84**
6.	HFD: Normal Control	96 ±8.3	140±4.71	135±6.34	163±5.42
7.	HFD: STZ Control	100±6.72	106±6.59	241±13.68	257±14.87
8.	HFD: STZ +Fenofibrate(300)	98±9.51	109±7.38	117±10.54***	105±6.66***
9.	HFD: STZ+A001(250)	97±7.28	96±4.72	146±12.45***	122±11.59***
10.	HFD: STZ+A002(250)	101±4.77	107±7.21	145±11.84***	130±15.07***

n(Number of animals) : 6

*** $p < 0.001$; ** $p < 0.005$ compared to HFD: STZ Control.

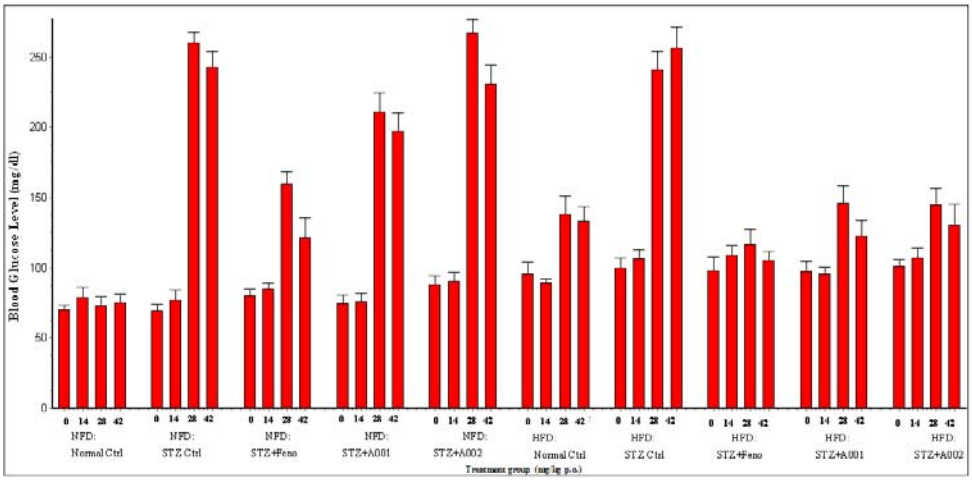

Fig. 2: Effect of RJM-P01-A001& RJM-KP-A002 on blood glucose in high fatty diet fed, STZ treated rats

DISCUSSION

Obesity is increasing problem worldwide and induces many diseases like diabetes, atherosclerosis and other metabolic syndrome. So the knowledge about the process of adipogenesis and formation of adipose tissue is very important. For the better understanding of these processes, at least the study should be conducted on *in-vivo* model. For *in vivo* experiments, the high fatty diet, STZ treated rats have been used as a model to evaluate the effect of test products on lipid and fats metabolism and insulin resistance. To develop the antidiabetic drugs, the lipid centric approach has been widely used. The present study has been envisaged to evaluate the herbal extracts in these two experimental conditions.

The main finding of the study on the test plant extracts was that RJM-P01-A001& RJM-KP-A002 prevented a rise in blood glucose level in glucose primed rats and STZ treated diabetic animals. Based on the observations made from these studies, it is concluded that, it is the blood glucose of the organism bears an equal relevance to diabetes.

Over the past two decades, the interest in medicinal plants has grown enormously leading to routine scientific investigation of numerous plant extracts for their biological effects and potential therapeutic properties in human. A detailed investigation of plants used in local health traditions and pharmacological evaluation of these plants and their taxonomical relatives can lead to the development of invaluable plant drug for many dreaded diseases including diabetes mellitus. Thus based upon the background of diversified therapeutic values and uses in diabetes mellitus and its complications in folklore, available plants *Sarcococca saligna* was taken up for present investigation. The aim of the present study was to evaluate the antidiabetic potential of these test plants. In the present investigation, the *in vitro* and *in vivo* experimental models available, with a focus on the therapeutic targets for diabetes mellitus, were used to investigate the test extracts for their antidiabetic efficacy and pre-clinical safety. *Sarcococca saligna* unknown for antidiabetic activity were taken up for lipids profiling for diabetes mellitus.

REFERENCES

1. Sachan N. K., Kumar Y., Pushkar S., Thakur R.N., Sudhir S. Gangwar,V.K. ;Antidiabetic potential of alcoholic and aqueous extracts of *Ficus recemosa* Linn. bark in normal and alloxan induced diabetic Rats, *International Journal of Pharmaceutical Sciences and Drug Research*, 2009; 1(1):24-27.
2. Frank B; The endocrine glands. In: Canine Medicine. The work of forty three authors, (ed.). *American Vet. Publ Inc*., California 1962.
3. Nelson R W; Disorders of glucose metabolism in the dog-1. Diabetes mellitus *Vet. Med.,* 1985; 27-37.
4. Botazzo G F, Doniach D, Pouplard A; Humoral autoimmunity in diabetes mellitus. *Acta Endocrinol*, 1998; 83: 55-64.

5. Rastogi D P, Singh D M and Kumar S; Elucidation of therapeutic efficacy of potentized microdoses of homeopathic drugs: An experimental approaches with alloxan as model vis-à-vis its antidiabetic activity. *Hom Herit*, 1998; 23:83-88.
6. McGarry J"Banting lecture: dysregulation of fatty acid metabolism in the etiology of type 2 diabetes". *Diabetes*, 2001; 51(1): 7–18.
7. Rahman, A., Choudhary, M. I., Khan, M. Riaz., Anjum, S., Farooq A., and Iqbal, M., New Steroidal Alkaloids from *Sarcococca saligna, J. Nat. Prod.,* 2000; 63: 1364-1368.
8. Moghaddam, K. M., & Mohammad, A., Rafique, J., Rezaee, S., Fesharaki, P. J., Gohari, A. R.& Shahverdi, A. R.,: The Antifungal Activity of Sarcococca saligna Ethanol Extract and its Combination Effect with Fluconazole against Different Resistant Aspergillus Species, *Appl Biochem Biotechnol*; 2009; 009-8737-2.
9. Bonner-Weir, Morphological evidence of pancreatic polarity of beta cells within islets of langerhans. Diabetes, 1988; 37:616-21.
10. Henry, J. B., Clinical Diagnosis & management by Laboratory methods, W.B. Saunders, Philadelphia 18th ed, 1991; 204-211.
11. Mohamed, B., Said, B., Wafaa, B., Abdelkhaleq, L., Abderrahim, Z. and Hassane, M. Antidiabetic Activity Assessment of *Argania spinosa* Oil, *Journal of Complementary and Integrative Medicine*: 2008; Vol. 5: Iss. 1, Article 32.
12. Reed, M.J. , Meszaros, K., Entes L.J., Claypool M.D., Pinkett, A New Rat Model of Type 2 Diabetes: The Fat-Fed, Streptozotocin-Treated Rat Metabolism, 2000;49; 1390-1394.
13. Ahmed, Z., M Z Chishti, Johri, R. K. Bhagat, A., Gupta, K. K., Ram G, Antihyperglycemic And Antidyslipidemic Activity Of Aqueous Extract Of Dioscorea Bulbifera Tubers, Diabetologia Croatica,2009; 38-3,63-72.
14. Barham, D., and Trinder, P,An improved color reagent for the determination of blood glucose by the oxidase system. Analyst, 1972; 97, 142–145.
15. Buccolo, G., David, H, Quantative determination of serum triglycerides by the use of enzymes. Clin Chem; 1973;19, 76–480.
16. Annoni, G., Bottasso, B.m., Ciaci, D., Donato, M.F., Tripodi, A, Evaluation of a new enzymatic colorymetri method for Triglyceride estimation. Res. Lab. Med. 1982, 9, 115.
17. Lopes-Virella MF, stone p, Ellis S, Colwell JA, Cholesterol determination in HDL separated by three different methods. Clin Chem; 1977; 23. 882-84.
18. Lopes-Virella, M.F, Shere, G.K, Lees, A.M., Wohltmann, H., Mayfield, R., Sagel, J., LeRoy, E.C., Colwell, J.A; Surface binding, internalization and degradation by cultured human fibroblasts of LDL isolated from type I (Insulin-depedent) diabetic patients: Changes with metabolic control. Diabetologia, 1982; 22, 430-436.
19. Friedawald,W.T., Levy, R.I., Fredrickson, D.S; Estimation of the concentration of low- density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin. Chem; 1972; 18, 499-502.
20. Castelli, W.P., Doyle, J.T., Gordon, T., Hames, C.G., Hjortland, M.C., Hulley, S.B., Kagan, A., Zukel, W.;. HDL cholesterol and other lipids in coronary heart disease. The cooperative lipoprotein phenotyping study. Circulation, 1977; 55, 767-772.
21. Allain, C.C, Poon, L.C., Chan,C.S., Richmond, W., Fu, P.C.,. Enzymatic determination of total serum cholesterol. Clinical Chemistry. 1974; 20, 470-475.
22. Miller, N.E., Forde, O.H., Thelle, D.S., Mjos, O.D; High-density lipoprotein and coronary heart-disease. A prospective case-control study. Lancet, 1977; 1, 965-968.
23. Ren, A. J. Guo, Z. F. Wang; Inhibitory effect of obestatin on glucose-induced insulin secretion in rats 2008.
24. Tinnikov, A. A., Boonstra, R.: Colorimetric micro-determination of free fatty acids in plasma using microplate readers, *Clinica Chimica Acta,* 1999; 281 159 –162.